

## ORIGINAL ARTICLE

## EGFR Immunohistochemical Expression in Head and Neck Squamous Cell Carcinoma and its Prognostic Significance

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## ABSTRACT

**Background:** Head and neck Squamous cell carcinoma (HNSCC) is a highly rapidly spreading invasive malignant tumour of the epithelium and in the entire world, it is the sixth most common malignant neoplasm. EGFR protein is crucial for several processes that impact tumor genesis, growth, differentiation, suppression of apoptosis and metastatic development. Increased expression of EGFR is linked to poor clinical outcomes and resistance to radiation therapy.

**Aim:** Evaluation of expression of EGFR is done in HNSCC and it is correlated with various parameters like histological grade, staining intensity, percentage of tumor cells stained and its prognostic significance is determined.

**Materials and methods:** Total number of 80 cases were studied and statistically p value is determined.

**Results:** Females holds the majority of 51cases (63.75%) and males were 29 cases (36.25%). Age group 41 to 50 years has highest occurrence which is followed by 51 to 60 years. 63.75% of patients have positive smoking history. 71.25% have history of tobacco use. Most common site of tumor is oral cavity (95%). Majority of the cases were conventional Squamous Cell Carcinoma (95%) and verrucous carcinoma comprising (5%) cases. Most of the patients had grade I tumors (53.75%). 51.25% of cases have strong intensity in staining. Greater than 50% of cells are stained by EGFR in 56 cases and p value is statistically significant.

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**Conclusion:** High EGFR expression is a helpful diagnostic and prognostic immunohistochemical marker especially in advanced cancers. EGFR targeted therapy along with radiotherapy, chemotherapy can be used for locally advanced, metastatic and recurrent Head and Neck squamous cell carcinomas.

## KEYWORDS

• Head and Neck • Squamous cell carcinoma • EGFR • Grade • Intensity  
• Prognosis

## INTRODUCTION

Head and neck Squamous cell carcinoma (HNSCC) is a highly rapidly spreading invasive malignant tumor of the epithelium and in the entire world, it is the sixth most common malignant neoplasm.<sup>1</sup> Resistance to treatment is frequently linked to highly tumorigenic expression of specific carcinoma variants, and its progression to locally invasive carcinoma, metastatic and recurrent tumor development.<sup>2</sup> EGFR protein is crucial for tumor genesis, growth, differentiation, suppression of apoptosis and metastatic development.<sup>3</sup> Elevated level of EGFR immunohistochemical expression is a poor prognostic factor and cause resistance to radiation therapy.<sup>4</sup> The molecular target for the therapy of HNSCC was EGFR, according to many studies.<sup>5</sup> The main aim of the present study is to evaluate the expression of EGFR in HNSCC and it is correlated with various parameters like histological grade, staining intensity of the tumor, percentage of tumor cells stained and its prognostic significance is determined. By assessing the risk stratification of the patients, they can be benefited from targeted therapy with EGFR inhibitors along with chemotherapy and radiotherapy in cases of locally advanced, metastatic and recurrent carcinomas.

## MATERIALS AND METHODS

The current study was done for a period of 2 years. All the epithelial malignancies of Head and Neck carcinomas sent as small biopsies and specimens by ENT and General Surgery Departments were included in the study. Total number of 80 cases were collected. Comprehensive clinical history and findings from pertinent diagnostic investigations were obtained from the patient's medical records.

**Inclusion criteria:** All biopsies and specimens diagnosed as epithelial malignancies of Head and Neck.

**Exclusion criteria:** Tumors with significant necrosis and not enough viable tissue Autolysed tissue and Non neoplastic lesions Histopathological examination and EGFR immunohistochemistry is done and its expression is studied.

## Immunohistochemical Evaluation

Immunohistochemical staining for EGFR was done on paraffin-embedded sections of the tissue by using a super sensitive polymer HRP system, which employs non-biotin polymer-based technology. Tissue sections of 4 microns thickness were obtained and was mounted on the slides coated with gelatin. Antigen retrieval was achieved through heat-induced epitope retrieval. EGFR detection was performed using a rabbit monoclonal primary antibody, followed by a secondary antibody conjugated with horse radish peroxidase-polymer, and visualized by using a diaminobenzidine (DAB) chromogen.

## Interpretation and Scoring:

The immunohistochemically stained sections were evaluated for the presence of staining, subcellular localization, percentage of positively stained cells, and staining intensity. For EGFR, the intensity of membranous staining was specifically assessed. To establish a clearer correlation between EGFR expression and prognostic parameters, a semi-quantitative evaluation was conducted. EGFR-positive cells were analyzed across five to ten high-power fields.

