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Original Article

Immunohistochemical Expression of EGFR and Her2; and its Correlation with WHO Grades of Head and Neck Squamous Cell Carcinoma

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ABSTRACT

Background: Head and neck squamous cell carcinoma is the seventh most common malignancy in the world. EGFR is over expressed in approximately 80% to 90% of all cases of squamous cell carcinoma of head and neck (HNSCC), whereas HER2 overexpression ranges from 0 to 53%.

OBJECTIVES: To identify the expression of EGFR & HER2 and their correlation with WHO grades in Head and neck squamous cell carcinomas

Materials and Methods: This Cross-sectional comparative study was carried out in Department of Pathology, PIMS, Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Islamabad, from December 2017 till November 2018. Mucosal biopsies were processed and stained with Hematoxylin and Eosin (H&E). Slides were examined and histological sub-typing of HNSCC was done. Immunohistochemistry was performed on selected sections to evaluate immuno-expression of EGFR and HER2. Immunohistochemical scoring was done taking into account both staining intensity and percentage of positive cells.

Results: Out of 74 cases, 47 were males and 27 were females. 38 cases were G1 well differentiated, 19 were G2 moderately differentiated and 17 were G3 poorly differentiated SCC. Most frequent site of SCC was oral cavity followed by larynx and pharynx respectively. EGFR was positive in 60 (81.08%) cases and HER2 in 33(44.59%) cases. All HER2-positive cases were also positive for EGFR, except for one poorly differentiated carcinoma.

Conclusion: A statistically significant correlation is seen between IHC immunoexpression of EGFR and HER2. Also, gender shows a clinically significant correlation with anatomical sites of HNSCC. Age groups and anatomical sites are also significantly correlated with the WHO grades of tumor.

Key Words: Head and Neck, Squamous Cell Carcinoma, WHO Grades, Immunohistochemistry, HER2, EGFR

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer globally.[1] Approximately 600,000 new cases are reported each year. [2, 3] Oral cancers are the second most common among HNSCC cancers in the Indian population. [4]This may be attributed to excessive utilization of gutka and tobacco in this zone. [5]

Pakistan stands among the top high risk regions for head and neck malignancies regarding cancer landscape.

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In an epidemiological survey in 2006 done in Karachi, HNSCC accounted for 21% of all cancers in males and 11% of all cancers in females. In both genders oral cavity was the most affected site followed by larynx. Mean age was 53 years and predominant histological grade was G2 (47%) and most had advanced stage (III or IV) with distant metastasis (65%) at the time of diagnosis.[6]Overall five year survival rate for HNSCC is 40 to 60% while two year survival in patients with metastasis or recurrent disease is 28 to 55 %.[7]

HNSCC arises from mucosa of paranasal sinuses, oral cavity, nose, pharynx and larynx.[8] Grading of HNSCC is done according to WHO guidelines (Barne's et al., 2005) which is based on Broder's system of grading developed in 1920 for oral squamous cell carcinoma and is now applied to all sites in head and neck.[9] This system is based on differentiation grade, keratin

exhibition, mitotic count and extent of pleomorphism.[10]

Epithelial growth factor receptor (EGFR) or ERBB1 is a representative of HER protein Family and is expressed normally in all cells of epithelial origin. It is a transmembrane glycoprotein and a tyrosine kinase receptor. EGFR is a promoter for proliferation of cells and inhibitor of apoptosis. EGFR mutation or amplification can lead to activation and an aberrant over-expression. [11, 12]. EGFR shows a significant link with tumor size, stage, distant metastasis and reduced overall survival.[13]Targeting EGFR with concurrent chemotherapy has shown to be superior in terms of patient outcome as compared to radiation alone.[14]

Human Epidermal growth factor receptor 2 (HER2) or ERBB2 is also one of the components of the same HER protein Family. It is also a transmembrane tyrosine kinase receptor involved in proliferation and growth of cells. HER2 overexpression is widely seen and studied in breast, gastro-esophageal, urothelial, ovarian, endothelial, endometrial, pancreatic and non-small cell lung cancers. HER2 primarily functions as a heterodimerization partner for EGFR. It is a validated poor prognostic marker in HNSCC. It shows a variable immuno-expression in HNSCC cases and its overexpression is significantly linked with the advanced clinical stage of disease. Many studies have shown that HNSCC patients respond well to anti-HER2 chemotherapy with which the overall survival is improved .[15]

