ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-2 (HER-2) IN COMMON SALIVARY GLAND CARCINOMAS

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Background: Carcinomas of the salivary gland are known to be aggressive in nature, making them difficult to manage. The therapeutic options offered include excision of the gland (maxillectomy in cases of palatal tumours), with or without lymph node dissection, proceeded with radiotherapy. Chemotherapy has not produced promising outcomes and has a minimal impact as a therapeutic alternative. Targeted therapy against human epidermal growth factor receptor 2 (HER-2), which is a commonly used treatment modality for their mammary analogues, is not being offered to these patients since scant literature is available showing its usefulness and no promising evidence is present regarding their efficacy and efficiency in such cases. The study aimed to evaluate and quantify the immunohistochemical expression of HER-2 in cases of adenoid cystic carcinoma (AdCC), mucoepidermoid carcinoma (MEC) and salivary duct carcinoma (SDC), which are analogues of similar tumours arising in breast tissue. **Methods:** A retrospective, cross-sectional study was carried out in the department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, duration of which was six months. A total of 45 cases (15 of each tumour) were taken, and sampled using non-probability convenience technique. The immunohistochemical marker, monoclonal HER-2 antibody (Leica microsystem Germany) was applied on appropriate blocks of all included cases. The staining pattern and intensity were recorded after visualizing the slides under a light microscope. **Results:** Seven cases of salivary duct carcinoma and a single case of mucoepidermoid carcinoma expressed positivity for HER-2, while no expression could be seen in the case of adenoid cystic carcinoma. A statistically significant difference was seen when HER-2 expression was compared among the aforementioned tumours. **Conclusion:** The use of targeted therapy against HER-2 is limited to patients of salivary duct carcinoma and a fraction of patients suffering from mucoepidermoid carcinoma.

Keywords: Adenoid cystic carcinoma; Human epidermal growth factor receptor 2; Immunohistochemistry; Mucoepidermoid carcinoma; Salivary duct carcinoma; Salivary gland

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INTRODUCTION

Breast cancer is the most common malignancy among women in Pakistan. It has a five-year prevalence of 56.39 per 100,000.[1] It has been a long since targeted therapy was introduced for these malignancies, which is provided based on the receptor they express on immunohistochemistry. About 10–30% of over-express HER-2.[2] HER-2 positive tumours are treated with trastuzumab. Treatment with this drug has shown excellent results and increased overall survival rate.[3]

Salivary gland carcinomas are becoming common with every passing day. These tumours are known to be relentless and aggressive, making them very difficult to treat. The treatment options currently available are limited and include, excision with clear margins (maxillectomy in cases of palatal tumours) and radiation therapy. Lymph node dissection is done in cases with evidence of nodal metastasis. The results of chemotherapy in patients with salivary gland cancer are not promising.[4] Targeted therapy is not being considered as a treatment modality as there is very scant evidence proving its benefit. Salivary glands are analogues of breast tissue. Both share the same morphology as they arise from tubular-acinar exocrine tissues.[5] Our study included the most commonly occurring, AdCC and MEC, which are known to arise in both, breast and salivary gland.[6] Salivary duct carcinoma being an analogue of high-grade mammary duct carcinoma, was also studied.[7] Aquino *et al*. in 2018 claimed in his paper that these malignancies are the commonest and often, the most aggressive.[8]

According to World Health Organization (WHO), mucoepidermoid carcinoma is a glandular malignancy of epithelial origin comprising mucus,

intermediate and squamoid cells arranged in cystic and solid patterns.[9] It is composed of an admixture of mucous, squamous and intermediate cells arranged in a cystic pattern.[10]

WHO defines adenoid cystic carcinoma as a biphasic tumour comprising epithelial and myoepithelial cells arranged in cribriform, tubular and solid patterns.[9] On histopathological examination, an admixture of polygonal epithelial cells and basaloid myoepithelial cells are seen arranged in the three previously mentioned patterns. High-grade AdCC shows marked cytological atypia, necrosis and frequent mitoses.[11,12]

