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Immunohistochemical Expression of Estrogen Receptor α and Epidermal Growth Factor Receptor in Head and Neck Cancer: Assessing their Prognostic Significance

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KEYWORDS

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ABSTRACT:

Background: Head-and-neck squamous cell carcinomas (HNSCC) comprise 30% of all cancer cases in India, and shows male preponderance. Risk factors include smoking, betel/areca nut chewing, alcohol intake, and genetic factors. The epidermal growth factor receptor (EGFR) and estrogen receptor α (ERα) play pivotal roles in HNSCC progression, making them potential therapeutic targets.

Objectives: This study aimed to investigate the expression of ERα and EGFR in HNSCC, assessing their potential as prognostic indicators. The research analyzed their expression in correlation with clinicopathological parameters thus aiming to contribute insights for personalized treatment strategies.

Methods: A one-year descriptive study (August 2022 to July 2023) included 30 histologically proven HNSCC cases. Immunohistochemistry staining for EGFR and ERα was performed, and a scoring system (H-Score) was used for quantification. Data analysis utilized SPSS 22.0 software.

Results: There were 80% males, with 43% exhibiting moderate tumor differentiation. The lateral border of the tongue was the most common anatomical site (33.3%). Clinicopathologic factors were analyzed in relation to H scores, showing varying patterns across histological grade, lymphocytic infiltration, lymphovascular invasion, metastasis, and two-year disease-free survival.

A significant association was observed between two-year disease-free survival and recurrence (p value =0.042).

Discussion: The study contributed to understanding HNSCC by exploring epidemiology, clinical characteristics, molecular factors, and prognostic markers. The male predominance, common tumor sites, and lymphocytic infiltration findings aligned with previous studies. ERα expression was absent, while EGFR expression correlated with advanced tumor grade and metastasis, with a significant association to improved two-year disease-free survival.

Conclusion: This study shows that HNSCC is complex, and underlines the importance of EGFR for prognosis, emphasizing the need to consider molecular factors for personalized HNSCC treatment. The lack of ERα expression suggests more research are required.

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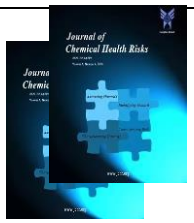

I. INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) are a highly prevalent and significant form of cancer in the head and neck region, accounting for over 90% of all cases. In India, HNSCC stands out as the most commonly diagnosed cancer among males, with a high incidence rate and significant mortality.**1, 2** The escalating number of cases and the advanced stage at which they are frequently detected pose a major concern for public health in India. **3,4**

Several risk factors contribute to the development of HNSCC, with smoking, betel/areca nut chewing, and alcohol intake being the most prevalent ones. These habits are widespread in the Indian population and play a substantial role in the high incidence of HNSCC. Additionally, genetic factors, including Fanconi anemia and specific gene polymorphisms, have been associated with an increased risk of developing HNSCC. **3,5**

The epidermal growth factor receptor (EGFR) emerges as a key molecular player in HNSCC. Belonging to the ErbB/HER family of tyrosine kinase receptors, EGFR is frequently overexpressed in HNSCC cases. **6** Dysregulation and abnormal activation of EGFR have been linked to heightened tumor proliferation, angiogenesis, metastasis, and poor prognosis. Consequently, targeting EGFR holds considerable promise as a therapeutic approach for HNSCC treatment. EGFR can be activated through mechanisms independent of EGFR itself, involving the activation of other receptors or signaling pathways. This activation leads to increased proliferation and angiogenesis, fueling tumor growth and progression. Dysregulation of EGFR signaling pathways plays a critical role in HNSCC development and is closely associated with a poor prognosis. High expression of EGFR is correlated with heightened metastasis and reduced survival rates in HNSCC patients. **7**

Another significant molecular player in HNSCC is the estrogen receptor α (ERα). As a nuclear hormone receptor, ERα mediates the effects of estrogens in gene regulation. ERα and EGFR have the ability to cross-activate each other in HNSCC, forming a complex signaling network. Tumors exhibiting high expression of both receptors have been associated with diminished progression- free survival, underscoring the clinical significance of their cross-activation. **8,9**

The overexpression of both EGFR and ERα in HNSCC is closely associated with invasion and poor prognosis. Their presence indicates a more aggressive tumor phenotype, highlighting the pressing need for targeted therapies that can effectively inhibit these receptors.