In this study we have studied the IHC expression of EGFR and HER2, both of which are markers of poor prognosis. [16]

HNSCC at an early stage is treated well by surgery and radiation. Advanced stage HNSCC requires addition of chemotherapy which shows an overall improvement in reduction of distant metastasis.[17] Some cases that overexpress EGFR or HER2, fail to respond to their target therapy. Such refractory cases are seen responding very well when co-targeted with anti-EGFR and anti-HER2 drugs like Afatinib. The reason can be essential heterodimerization of HER2 with EGFR which is thought to mediate the disease progression.[18, 19]HER2 and EGFR activation causes increased invasiveness and motility in cancer cells by inducing epithelial to mesenchymal transition and shorter disease-free and over-all survival.

Through this study we aim to stratify potentially high risk cases so that a prompt action can be taken by the physician and target therapy is started as early as possible.

Materials and Methods:

This Cross-sectional comparative study was carried out in Department of Pathology, PIMS, Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Islamabad, from December 2017 till November 2018. Sample size was calculated by using WHO sample size calculator, using the formula as follows:

- n=Z (1-P) /d2 (where Z= Z1- α/2) taking following parameters:
- Confidence level: 95%
- Z2 = (1.96)2 for 95% confidence (i.e. α= 0.05)
- Anticipated population proportion (P): 10% or 0.1 (3)
- Absolute precision (d): 7% or 0.07
- Sample Size n = 74

Consecutive non-probability sampling technique was applied in the present study. Biopsies and resection specimens of all adult male and female patients that were received in the department of Histopathology, PIMS during study period with histopathological diagnosis of head and neck squamous cell carcinoma were included. All head and neck malignancies that do not fall into the category of squamous cell carcinoma and metastatic tumors to head and neck region were excluded. All those cases in whichbiopsy specimenwas inadequate were also excluded.

Approval from Ethical Review Board (ERB) Committee and Advanced Studies and Research Board (AS&RB), Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU) was taken. After fixation of the specimen in 10% formalin solution, gross examination was done according to AJCC protocols and submitted in tissue cassettes. Tissue was then processed in automated tissue processor, LIECA TP-1020, followed by cutting of 3 to 4 micron tissue sections. The sections were then mounted on glass slides and staining of the tissue with H&E in a tissue stainer Shandan Varistan 24-4 was done.

Slides were examined under Olympus CX 22 LED series microscope by post-graduate resident along with supervisor and diagnosis was recorded. Out of 74 cases 38 were well differentiated, 17 were moderately differentiated and 19 were poorly differentiated SCC. Patient's data and other relevant patient details were recorded on the proforma.

Three to four micron sections of selected 74 blocks were prepared. IHC was applied for EGFR and HER2. Positive and negative controls were run with each batch. The slides stained for EGFR and HER2 were examined under light microscope. Interpretation and

scoring was done according to the following criteria. (Table .1).

Table.1: Scoring criteria for HER2 and EGFR in HNSCC

HER2 IHC score	Percentage of cells showing expression + staining intensity	HER2 expression assessment
0	00%	Negative
1+	<10% weak incomplete	Negative
2+	<10% weak complete >10% moderate, intense incomplete	Positive
3+	>30% intense complete	Positive
0	00%	Negative
1+	<10% weak incomplete	Positive
2+	<10% weak complete >10% moderate, intense incomplete	Positive
3+	>30% intense complete	Positive

Data was entered in computer, analyzed using SPSS version 20.0.Mean and standard deviation was calculated for quantitative variables like age and immunohistochemical scores of both markers. Chi-square test was applied to compare histopathological subtypes on the basis of grade and immunohistochemical markers (HER2 and EGFR).P value <0.05 was taken as significant.

Results:

Out of 74 cases of HNSCC, 38 were well differentiated, 19 moderately differentiated and 17 poorly differentiated squamous cell carcinomas.

Out of 74 cases, 47 (63.5%) were males and 27 (36.5%) were females with a male to female ratio of 1.7:1. Out of 47 male cases, 24 (51.06%) were well differentiated, 9 (19.14%) were moderately differentiated and 14 (29.78%) were poorly differentiated. Out of 27 female cases, 14 (51.85%) were well differentiated, 10 (37.03%) were moderately differentiated and 3(11.11%) were poorly differentiated. In both genders, the predominant histological subtype is well differentiated SCC. In our study the correlation of gender with WHO grades of HNSCC was statistically insignificant with a p-value of 0.093.