Salivary duct carcinoma is defined as an aggressive epithelial malignancy that closely resembles high-grade mammary ductal carcinoma.[9] It can either occur as a component of carcinoma ex pleomorphic adenoma or may arise de novo. Microscopy reveals an epithelial malignancy that possesses pleomorphic nuclei and conspicuous nucleoli. The cytoplasm of these cells is eosinophilic. Mitoses are frequent. Areas of comedo necrosis are common.[13] HER-2 is a common target against which therapy is given to treat breast cancers. Tumours that are positive for HER-2 upon application of immunohistochemistry are treated with Trastuzumab.[3] Since breast and salivary tissue are similar, it would not be implausible to expect the same treatment response against HER-2 in patients of salivary gland cancers however, literature regarding HER-2 expression in salivary gland malignancies is scarce.

MATERIAL AND METHODS

After obtaining ethical approval from the review committee, a retrospective cross-sectional study was carried out in the Department of histopathology, Armed Forces Institute of Pathology, Rawalpindi, the duration of which was six months. The sample size was calculated using the WHO sample size calculator.[14] A total of 45 samples (15 samples of each tumour) were taken. Cases of AdCC, MEC and SDC reported during January 2018 and December 2021 were retrieved till the desired sample size was achieved. Samples from both genders and all age groups were considered. Specimens with scanty tissue, extensive necrosis, poor fixation and samples demonstrating an equivocal score of HER-2 were excluded from the study. Paraffin-embedded blocks were retrieved from the records of histopathology department, AFIP. Fresh haematoxylin and eosin-stained slides were prepared with the assistance of laboratory staff and analyzed by two histopathologists. Appropriate sections were selected and the immunohistochemical marker, HER-2 was applied using indirect technique. The sections were deparaffinized and rehydrated after which the antigen retrieval was carried out. Endogenous peroxidase was blocked and an unlabelled primary antibody was applied. This was followed by the introduction of labelled secondary antibodies. The chromogen was applied after which the sections were counter-stained, dehydrated, cleared and mounted.[15] The results were interpreted using the given criteria.

Table-1: Quantification of HER-2[16]

Staining pattern	Score	HER-2 protein over-expression assessment
No staining or membranous staining in less than 10% of tumour cells	0	Negative
Faint partial membranous staining in more than 10% of tumour cells	1+	Negative
Weak to moderate complete membranous staining in more than 10% tumour cells/strong complete membranous staining in less than 10% tumour cells	2+	Equivocal
Strong complete membranous staining in more than 10% of tumour cells	3+	Positive

The images of HER-2 intensity (weak/moderate/severe) by Guo-Dong Cao were used as a reference while evaluating the results.[17]

Scores of 0–1+ were considered negative, 2+ or equivocal cases were excluded and only cases with a score of 3+ were considered positive.

Positive control was obtained on breast carcinoma known to express HER-2 positivity while negative control was achieved on brain tissue.

RESULTS

A total of 45 cases were studied which included 15 cases of each tumour, namely MEC, AdCC and SDC. Haematoxylin and eosin-stained slides of each case were thoroughly studied, blocks were carefully selected and an immunohistochemical marker, HER-2 was applied.

Out of 45 patients, 27 (60%) were males and 18 (40%) were females. Among 15 patients of mucoepidermoid carcinoma, 10 were males and 5 were females. Samples from 7 males and 8 females were studied in the case of adenoid cystic carcinoma. Salivary duct carcinoma followed the same gender distribution trend as mucoepidermoid carcinoma.

The mean age of patients who participated in the study was 51.87±16.39 (mean±standard deviation). The oldest participant was 90 years of age while the youngest one was 21 years old. The mean age in the case of mucoepidermoid carcinoma was 44.40±13.79. In patients with adenoid cystic carcinoma, the average age was calculated to be 48.40±15.18. Salivary duct carcinoma patients had an average age of 62.80±14.93. A total of 23 out of 45 cases (51.1%)

of the three carcinomas were found to arise in the parotid gland, making it the commonest site followed by the soft palate and hard palate. The most frequent site of occurrence in the case of MEC and SDC was parotid while AdCC was found to arise most frequently on the soft palate. HER-2 expresses membranous staining. Cases were scored according to a set criterion already mentioned in Table-1.[16] Only cases with strong complete membranous staining in more than 10% of cells were scored 3+ and considered positive. 2+ was kept as an exclusion criterion while 1+ score was considered negative. A single case (6.7%) of mucoepidermoid carcinoma and 7 cases (46.7%) of salivary duct carcinoma were positive for HER-2.

