Evaluation of epidermal growth factor receptor expression by a new scoring system in head-and-neck squamous cell carcinoma and its association with various pathological prognostic factors

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Abstract

Context: Squamous cell carcinoma is an aggressive epithelial malignancy of the upper aerodigestive tract comprising 90% of all head-and-neck squamous cell carcinoma (HNSCC). It is the sixth leading cancer worldwide, with approximately 600,000 cases reported annually. It is one of the most common cancers in India.

Aims and Objective: Epidermal growth factor receptor (EGFR) being the most promising marker has potentially offered new methods to prognosticate and plays an essential role in early diagnosis and treatment apart from tumor, node and metastasis staging which has been used till now. Therefore, this study has been undertaken to evaluate the expression of EGFR in HNSCC cases, according to the new scoring system and find its association with various pathological prognostic factors.

Materials and Methods: Forty-eight resected specimens of oral squamous cell carcinoma were received. Cases were appropriately staged, and paraffin-embedded tumor sections, stained with hematoxylin and eosin, were graded. EGFR expression was evaluated as extent score, intensity score and total score (TS).

Statistical Analysis: Data obtained were transferred on to an excel sheet. Chi-square test with and without Yate’s correction was employed to compare various parameters. P ≤ 0.05 was taken as critical level of significance.

Results: A significant association was observed between TS of EGFR expression and tumor grade but not with tumor stage or lymph node metastasis.

Conclusion: A significant association of EGFR expression exists with tumor grade as per the new scoring system adopted. High EGFR expression suggests uncontrolled growth which depicts that EGFR upregulation may be an early event during HNSCC carcinogenesis.

Keywords: Epidermal growth factor receptor, expression score, head-and-neck squamous cell carcinoma, intensity score, total score
INTRODUCTION

Head-and-neck cancer consists of a heterogeneous group of lesions that arise in the upper aerodigestive tract. It is the sixth leading cancer worldwide, with approximately 600,000 cases reported annually.[1] Oral cancer is known to account for 2%–4% of all cancers worldwide, among which oral squamous cell carcinomas (OSCCs) are responsible for 90% of the cancers. Incidence is much higher in many developing countries.[2] Prevalence of oral cancer in India is around 45%.[3,4] The incidence of cancer in India is known to increase from 1 million in 2012 to >1.7 million in the year 2035 as predicted by the International Agency for Research on Cancer.[4] This depicts that death rate in this period caused due to cancer will also increase from 6.8 lakhs to 1 million.[5] Uttar Pradesh, Jharkhand and Bihar have reported an increased risk of oral cancer in India.[5]

Tobacco use (both smoking and chewing betel quid) and alcohol consumption are independent risk factors for development of oral cancer. However, with combined intake, they have a synergistic effect, in the development of head-and-neck squamous cell carcinoma (HNSCC).[6,7] Tobacco use is expected to cause 8.4 million deaths by 2020 and 70% of these will be in developing countries.[8] Incidence of oral cancer is 8.4 times higher in patients who have tobacco smoking and chewing habits.[9] In Uttar Pradesh, cancer of buccal mucosa and cheek exceeds other oral cancers.[10] About 7% of all the deaths in India (≥30 years of age) are due to tobacco intake as per the WHO Global Report on “Tobacco Attributable Mortality” 2012.

OSCCs are histologically graded as well, moderate, or poorly differentiated carcinomas. Though histological grading system is essential for the classification of HNSCC, it is not necessary for the treatment protocol. This is due to the fact that clinical outcome or treatment response is not strongly associated with differentiation grade.[11]

Tumor, node and metastasis (TNM) staging system is essential in predicting prognosis and helps to classify HNSCC patients based on the clinical, radiological and pathological examination. However, it is noted that patients with same clinicopathological stage of tumor do not have similar disease progression, response to therapy and rate of disease recurrence and survival.[1,11] This is because molecular heterogeneity of HNSCC is not incorporated in conventional TNM classification. Therefore, there is a dire need to understand the prognostic relationship of HNSCC with various molecular markers that have been discovered in the last few years. Among various markers that are relevant in HNSCC, it seems that epidermal growth factor receptor (EGFR) is most beneficial to prognosticate and also to design the treatment protocol. There is no established criterion that is universally used to evaluate EGFR expression. We have extensively explored the available review of literature and thereafter have used a scoring method by Sarkis et al. (2010) and Young et al. (2011) to evaluate the extent score (ES), intensity score (IS) and total score (TS) of EGFR expression.