In recent years, targeted therapies have shown promise in the treatment of HNSCC. Drugs such as cetuximab, which inhibits EGFR, and tamoxifen, which targets ERα, have demonstrated encouraging results in reducing HNSCC. These targeted approaches offer a potential avenue for improving treatment outcomes for patients with this challenging malignancy.**10**

The rationale for this study stems from the high prevalence and impact of HNSCC, the crucial role played by EGFR and ERα in HNSCC progression, and the potential of targeted therapies to enhance treatment outcomes. By investigating the molecular mechanisms and therapeutic potential of EGFR and ERα in HNSCC, this study aims to contribute to the development of more effective and personalized treatment strategies for patients affected by this aggressive cancer.

Aims and Objectives: The aims and objectives focused on investigating the expression of ERα and EGFR in Head and Neck Cancer and their potential role as prognostic indicators. The study analyzed the score of the expression levels of ERα and EGFR in a cohort of Head & Neck Cancer patients. Subsequently, the research also analyzed the relationship between ERα and EGFR expression and various prognostic indicators, such as TNM stage, tumor size, histological type, vascular & lymphatic embolization, mitotic rate, and lymphocytic infiltration and the two-year disease-free survival rate in patients. By elucidating the significance of ERα and EGFR as potential prognostic markers, the research aimed to contribute valuable insights to enhance patient outcomes and tailor treatment strategies for Head and Neck Cancer.

II. MATERIALS AND METHODS

The present study is a descriptive research design with a duration of one year, from August 2022 to July 2023. The sample size consisted of 30 cases of histologically proven

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squamous cell carcinoma (SCC) in Head & Neck lesions.

Cases with premalignant and benign lesions, sarcomas, and those already undergoing chemotherapy were excluded from the study.

Data collection involved retrieving relevant clinical and demographic details from the available case sheets. Two-year disease-free survival was defined as the duration from surgical resection until the first recurrence, patient's death, or last medical follow-up.

The present study utilized immunohistochemistry staining for EGFR and ERα, and a scoring system was devised to quantify the staining intensity and percentage of positive cells.

The biopsy tissue was preserved in 10% formal-saline. The sections were taken and stained with Hematoxylin & Eosin (H&E) to study morphological changes. Immunohistochemistry staining involved antigen retrieval, blocking endogenous peroxidase, incubation with primary antibodies (EGFR and ERα), linking with secondary antibodies, enzyme labeling, and staining with Diaminobenzidine (DAB). Counterstaining was done with Hematoxylin. The H-Score calculation was used to quantify the immunohistochemistry staining results

Data Analysis was performed using SPSS 22.0 Software

The H-Score was obtained by multiplying the percentage of cells showing different staining intensities (0, 1, 2, or 3) with their respective intensity values and adding the scores together. The final score ranged from 0 to 300.

H-Score Calculation

Intensity of staining	Percentage of different intensity cells
No staining =0	
Weak staining intensity = 1	% of cells showing weak staining-P1
Moderate staining intensity = 2	% of cells showing moderate staining-P2
Strong staining intensity = 3	% of cells showing strong staining-P3

H-Score = (P1 × 1) + (P2 × 2) + (P3 × 3)

Where, P1 = Percentage of cells showing weak staining intensity (score 1) P2 = Percentage of cells showing moderate staining intensity (score 2) P3 = Percentage of cells showing strong staining intensity (score 3)

The Final Score ranges from 0 to 300 H-Score Ranges and Interpretation

Ranges	Interpretation
0-50	Negative
51-100	Low Positive
101-200	Moderate Positive
201-300	Strong Positive

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ER α:

0 No staining.

1 + Faint membranous in >10% of tumor cells.

2 + Moderate membranous in >10% of tumor cells.

3 + Strong membranous in >10% of tumor cells.

In the present analyzed various parameters, such as age-wise distribution, gender-wise distribution, histological grade, site of the tumor, lymphocytic infiltration, lymphovascular invasion, mitotic figures, tumor size, lymph node status and metastasis. Additionally, correlations between immunohistochemical expression of EGFR (H-score) and various clinicopathological parameters were studied, including grade, lymphovascular invasion, lymphocytic infiltration, tumor size, lymph node status, metastasis, and two-year disease-free survival.