Out of a total of 74 cases of HNSCC, 50 cases (67.5%) were from oral cavity, 14 cases (18.9%) were from larynx and 10 (13.5%) were from pharynx. Anatomical site of tumor and WHO grades were also significantly correlated**.**

Anatomical sites for HNSCC show a clear link with the gender in our study. Oral cavity was the most frequent anatomical site involved in our study. In males' second most involved site was larynx followed by pharynx, whereas opposite was seen in case of females i.e second most involved site was pharynx followed by larynx.

Anatomical site is significantly correlated with the Female gender having p value of 0.006 (Table .2)

Table .2: Correlation of Anatomical Site of tumor with WHO Grades

WHO Grade		Site			Total	
		Larynx	Oral cavity	Pharynx		
Grade	Well	7(18.4%)	30(78.9%)	1(0.02%)	**38**	p=0.023
	Moderate	2(10.5%)	11(57.8%)	6(31.5%)	**19**	
	Poor	5(29.4%)	9(52.9%)	3(17.6%)	**17**	
Total		14	50	10	**74**	
Gender		**Site**			**Total**	
		Larynx	**Oral cavity**	**Pharynx**		
Gender	**Female**	1(0.03%)	19(70.3%)	7(25.9%)	**27**	p= 0.006
	Male	13(27.6%)	31(65.9%)	3(0.06)	**47**	
Total		14	50	10	**74**	

Regarding the distribution of anatomical site in various age groups, the results showed no evident statistical relationship with a p value of 0.245.

Out of 74 cases, EGFR was positive in 81.08% (60 cases) and HER2 was positive in 44.59% (33 cases).In this study there is no significant correlation of HER2 and EGFR immunoexpression with gender and age groups. Both markers do not show any significant correlation with the histopathological grades (Table .3).

Table.3: EGFR and HER2 Immunoexpression, their correlation with WHO Grades

WHO Grade		EGFR		HER2		Total	P value
		Negative	Positive	Negative	Positive		
Grade	Well	8(21%)	30(79%)	21(55.2%)	17(44.7%)	38	p (HER2)=0.229 p(EGFR)=0.882
	Moderate	3(15.7)	16(84.2%)	8(42.1%)	11(57.8%)	19	
	Poor	3(17.6)	14(82.3%)	12(70.5%)	5(29.4%)	17	
Total		14	60	41	33	74	

Table4: Distribution of EGFR and HER2 Immunoexpression and their correlation

HER2		EGFR		Total	
		Negative	Positive		
HER2	Negative	13 (31.7%)	28 (68.2%)	**41**	p=0.002
	Positive	1 (3.03%)	32 (96.96%)	**33**	
Total		14	60	74	

There is a significant correlation of HER2 immunoexpression with EGFR immunoexpression in our study with a p value 0.002 (Table 4).

Discussion

In this study male to female ratio is 1.7:1. A study by Brown et al (USA) showed that the pharyngeal SCC incidence is exceedingly rising among the female.[23]

Well differentiated grade was predominant in our study which is also seen in many other studies including Yun et al. (Spain, G1=62.9%), Vats et al. (India, G1=78.5%) and Xia et al. (China, G1=72.9%).[14]

In our study, EGFR positive immunostaining was seen in 81.1% cases (60 cases). A study conducted by Kriegs et al. in Ukraine reported 80% overexpression in their cohort. [24] We have scored EGFR in HNSCC as it is scored in ESCC (esophageal SCC), where greater than 10% cells showing weak complete membranous staining is taken as cut off for positivity. [25.] In literature no separate scoring criteria or system is designated for EGFR in HNSCC.In another study conducted in Iraq EGFR expression was positive in 87.5% and its expression was 90% in another study carried out in North Carolina.[25, 26] In a study done in Pakistan EGFR was over expressed in 86.5% patients.[26] So the expression of EGFR is almost constant worldwide ranging between 80% to 90%.

The expression of HER2 shows a significant variability. In our study Her2 was over expressedin 43.2% of patients. We took both 2+ and 3+ as positive scores for HER2 so that more patients may benefit from target therapy. However, all cases with score 2+ should be considered in grey zone and confirmed by FISH before starting the targeted therapy which is quite expensive. In this study we could not confirm the cases by FISH due to limited availability of resources and financial constraints.