EGFR plays an important role in numerous processes that affect cell cycle progression which leads to tumor development, growth, progression, differentiation and development of metastasis.[12] In many studies, it has been shown to correlate with a poor prognosis[12,13] and also resistance to radiotherapy in a variety of cancers, especially SCC.[14] Therefore, this study has been undertaken to evaluate the expression of EGFR in HNSCC cases and find its association with various pathological prognostic factors including grade, stage and lymph node metastasis, hoping that these findings could provide prognostic assessment of the disease and help in designing more appropriate and effective treatment strategies for OSCC. Thus, limited resources available to the patients can be conserved and undue treatment can be avoided.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology in collaboration with the Department of Otorhinolaryngology, Motilal Nehru Medical College, Allahabad. Forty-eight cases diagnosed and operated for HNSCC were specifically focused on. Cases were mainly confined to the oral cavity due to the extremely high prevalence of oral tobacco (pan, khaini, zarda and gutka) consumption where the study was conducted.

Specimens that we received for routine histopathological examination were hemimandibulectomy, segmental mandibulectomy or glossectomy specimens along with unilateral or bilateral neck dissection of lymph nodes, as per the tumor site and adjacent area of involvement.

Cases in which only a biopsy or limited surgery was done, cases diagnosed other than SCC and tumors with extensive necrosis with insufficient viable tumor cells for accurate evaluation were all excluded from the study.

Specimens received were fixed in 10% (v/v) formalin. In each case, the standard American Joint Committee on Cancer protocol for grossing of the surgical specimens was followed. After conventional processing, paraffin sections of 3–4 μm thickness were stained by hematoxylin and eosin for diagnosing, grading and pathological staging of the tumor. Tumours were graded according to Broder’s criteria into well, moderate and poorly differentiated [Figures 1–3].[15] In
addition, 4-μm sections were cut from a paraffin-embedded tumor tissue and taken on glass slides precoated with adhesive (silane) for immunohistochemistry (IHC).

**Immunohistochemistry**

IHC was carried out using polyclonal Rabbit anti-EGFR (Bio Genix Fremont CA). The rabbit antibody to EGFR (LRVAP) reacts with the 170 kD EGFR transmembranous glycoprotein and binds specifically to the intracellular region regardless of phosphorylation state. The extracellular domain binds EGF as a proliferation signal. The antibody is diluted in phosphate-buffered saline of pH 7.6 containing 1% bovine serum albumin and 0.09% sodium azide. Tumor cells showed both membranous and cytoplasmic staining but cytoplasmic staining was neglected and membranous staining was considered as positive. Normal skin was taken as control which showed brown membranous staining. The scoring criteria for EGFR immunoreactivity were based on previous scoring methods.\[^{15,16}\] The staining intensity was compared with IHC-stained sections of normal skin section taken as positive control and recorded as IS. ES was evaluated and TS was calculated by the following formula:

\[
TS = ES \times IS
\]

**RESULTS**

Average age of the patients was 46.6 ± 11.9 standard deviation, ranging from 25 to 74 years. Peak incidence was between 51 and 60 years. Thirty-six cases were male and 12 were female, with a sex ratio of 3:1. Most of the cases (22 [46%]) were located in buccal mucosa, followed by 12 (25%) in tongue, 6 (13%) cases in lip, 5 (10%) in gingivo-buccal sulcus and the rest 3 (6%) included cases from parotid, retromolar trigone and chin.

Out of 48 cases, 34 (71%) were Grade I, 13 (27%) were Grade II and 1 (2%) was Grade III. Similarly, majority of the cases (12 [25%]) were in Stage II, 10 (20.8%) in Stage I, 10 (20.8%) in Stage III and 17 (35.4%) in Stage IV.

Twenty-one out of 48 (43.75%) cases showed lymph node metastasis while 27 (56.25%) showed absence of metastasis.

EGFR expression was analyzed based on TS, and it was found that out of 48 cases 12 (25%) showed strong

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**Table 1: Extent of EGFR expression (ES)**

| Percentage of cells stained (ES) | Extent of expression |
|----------------------------------|----------------------|
| <10 (1)                          | Negative weak extent |
| 10‑50 (2)                        | Weak extent          |
| 51‑80 (3)                        | Moderate extent      |
| ≥80 (4)                          | Severe extent        |

**Table 2: Intensity of EGFR Expression (IS)**

| IS | Intensity of staining |
|----|-----------------------|
| 0  | Negative              |
| 1  | Mild                  |
| 2  | Moderate              |
| 3  | Severe                |

**Table 3: Total Score of EGFR expression (TS= ES x IS)**

| TS (points) | Grading of expression |
|-------------|-----------------------|
| 0-4         | + (weak)              |
| 5-8         | ++ (intermediate)     |
| 9-12        | +++ (strong)          |