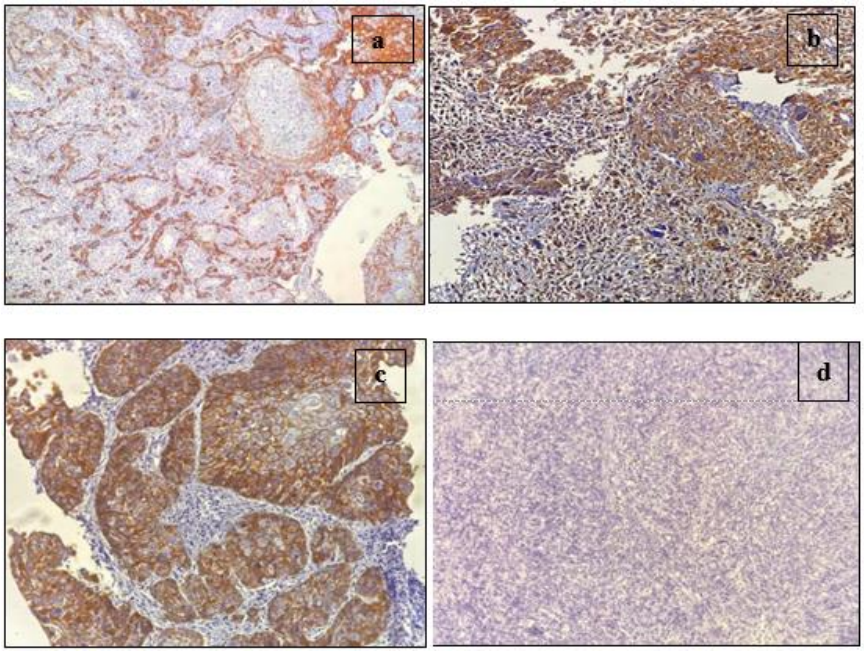

Fig 1a. Weak (+1) epidermal growth factor receptor staining observed in oral squamous-cell carcinoma cases (EGFR; 40X). **b.** Marked (+3) epidermal growth factor receptor (EGFR) staining seen in oral squamous-cell carcinoma (EGFR; 40X**)**. **c**. Marked (+3) immunohistochemical expression of epidermal growth factor receptor (EGFR) observed in oral squamous-cell carcinoma (EGFR; 40X). d. Negative ERα staining in OSCC. (ERα; 40X)

Results: In the present study out of 30 cases, 24 cases (80%) were males while the remaining 6 cases (20%) were females. There was male predominance. Out of these 30 cases, most cases (43%) displayed moderate tumor differentiation, with a minority being well (47%) or poorly (10%) differentiated. The lateral border of the tongue was the most common anatomical site (33.3%), followed by the buccal mucosa (23.3%), with various other sites accounting for the remainder of cases.

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Clinico-pathologic	H Score								P value
	0-50		51-100		101-150		151-200		
Factors	No. of cases (n)	Percentage (%)	No. of cases (n)	Percentage (%)	No. of cases (n)	Percentage (%)	No. of cases (n)	Percentage (%)	
Histological Grade of the tumor									
Well Differentiated (12)	2	16.6	3	25	5	41.6	2	16.6	0.63
Moderately Differentiated (15)	1	6.66	4	26.6	4	26.6	6	40	
Poorly Differentiated (3)	1	33.3	0	0	1	33.3	1	33.3	
Lymphocytic Infiltration									
Mild (6)	1	16.6	1	16.6	1	16.6	3	50	0.84
Moderately (10)	1	10	4	40	3	30	2	20	
Intense (14)	2	14.2	2	14.2	5	35.7	5	35.7	
Lymphovascular Invasion									
Well Differentiated (12)	2	16.6	3	25	5	41.6	2	16.6	0.96
Moderately Differentiated (15)	1	6.66	4	26.6	4	26.6	6	40	
Poorly Differentiated (3)	1	33.3	0	0	1	33.3	1	33.3	
Metastasis									
M0 (25)	3	12	6	24	9	36	7	28	0.61
M1 (5)	1	20	1	20	0	0	3	60	

Two-Year Disease-Free Survival									
Survival Recurrence was noted (n)	0	0	2	14.2	0	0	0	0	0.042
Two-Year Disease-Free Survival Recurrence was noted	3	21.4	7	50	2	14.2	0	0	

Table 1: Epidermal growth factor receptor expression and its association with demographic, clinical and histopathological parameters in oral squamous-cell carcinoma

In this study, clinicopathologic factors were analyzed in relation to H scores, with a focus on histological grade, lymphocytic infiltration, lymphovascular invasion, metastasis, and two-year disease-free survival. Well-differentiated tumors exhibited varying H scores, while poorly differentiated tumors had higher lymphocytic infiltration. Lymphovascular invasion and metastasis showed distinct patterns across tumor grades. A statistically

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significant association was observed between two-year disease-free survival and recurrence. (Table No. 1) These findings suggest potential prognostic implications, emphasizing the importance of considering these factors in the evaluation and management of tumors.