For interpretation in HNSCC, most studies take scoring criteria used for interpretation of HER2 expression in breast carcinoma. So we have also used the same scoring criteria. We have considered less than 10% cells with intense complete staining or greater than 10% cells with moderate to intense incomplete staining as cut off for positive HER2 immunoexpression. Frequencies have been very variable in different geographical zones e.g; Bernardes et al (Brazil) reported 2% positivity, Cavalot et al (Italy) 39%, Xia et al (China) 51.3%, Vats et al (India) 20% and Safoura Seifi et al (Iran) 17% positivity of HER2 immunoexpression.[27]To date, no study has been done on Pakistani population of HNSCC to evaluate HER2 immunoexpression for prognostic or therapeutic purpose. However, the immunoexpression of EGFR and HER2 does not show any significant correlation with gender or age groups.

It seems that EGFR and HER2 are amongst the most beneficial markers that are relevant in HNSCC to prognosticate and design the treatment protocol. In recent years, researchers tried to find and evaluate the IHC markers including EGFR and HER2 can be used either independently or in conjugation with grading and staging, to predict the outcome of head and neck cancers. [28]

Few studies have also shown a significant correlation of overexpression of EGFR and HER2 with the grades of HNSCC, some of the studies favor that the overexpression is strongly seen with poor differentiation[27], whilst some other studies show that the overexpression of these immunomarkers is seen as tumor becomes more differentiated.[29] Yet many studies across the globe including Hashmi et al.

(Karachi) and Doescher et al. (Finland) have failed to show any significant correlation of these markers with the grades of HNSCC [26, 27]. Our results are in concordance with most studies that show an insignificant correlation between the two parameters.

As a fact, it is observed that both molecular markers frequently show a co-expression in HNSCC, reason being the heterodimerization phenomenon which is a critical event for the oncogenic activation of HER2. Though EGFR can overexpress in the absence of HER2 overexpression, but HER2 essentially heterodimerizes with EGFR for activation. Upon ligand binding they undergo heterodimerization and autophosphorylation with subsequent transduction of a malignant proliferation signal through activation of RAS and MAPK pathways [13].

EGFR and HER2 are well established molecular markers of poor prognosis. FDA approved therapeutic drugs are available against them including Monoclonal Antibodies and Tyrosine Kinase Inhibitors. Various review studies on squamous cell carcinoma of the head and neck report that the overexpression of EGFR and HER2 is significantly linked with the clinicopathological grade, clinical stage and outcome of the disease.[30] Literature shows that EGFR is widely expressed in normal epithelial tissue of head and neck and its overexpression, which is seen in almost 90% of all HNSCC cases according to literature reviews, is related to an ongoing potentially oncogenic process.[3] Studies show that HER2 overexpression in HNSCC is very variable ranging from 0 to 58 %. [31] Research favors co-targeting EGFR and HER2 in HNSCC for maximum therapeutic benefit. It has been shown that co-targeting with anti-HER2 agents augment Cetuximab (an anti-EGFR antibody) responses and overcomes therapeutic resistance. Afatinib is a recent drug which co-targets both immunomarkers. [32]

In our study, the overexpression of both markers is significantly a 'simultaneous phenomenon' so the patients resistant to EGFR targeted therapy can benefit from HER2 targeted therapy and vice versa (as in most cases HER2 hetrodimerizes with EGFR). This view is supported by some recent studies as well. Pauw and Lardon et al. showed that most cancers that are resistant to Cetuximab, an EGFR inhibitor, harbor HER2 mutation too. Afatinib effectively overcomes this resistance by co-targeting them.[32] This concept can help overcome drug resistance by unmasking the co-existing oncogenic molecular pathways.

These factors can help in the stratification of our patients on basis of the intensity of EGFR and HER2 immunoexpression. It is expected that this would help a significant percentage of HNSCC patients and improve the patient outcomes.

Conclusions

EGFR immuno-expression is significantly associated with HER2 immuno-expression, therefore co-targeted therapy can be given in a significant set of HNSCC patients to overcome the underlying drug resistance pathways.

Conflict of Interest: Authors declare no conflict of interest.

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

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