EGFR scoring in head and neck squamous cell carcinoma and its association with clinicopathological variables

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Abstract

Background: Head and Neck squamous cell carcinoma (HNSCC) is the seventh most common cancer in the world with poor overall survival rate which is unchanged during the last two decades. **Aim:** Aim of our study is to measure the level of Epidermal Growth Factor Receptor (EGFR) expression in HNSCC by immunohistochemistry (IHC) and to correlate EGFR with clinicopathological variables. **Settings and Design:** Cross sectional study from 1st October 2012 to 31st of March 2014 was performed. **Materials and Methods:** After taking detailed history and a thorough examination, biopsy/ specimen of HNSCC region were evaluated to confirm the diagnosis of HNSCC. Paraffin blocks of such tumors were processed for EGFR staining. Staining intensity was evaluated by using scale from 1 to 4. **Statistical Analysis:** Chi-square test was used as appropriate for data analysis. **Results:** In the present study 38/50 (78%) patients were diagnosed as well differentiated, 12/50(24%) were diagnosed as moderately differentiated. For EGFR staining, 24/50 (48%) scored as +2, 16/50(32%) as +3, 8/50(16%) as +1 and 2/50(4%) scored as 0. 23/38(60.5%) well differentiated SCC cases presented as +2, 5/38(13.2%) as +3, 8/38(21.1%) as +1 and 2/38(5.3%) as 0. 11/12(91.6%) moderately differentiated SCC cases were scored as +3, 1/12(8.3%) as +2. p value 0.001, which is highly significant. However, correlation of EGFR scoring with patients age, sex, addiction history, site of the tumor was insignificant. **Conclusion:** EGFR was highly expressed in HNSCC. The result of our study showed that, high EGFR scoring was associated with high grade of the tumor. There was no significant relationship between EGFR scoring and clinicopathological variables.

Key words: Epidermal growth factor receptor, Immunohistochemistry, Head and Neck squamous cell carcinoma.
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Introduction

Head and Neck squamous cell carcinoma (HNSCC) is a heterogeneous and complex disease, having a severe impact on quality of life of patients and survivors. At the time of diagnosis 60-70% of patients present with advanced disease affecting survival of the patients negatively [1]. Oral cavity, oropharynx, hypopharynx and laryngeal cancers, when grouped together as head and neck cancer, constitute seventh most common cancer in the world [2]. HNSCC is the most common cancer in developing countries. It is the most common cancer among males in India and the fifth most common in

females [3]. It causes devastated effects on communication and swallowing. The overall five years survival rate is among the lowest of the major cancers and has not changed during the past two decades [4,5].

Many factors interplay in the phenomenon of carcinogenesis. These include hereditary factors, hormones, ageing, immune status and background radiation. In Indian subcontinent, chewing tobacco in the form of betel quid, bidi smoking and drinking locally brewed crude alcoholic drinks are the major causative factors [6,7]. All normal cells require stimulation on the basis of signals to undergo growth, differentiation and proliferation,

many of which are carried by growth factors. Epidermal Growth Factor Receptor (EGFR) plays an important role in the differentiation and morphogenesis of most organs and proliferation and survival in mammalian cells [8]. In HNSCC, either over expression or mutation of EGFR has been found in 80- 100% of patients, and both are associated with poor prognosis and decreased survival [9]

Despite recent advances in our understanding of the role of molecular and genetic abnormalities in the pathogenesis and clinical course of HNSCC, this disease remains one of the most significant cause of morbidity and mortality among malignancies worldwide [10]. Only 50% of HNSCC patients, the current conventional treatment strategies, including surgery, chemotherapy and radiation, are effective, underscoring the need for new approaches to treat this malignancy [1].

Overexpression of EGFR in all HNSCCs led to development of pharmacotherapy directed against this cell-surface receptor [10]. The rationale for the development of EGFR-targeted therapies for treatment of HNSCC includes the following: 1) EGFR is highly expressed in many head and neck cancers; 2) EGFR overexpression in HNSCC is associated with reduced survival in several independent studies, and 3) EGFR-targeting in HNSCC preclinical models demonstrated anti-tumor efficacy [10,11]

However, correlation of EGFR scoring with grading of HNSCC has not been well established in previous studies. The purpose of this study is to better understand the role of EGFR expression in head and neck tumorigenesis and to compare EGFR scoring by IHC with different clinicopathological variables.