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**Figure 1:** Section of well-differentiated squamous cell carcinoma showing mildly pleomorphic cells with abundant keratin production

**Figure 2:** Section of moderately differentiated squamous cell carcinoma showing moderately pleomorphic cells with scant keratin
EGFR expression between 9 and 12, 29 (60%) showed intermediate expression between 5 and 8, 6 (13%) showed weak expression between 1 and 4 and 1(2.1%) showed no expression i.e. score 0.
Verma, et al.: Evaluation of EGFR expression scoring system in Head and Neck Squamous Cell Carcinoma and its association with various pathological prognostic factors

Correlation of epidermal growth factor receptor expression with tumor grade

Association between TSs of EGFR expression and tumor grade was analyzed, and we found that out of 34 Grade I cases, 21 showed intermediate and 7 showed strong EGFR expression. Similarly, out of 13 Grade II cases, most of the cases showed intermediate expression. We observed that the association between TSs of EGFR expression and histological grades just touched the line of statistical significance ($P = 0.055$) [Table 4 and Figure 9].

Correlation of epidermal growth factor receptor expression with tumor stage

Association between TSs of EGFR expression and tumor stage was evaluated, and we found that out of 17 Stage IV cases, majority showed intermediate EGFR expression. Similarly, most of the Stage I and II cases showed intermediate degree of EGFR expression.

The result showed that association between TSs of EGFR expression and tumor stage was not statistically significant ($P = 0.6$) [Table 5 and Figure 10].

Correlation of epidermal growth factor receptor expression with and without lymph node metastasis

We analyzed TS of EGFR expression with and without lymph node metastasis and found that out of 21 cases, 13 (61.9%) cases with lymph node metastasis and 16/27 (59.2%) cases without lymph node metastasis showed moderate staining intensity. The difference was not statistically significant ($P = 0.66$) [Table 6 and Figure 11].

DISCUSSION

Oropharyngeal cancers are a heterogeneous group of malignancies in terms of etiology, biological behavior and prognosis. Over the years, TNM staging has been the most useful indicator to predict the prognosis in patients with oropharyngeal carcinoma. In the recent years, several attempts have been made by researchers, across the globe, to find immunohistochemical markers that can be used either independently or in conjunction with TNM staging, to predict the outcome of these cancers. Among the various markers explored, EGFR has been the most promising. We extensively searched the literature but could not find any study which has used this scoring system of EGFR expression for comparison with the parameters that we have used in our study. Considering this fact, the study was conducted and we therefore evaluated EGFR expression in OSCC cases as per this scoring system and found its association with various pathological prognostic factors such as grade, stage and lymph node metastasis.

The overexpression of EGFR has been observed in 40%–80% of cancers of the head and neck. The nature of this overexpression seems to be due to increase of the transcription of EGFR, although its amplification has also been observed. Numerous works attribute a negative independent prognostic value to EGFR and point out that there is a greater probability of posttherapeutic secondaries and a lower survival rate, if the tumor expresses EGFR.

There is no established protocol that is universally used to evaluate EGFR expression. We have extensively explored the benefits and limitations of various scoring systems and thereafter used the scoring methodology similar to that used by Young et al., 2011, as we found it to be the most comprehensive scoring method for evaluating EGFR expression, in HNSCC.

In this study, we found that out of the total 48 cases, majority of the cases (29 [60%]) showed intermediate EGFR
expression, followed by 12 (25%) cases which showed strong EGFR expression. Six (13%) cases showed weak expression with one case (2.1%) showing no expression at all.

Overexpression of EGFR has been observed in 40%–80% of cancers of the head and neck. Similarly, our study showed high percentage of EGFR overexpression. A study conducted by Sarkis et al. showed that high EGFR expression suggests an uncontrolled growth which may be mediated by abnormal EGFR expression.

Maiti et al. conducted a study on the gene amplification, microRNA (mRNA) expression and protein overexpression of EGFR. It was observed that there was a significant correlation between gene amplification and mRNA expression, whereas protein overexpression did not correlate with mRNA expression. This suggested that EGFR expression is not regulated transcriptionally. It is possible that other mechanisms besides gene amplification/mutations might be responsible for observed overexpression of this protein in HNSCC tumors.

We evaluated the significance of association between TSs of EGFR expression and tumor grade and found that it was statistically significant marginally ($P = 0.055$) [Table 4]. This could be as a result of scoring method that is taken into consideration or might be a result of heterogeneous distribution of cases with respect to grades, as only single case of poorly differentiated carcinoma, was reported.