The staining was graded as follows:

(+++): 3: Strong membranous positivity in 50–100% of cells

(++) 2: Moderate membranous positivity in 10–50% of cells

(+) 1: Faint membranous positivity in < 10% of cells

(0): No detectable staining in cells

**Statistical Analysis:** Data analysis was done by SPSS VER 25.0 SOFTWARE. Qualitative data analysis was done by applying chi-square test and quantitative data analysis by z-test.

## RESULTS

Among 80 specimens, females holds the majority 51cases (63.75%) and males were 29 cases (36.25%). The male to female ratio was 0.5:1 indicating female preponderance. The patient's ages ranged from 23 to 80 years, with a mean age of 55 years ( $\pm 12$  SD). Age group of 41 to 50 years has the highest occurrence which is followed by 51 to 60 years.

51 cases (63.75%) of the patients have positive smoking history. 29 cases (36.25%) of the patients were non-smokers. 71.25% (57cases) of the patients reported a history of tobacco use, while 35% (28 cases) of the patients were known alcoholics. The tumor size ranged from 2.1-4 cm in the majority of 38 cases (47.5%), 22(27.5%) cases has the tumor size >4.1cm followed by 20 (25%) cases has the tumor size <2 cm. Most common site of tumor was oral cavity in 76 (95%) cases, Pharynx and larynx has 1 (1.25%) case each, other sites such as nasal cavity and maxillary sinus has 2 (2.5%) cases.

In the current study, highest number of the cases were Squamous cell Carcinoma, with 76 (95%) cases being conventional Squamous cell Carcinoma and the verrucous carcinoma comprising about 4(5%) cases. 43 cases (53.75%) had grade I tumors, which were followed by 35 (43.75%) cases with grade II. Grade III carcinoma cases were 2 (2.5%) only. *Table 1-3.*

**Table 1:** EGFR staining intensity of the Tumor cells

Intensity of Staining	Cases	Percentage (%)
Negative	10	12.5%
Weak	6	7.5%
Moderate	23	28.75%
Strong	41	51.25%
<b>Total</b>	<b>80</b>	<b>100.0%</b>

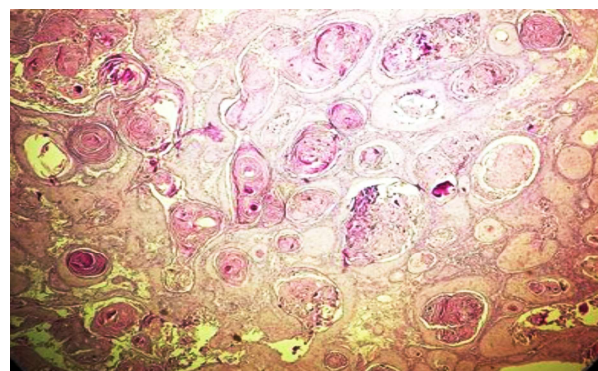
**Table 2:** Variation in EGFR Staining Intensity by Tumor Histological Grade

Tumour Grade	EGFR Staining Intensity				
	Absent	Weak	Moderate	Strong	Total
	Cases	Cases	Cases	Cases	Cases
Grade-I	9	3	14	17	43
Grade-II	1	3	8	23	35
Grade-III	0	0	1	01	02
<b>Total</b>	<b>10</b>	<b>6</b>	<b>23</b>	<b>41</b>	<b>80</b>

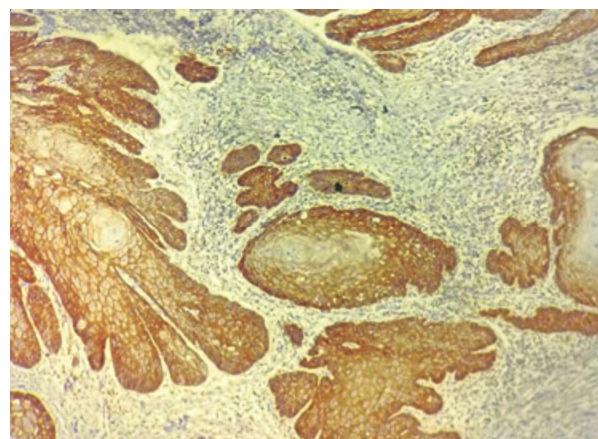
**Table 3:** Percentage of Tumor cells stained by EGFR with relation to intensity of staining

Percentage of Tumour Cells Stained	Intensity of EGFR Staining (No. of cases, Percentage)				
	Absent	Weak	Moderate	Strong	Total
<50%	9 (37.55%)	3 (12.5%)	8 (33.3%)	4 (16.7%)	24 (30%)
>50%	1 (1.8%)	3 (5.4%)	15 (26.8%)	37 (66.1%)	56 (70%)
<b>Total</b>	<b>10 (12.5%)</b>	<b>6 (7.5%)</b>	<b>23 (28.75%)</b>	<b>41 (51.25%)</b>	<b>80 (100%)</b>

p=0.00 (significant)