III. DISCUSSION

The present study aimed to provide understating into HNSCC, by establishing correlation between various aspects such as epidemiology, clinical characteristics, molecular factors, and prognostic markers with immunohistochemical findings of ERα and EGFR. A total of 30 patients participated in the study, with ages ranging from 27 to 72 years and a mean age of 50.1±12.26 years, consistent with previous findings by Johnson DE *et al*. [11] indicating a median age of diagnosis at 66 years.

The male-to-female ratio in the present study was 4:1, aligning closely with Stoyanov GS *et al.'s* study, reporting a ratio of 3.24:1, indicating a higher incidence of HNSCC in males. [12] The most common site for HNSCC was the lateral border of the tongue, representing 33% of cases, which coincided with Selvamani M *et al.'s* study, [13] where over 80% of cases exhibited cancer in the same location.

HNSCC is a diverse group of cancers originating from the upper aerodigestive tract's epithelial lining, and understanding its molecular factors is crucial for treatment strategies. Lymphocytic infiltration within the tumors revealed that a substantial number of cases exhibited intense lymphocytic infiltration, a potential prognostic marker, as tumor-infiltrating lymphocytes (TILs) have shown promise in predicting cancer outcomes.

We also evaluated the expression of ERα in HNSCC. ERα expression was not detected in any case of our cohort. Doll *et al*., in their study noted rare expression of ERα in OSCC, particularly in males, is linked to a significant reduction in overall survival and advanced tumor staging [14]. The findings from their study underscored the potential relevance of ER-based therapeutic strategies for improving outcomes in ERα-positive OSCC patients have explored ER and androgen receptor (AR) expression in various cancers, including head and neck cancer. Some studies found associations between ER expression and patient outcomes, particularly in males, while others focused on AR expression and its gender-based differences.

In our study in HNSCC uncovered robust lymphocytic infiltration, rare lymphovascular embolization, and minimal mitotic activity, indicating subdued proliferative behavior. Higher EGFR expression correlated with advanced tumor grade and metastasis though was not statistically significant, while showing a significant association to improved two-year disease-free survival. The study on 170 HNSCC patients revealed that 88.82% exhibited EGFR expression, linked to poor prognosis. Significant associations were found with patient age, histological grade, perineural invasion, and recurrence site. EGFR expression correlated with lower survival rates, suggesting its value as a prognostic marker and potential therapeutic target in HNSCC treatment, emphasizing the potential benefits of anti-EGFR therapy combined with radiotherapy for improved outcomes. Hashmi *et al*., [16] expanded their study to include 115 cases, revealing a strong correlation between EGFR expression and tumor stage, but no significant correlations with age, gender, and grade of tumor, risk factor, or stage of the tumor. Verma *et al*., examined 48 cases, discovering significant correlations between EGFR expression and tumor grade, tumor stage, and lymph node metastasis [17]. Johnson DE *et al*., analyzed 59 cases and reported that EGFR expression significantly correlated with age, gender, tumor grade, and risk factors [11].

It's evident that understanding the role of EGFR in HNSCC requires considering multiple variables and might vary across different patient populations and clinical contexts. These findings align with previous studies that have also investigated EGFR expression and its associations with various clinical factors, providing valuable insights into potential prognostic markers for HNSCC. Overall, this study contributes to the understanding of HNSCC's multifaceted nature and highlights the potential significance of molecular markers like EGFR and TILs in predicting patient outcomes and guiding treatment decisions.

IV. CONCLUSION

This study investigates the immunohistochemical expression of ERα and EGFR in HNSCC, shedding light on their potential prognostic significance. Our study revealed intense lymphocytic infiltration, infrequent lymphovascular

embolization, and low mitotic counts in HNSCC, suggesting a less aggressive proliferative activity. While higher EGFR expression was associated with higher tumor grade and metastasis, no significant correlation with lymphovascular invasion or lymphocytic infiltration was observed. EGFR expression showed a significant association with two-year disease-free survival. However, ER expression was absent in all cases. The study underscores the need for further research to confirm these results and explore the mechanisms underlying this association, suggesting a potential avenue for ER-based therapeutic approaches in the future management of ERα-positive HNSCC patients.

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