To compare HER-2 expression among tumours, Fisher's exact test was run, which gave a *p*-value of 0.003, rendering it significant.

Test of normality, the Shapiro-Wilk test was applied that indicated the data is non-parametric. For comparison of HER-2 scores in tumours, the Kruskal-Wallis test was used which gave the hypothesis summary as given in table-3.

A *p*-value of 0.003 was achieved, suggesting that HER-2 scores are not the same across all tumours, since salivary duct carcinoma had a remarkably increased expression as compared to mucoepidermoid carcinoma and adenoid cystic carcinoma.

Kruskal-Wallis test with post-hoc Tukey test was carried out for pair-wise comparisons in the case of HER-2 to determine which tumour groups differ.

Pairs of salivary duct carcinoma with adenoid cystic carcinoma and mucoepidermoid carcinoma showed a significant value however, comparison of immunohistochemical expression of HER-2 among MEC and AdCC was insignificant since both showed minimal positivity for the aforementioned immunohistochemical marker. This suggests that targeted therapy can primarily be used for the treatment of salivary duct carcinoma.

Table-2: Expression of HER-2 in MEC, AdCC. and SDC.

Immunohistochemical marker	Interpretation	MEC	AdCC	SDC	*p*-value
HER-2	Positive	1 (6.7%)	0 (0%)	7 (46.7%)	0.003
	Negative	14 (93.3%)	15 (100%)	8 (53.3%)	

Table-3: Hypothesis summary

Null hypothesis	Test	Significance	Decision
The distribution of HER-2 score is same across the categories of tumours	Independent-sample Kruskal-Wallis Test	.003	Reject the null hypothesis

Table-4: Pair-wise comparison of carcinomas (HER-2)

Pair	Test statistic	Std. error	Std. test statistic	Sig.
Mucoepidermoid carcinoma – Adenoid cystic carcinoma	1.600	3.599	0.445	.657
Adenoid cystic carcinoma - Salivary duct carcinoma	-11.200	3.599	-3.112	.002
Mucoepidermoid carcinoma - Salivary duct carcinoma	-9.600	3.599	-2.668	.008

DISCUSSION

Salivary gland carcinomas are aggressive and relentless. These tumours are heterogenic, presenting with a variety of signs and symptoms, displaying a wide array of morphologies. About 20% of these carcinomas are malignant while a majority (80%) of these are benign. The most prevalent among these is mucoepidermoid carcinoma, followed by adenoid cystic carcinoma.[18,19] Salivary duct carcinoma may arise de-novo or as part of carcinoma ex pleomorphic adenoma.[20]

No definitive treatment options are available for these cancers. Currently, physicians and surgeons rely on the excision of the tumour, with or without lymph node dissection followed by radiotherapy. The use of chemotherapy is limited since it has not yielded promising results in the past. There is a dire need for the exploration of targets against which therapy can be provided for better outcomes and the survival of these patients.

The study aimed to quantify and determine the immunohistochemical expression of human epidermal growth factor receptor-2 in salivary gland carcinomas. Limited work has been carried out to evaluate the immunohistochemical expression of HER-2 in salivary gland carcinomas till now.

In our study, HER-2 was found to be positive in 1 case (6.7%) of MEC, a score of which was 3+. One case expressed a score of 1+ but was considered negative, in accordance with the set criteria. A total of 46.7% of cases (7 out of 15) of

salivary duct carcinoma expressed positivity and a score of 3+. Gene amplification of HER-2 in cases of SDC provides concrete evidence for its increased immunohistochemical expression in these cases.[9] No case of adenoid cystic carcinoma was found to be positive for HER-2.