In concordance to this, a study by Gitanjali et al. also had maximum cases (53.8%) in Grade I. However, it was seen that in the study by Reimers et al., Altuna Mariezkurrena et al., Issa and Hama et al., a majority of the cases were of Grade II. Whereupon our study was discordant to the study by Issa which concluded that high EGFR expression was associated with advanced tumor stage. In addition, the study by Reimers et al. suggested a correlation of EGFR expression with advanced tumor stage ($P = 0.051$). The discordance with the above-mentioned studies could be possibly because of relatively modest sample size of our study. Furthermore, as various criteria for assessment of EGFR are heterogeneous, therefore statistical discordance is because of varied inclusion and exclusion criteria.

We also interpreted the association of EGFR expression with lymph node metastasis. The association was not statistically significant ($P = 0.66$) [Table 6]. These findings were consistent with the study by Reimers et al. and Glazer et al. which suggested no correlation of EGFR expression with lymph node metastasis ($P = 0.54$). Whereas opposed to our study, Issa observed an association between EGFR expression and lymph node metastasis. This could be because of small sample size taken in this study.

**CONCLUSION**

Staging and grading are, till date, the most important parameters that help to understand the possible outcome of patients diagnosed with malignancy. In this study, we have tried to find the association of EGFR expression with various pathological prognostic factors such as grade, stage and lymph node metastasis by the TS method employed in the study by Young et al., 2011, which had used different

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**Table 5:** Distribution of cases based on total score of epidermal growth factor receptor expression with tumor stage

| EGFR (TS) | 0-4 (weak) | 5-8 (intermediate) | 9-12 (severe) | Total |
|-----------|------------|--------------------|--------------|-------|
| n (%)     | n (%)      | n (%)              | n (%)        |       |
| Stage I   | 1 (2.1)    | 6 (12)             | 3 (6.3)      | 10 (21)|
| II        | 3 (6.3)    | 8 (17)             | 1 (2.1)      | 12 (25)|
| III       | 1 (2.1)    | 4 (8)              | 4 (8.4)      | 9 (19 )|
| IV        | 2 (4.2)    | 11 (23)            | 4 (8.4)      | 17 (35)|
| Total     | 7 (15)     | 29 (60)            | 12 (25)      | 48 (100)|

**EGFR:** Epidermal growth factor receptor, **TS:** Total score

**Table 6:** Distribution of cases (percentage) based on total score of epidermal growth factor receptor expression with and without lymph node metastasis

| EGFR (TS) | 0-4 (weak) | 5-8 (intermediate) | 9-12 (strong) | Total |
|-----------|------------|--------------------|--------------|-------|
| n (%)     | n (%)      | n (%)              | n (%)        |       |
| LN mets   |            |                    |              |       |
| Present   | 3 (6.3)    | 13 (27.0)          | 5 (10.42)    | 21 (43.7)|
| Absent    | 4 (8.4)    | 16 (33.3)          | 7 (14.6)     | 27 (56.3)|
| Total     | 7 (14.7)   | 29 (60.3)          | 12 (25)      | 48 (100)|

**EGFR:** Epidermal growth factor receptor, **TS:** Total score, **LN:** Lymph node
parameters for comparison. EGF has shown to play a pivotal role in the molecular alteration in carcinogenesis. It is noteworthy that in many malignant tumors, high EGFR expression correlates with a more aggressive clinical course and is a very useful diagnostic and prognostic marker. In the recent years, EGFR has also been considered a promising target for monoclonal antibody therapy. We believe that these findings will be an important adjunct, along with staging and grading, to determine the prognosis and also to design the treatment options that would lead to lesser morbidity and increased survival of patients.

Acknowledgment

Though language is a poor substitute for sentiments, there is no way out other than to recourse it in words. I feel immense pleasure to acknowledge my teachers whom I interacted and shared my work during the course of this work. This piece of work could have never been completed without the unconditional efforts, support and encouragement from all of them.

I would like to express my sincerest gratitude to Dr. Vishal Dhingra, who not only helped in data analysis, but also in manuscript review, editing, intellectual content and framing of results. Also, I am extremely thankful to Dr. Sapan Srivastava, oncosurgeon, who provided us with a toll of cases.

I feel highly obliged and pay my utmost respect to the Professor and Head of Department of Pathology, Dr. Vatsala Misra, for her support, constant supervision and review of the manuscript. I pay my respect to Dr. Kachnar Varma for her cooperation at various steps in completion of this piece of work and also Dr. Shilpy in her unconditional help. Besides this, I owe my thanks to several people who have knowingly and unknowingly helped me in the successful completion of this project.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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