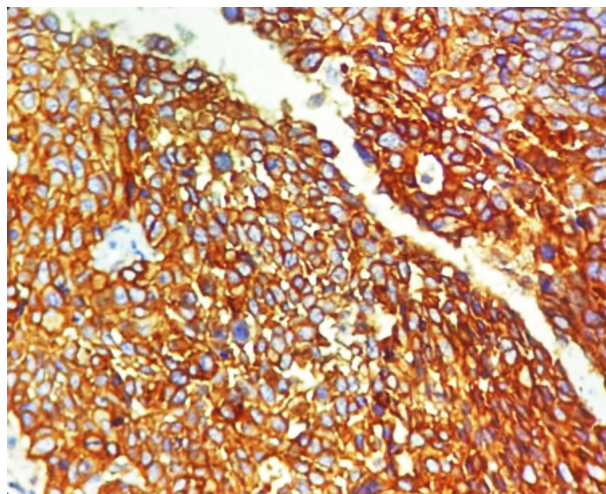


**Figure 1:** Photomicrograph showing well differentiated squamous cell carcinoma with keratin pearls (H&E, X100)



**Figure 2:** Photomicrograph showing moderate membranous EGFR positivity (IHC, X100)





**Figure 3:** Photomicrograph showing strong membranous EGFR positivity (IHC, X400)

## DISCUSSION

Over the past 50 years, Head and neck Squamous cell carcinoma patients has survival life span below 50% despite various advances in treatment, surgery procedures, radiation therapy and chemotherapy regimens.<sup>1</sup> Numerous biological indicators have been linked to head and neck tumors in recent years. The following indicators, however, have been given prognostic relevance in more recent studies: p53 mutations, over-expression of the EGFR, over-expression of cyclin D16 and TGF- $\alpha$ .<sup>3</sup> This could be attributed to the emergence of genetic and epigenetic changes in tumor cells, which lead to their uncontrolled proliferation, enhanced survival, invasive capabilities, and either acquired or inherent resistance to therapeutic interventions.<sup>2</sup>

Epidermal growth factor was one of the first identified growth factor, which was later demonstrated to induce mitosis by binding to the erbB1 or HER1/EGFR cell surface receptor.<sup>6</sup> The degree of expression of EGFR immunohistochemical marker on

head and neck carcinomas is enhanced relative to normal adjacent squamous mucosa in 83-100% of cases, making it as the most frequent molecular abnormalities in HNSCC. Higher receptor content is frequently linked to increased ligand production by HNSCC.<sup>5</sup> High EGFR expression in human tumors is a helpful diagnostic and prognostic immunohistochemical marker, correlating with more aggressive clinical outcome. EGFR has gained popularity as a potential target for monoclonal antibody treatment in recent years.<sup>7</sup>

Furthermore, signalling of EGFR is inhibited and the effects of radiation or chemotherapy are amplified by EGFR-targeted treatments include agents like the chimeric monoclonal antibody cetuximab (C225) and the quinolone-based inhibitor gefitinib.<sup>5</sup> This will benefit the prognosis of the patient and life span will be increased.

Out of 80 cases in the present study, mean age of presentation was 55yrs. The patients in the present study has mean age of presentation that was almost 10 years lower than that of Mariezkurrena *et al* study<sup>8</sup> and Willmore Payne CW *et al*,<sup>9</sup> studies respectively. Issa HI<sup>7</sup> study has mean age of 50 years which is slightly lower than the current study.

In the current study (n=80), 51 patients (63.75%) were female and 29 patients (36.25%) were male with a male to female ratio of 0.5:1, showing definite female Preponderance. In contrast, the majority of earlier studies by Chung *et al* (5.8:1),<sup>10</sup> Willmore Payne CW *et al* (11:1),<sup>9</sup> Ting Hsiung *et al* (2.2:1),<sup>11</sup> Keller J *et al* (3:1)<sup>12</sup> and Issa H (2.3:1)<sup>7</sup> reveals male predominance among the cases. This discrepancy may be brought on by women's greater use of cigarettes & betel nut chewing in the current system, which is more common in the lower socio-economic category. Table 4.

**Table 4:** Common primary tumor sites identified in various research studies

Study	Lip, oral cavity	Pharynx	Larynx	Others	Total cases
Mariezkurrena XA <i>et al</i> <sup>8</sup>	-	23 (52.0%)	18 (41.0%)	3 (7.0%)	44
Chung CH <i>et al</i> <sup>10</sup>	23 (30.6%)	25 (33.3%)	27 (36.0%)	-	75
Issa HI <sup>7</sup>	8 (26.7%)	9 (30.0%)	7 (23.3%)	6 (20.0%)	30
Hsiung DT <i>et al</i> <sup>11</sup>	10 (30.3%)	4 (12.2%)	19 (57.6%)	-	33
Hama T <i>et al</i> <sup>13</sup>	33 (40.2%)	22 (26.8%)	19 (23.3%)	8 (9.7%)	82
Present study	76 (95.0%)	1 (1.25%)	1 (1.25%)	2 (2.5%)	80