In 2020, research carried out by Hanna *et al*. in Massachusetts, USA declared that 34 out of 52 (65.3%) salivary duct carcinomas expressed positivity of HER-2 when I.H.C. was applied. All scores, 1+, 2+ and 3+ were positive in contrast to our study, where only a score of 3+ was considered positive in accordance with the widely used criteria. This led to the difference in our results.[21]

Similar studies carried out in Brazil by Santana *et al*. in 2019 and by Ryu *et al*., in 2018 showed promising results with HER-2 positivity in SDC.[22,23] The frequencies of positive responses were 62.5% and 64.2% respectively. Another study demonstrated that 13% of cases of mucoepidermoid carcinoma, 4% of adenoid cystic carcinoma and a majority (76%) of salivary duct carcinoma over-expressed HER-2.[24] These researchers confirmed all positive cases with in situ hybridization techniques. Equivocal cases, which could be positive if confirmed by FISH, were eliminated from our study due to budgetary restrictions hence, our outcome differed from the others.

The findings of our study and those of other pertinent research are only slightly different. Since the soft palate is the second most frequent site in our case while the submandibular gland was found to be a frequent site in other research, certain changes in trends may be noted. Additionally, ethnicity and sampling technique might be important factors in this context.

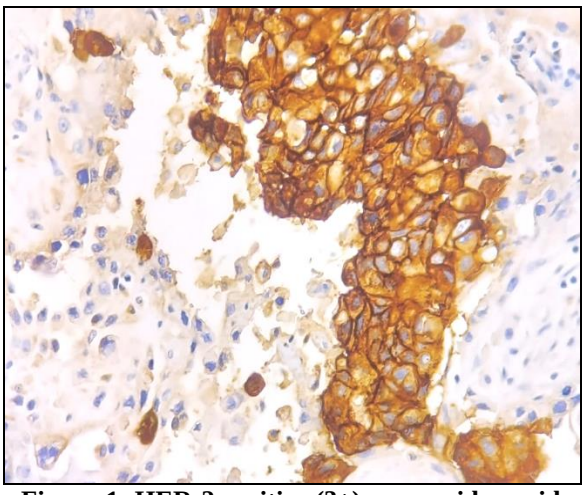

Figure-1: HER-2 positive (3+) mucoepidermoid carcinoma (40X)

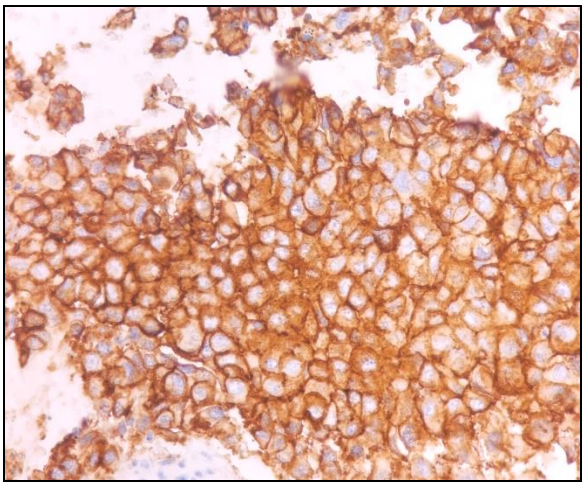

Figure-2: H.E.R.-2 positive (3+) salivary duct carcinoma (40X)

The limitation of the study is that the cases demonstrating a HER-2 score of 2+ were excluded from the project due to constraints of the budget. These cases could have a positive expression based on which, targeted therapy could be provided to such patients.

The recommendation for future studies is that cases of HER-2 with an equivocal expression should be confirmed using FISH, fluorescence in situ hybridization. Other salivary gland carcinomas should also be studied for possible HER-2 overexpression so patients can benefit from trastuzumab, if stained positive. Also, since scant literature is available, patients of salivary gland carcinomas who demonstrate HER-2 upon IHC and receive targeted therapy against it should be kept on long-term follow-up to assess the efficacy and effectiveness of targeted therapy as a potential treatment option.

CONCLUSION

Statistically significant differences in immunohistochemical expression and scores of HER-2 in salivary gland carcinomas favour the role of targeted therapy in patients of salivary duct carcinoma and a fraction of patients of mucoepidermoid carcinoma however, its use in adenoid cystic carcinoma is limited.

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AUTHOR'S CONTRIBUTION

ZS, NZ: Conceived, and designed the study and data analysis, write up and literature search, NA, SM: proofread the study, ZA, AH and ZSS: Data collection, literature search and final review.

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