A considerably greater percentage of cases in the current study (95%) had the initial tumor confined to the oral cavity as compared to other studies done by Mariezkurrena *et al.*<sup>8</sup> Chung *et al.*<sup>10</sup> and Hama *et al.*<sup>13</sup> According to Basu R *et al.*<sup>14</sup> in south central Asia, approximately 80% of head and neck carcinomas are diagnosed in oral cavity and oropharynx, whereas in other parts of world, carcinomas of larynx and nasopharynx constitute about 33% to 50% of all head and neck carcinomas.

Most likely, the habit of chewing tobacco in people, which is more common in our own country, is the cause for increased frequency of oral cavity Squamous cell carcinoma.

Tobacco chewing raises the incidence of HNSCC, as stated by Zhou *et al.*<sup>15</sup> The current study revealed a high prevalence of tobacco consumption (71%) that was comparable to

research work done by Maiti GP *et al.* (72%)<sup>16</sup> and Huang SF *et al.* (92%).<sup>17</sup>

In the present study, most of tumors are in Grade-I (53.75%) and Grade II (43.75%). Only 2 cases (2.5%) are in Grade III. Mariezkurrena *et al.*<sup>8</sup> study has tumors in Grade I (31.8%), Grade II (38.6%), Grade III (29.5%) with almost equal distribution. Hama *et al.* study<sup>13</sup> has more cases in Grade II (46.5%), when compared to Grade I (36.5%) and Grade III (17%). Issa HI<sup>7</sup> study has more cases in grade II (43.3%), followed by Grade III (36.7%) and grade I (20%).

Out of 80 cases of head and neck carcinomas in current study, EGFR staining were over expressed in 87.25% of cases. This results correlated with the research work done by Payne *et al.* (87.5%)<sup>9</sup>, Sarkis SA *et al.* (87.5%)<sup>5</sup>, Issa HI (100%)<sup>7</sup>, Maiti GP *et al.* (84%)<sup>16</sup>. Table 5

**Table 5:** EGFR intensity of expression in other studies

Studies	Absent	Weak intensity	Moderate intensity	Strong intensity	Total cases
Payne CW <i>et al.</i> <sup>9</sup>	2 (8%)	1 (4%)	8 (33%)	13 (54%)	24
Sarkis SA <i>et al.</i> <sup>5</sup>	5 (12.5%)	15 (37.5%)	14 (35%)	6 (15%)	40
Issa HI <sup>7</sup>	-	5 (16.6%)	17 (57%)	8 (26.6%)	30
Present study	10 (12.5%)	6 (7.5%)	23 (28.75%)	41 (51.25%)	80

EGFR expression was also mostly concentrated near the edges of tumor islands. Higher expression of EGFR shows that uncontrolled development may be mediated by aberrant EGFR expression, according to Sarkis SA *et al.*<sup>5</sup>

Higher EGFR percentage expression in tumor cells was associated with greater intensity of EGFR staining. This result is in line with the current study, where there is a significant link between EGFR percentage expression in tumor cells and intensity of staining (p value < 0.05) and it is statistically significant. According to Issa HI,<sup>7</sup> there is a correlation between high EGFR expression and advanced stage of tumor, distant metastasis, lymph node metastasis, poor differentiation and invasion in Squamous cell carcinoma of head and neck. It was discovered that staining of EGFR was comparatively elevated in tumor tissue and extremely little in normal neighbouring mucosa without tumor. The correlation between increasing dysplasia and an increase in intensity of staining may indicate that this is a precursor to the development of cancer.

## CONCLUSION

This is a hospital-based study, thus it might not accurately reflect the prevalence of the disease in the general population. It is difficult to properly treat head and neck Squamous cell carcinomas with advanced primary lesions while preserving the function of essential healthy structures. Patients who present at an advanced stage of tumor still have poor outcomes.

The expression of EGFR protein is a potential marker for targeted therapy in these patients, especially in those who present with locally advanced tumors. Most of the cases of Squamous cell carcinomas of head and neck showed overexpression of EGFR and majority showing moderate to strong intensity of staining in >50% of the tumor cells. The expression of this protein can be used in the treatment by EGFR targeted therapy in these patients, especially in those who present with locally advanced tumors.

In conclusion, EGFR targeted therapy plays a key role for anticancer therapy in Squamous

cell carcinomas of Head and neck and this will improve the prognosis of the patient. It is advantageous for the patient when Combination of EGFR-targeted therapy along with radiotherapy and chemo therapy are used in the treatment of locally advanced, metastatic and recurrent head and neck carcinomas.

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**Conflicting Interest:** Nil

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