Materials and Methods

Source of Data: Patients attending Out Patient Department and In-patients diagnosed or suspected to have squamous cell carcinoma, at Sri Siddhartha Medical College and Research Centre, Tumkur.

Study Design: Prospective case control study.

Participants: 50 biopsies received in the department from 1st October 2012 to 31st of March 2014.

Period of Study: 1st October 2012 to 31st of March 2014. (18 months)

Inclusion Criteria: Histologically proven cases of head and neck squamous cell carcinomas.

Exclusion Criteria

1) Benign lesions of head and neck region.
2) Malignancies other than squamous cell carcinoma.
3) History of previous oncological treatment.
4) Squamous cell carcinoma of other sites.

Clinicopathological Variables: Age, Sex, Addiction, Site of the tumor, Grading of the tumor.

Data Source: Before the commencement of study, we considered the ethical aspects and obtained ethical committee approval. After taking consent, detailed history of the patient was taken including the history of tobacco, alcohol, quid chewing and other environmental exposures. Complete physical examination was done.

Tissue samples from each tumor lesion were fixed for in 4% neutral buffered formalin, for 6 to 24 hrs according to the size of sample. After paraffin embedding, tumor specimens were cut into 5 µm sections and stained routinely with haematoxylin and eosin to confirm the diagnosis of HNSCC and to define representative tumor regions.

Histologically, the tumor was graded as well or moderate or poorly differentiated. The histologic tumor grade is based on the degree of squamous differentiation (keratinisation, pearl formation, and intercellular bridges), degree of cellular pleomorphism and mitotic index (number of visible mitotic figures in tumor cells) [12].

Paraffin blocks of such tumors were processed for EGFR staining. IHC staining was performed by using the EGFR Leica biosystem, according to the manufacturer's instructions and using the reagents supplied with the kit. Slides were counterstained with hematoxylin, dehydrated and mounted. In brief, sections of 5µm were mounted on sialinized charged slides and allowed to dry for one hour followed by one hour at incubator at 60ºc. After deparaffinization and rehydration, slides were incubated with proteinase K solution for five min. After washing procedure with distilled water, tissue

sections were covered for five minutes with 3% H_2O_2, to block endogenous peroxidise, followed by an additional washing procedure with the supplied buffer. Slides were placed in a humid chamber and incubated for 30 minutes with the primary mouse anti EGFR MAb, which binds to formalin resistant epitope near the ligand binding site on the extracellular domain of the EGFR. After two rinses in buffer the slides were incubated with the detection system for 30 minutes. Tissue staining was visualized with a DAB substrate chromogen solution. Slides were counterstained with hematoxylin, dehydrated and mounted. Control samples were run simultaneously. Although occasional cytoplasmic staining of the tumor cells was observed, which may result from either internalized or nascent receptor molecules, only staining of the tumor cell membranes was considered to be specific. Staining intensity was evaluated on paraffin embedded tumor sections by microscopy using a scale from one to four [13].

0)   No Positive Cells
+)   Up to 20% Cells Stained
++)   21 - 49% Cells Stained
+++)   >50% Cells Stained

Statistical analysis tests: chi square was applied to reject the null hypothesis, that the data are independent.
 p value, in our study, if found to be
<0.01= highly significant,
0.01-0.05= significant,
>0.05 insignificant.

Results

In our study, during the period of 18 months, from 1st October 2012 to 31st of March 2014, a total of 50 cases of head and neck squamous cell carcinoma cases were studied at a tertiary care centre. EGFR mutation analysis was done for all cases.

Age of the patient varied from 40 to 70 years. Maximum number of cases were in the age group of 50-59 years (20/50) comprising 40% of cases followed by the age group of 60-69 years (13/50). Youngest subject was 40 years old and the oldest was 80 years old. Overall mean of the age variable was 58 years (Table 1). p value for the EGFR scoring and age was 0.0632(Table 2), which was not significant statistically. Out of 50 HNSCC cases in our study 29(58%) and 21(42%) were males and females respectively, with male to female ratio of 1.38:1 (Table 1). For both males and females, majority of the cases were scored as +2(47.6% males and 48.3% females). But no association was found between sex and EGFR scoring (Table 2).

Majority of HNSCC cases gave history of addiction to quid chewing and smoking for 20 to 30 years. In the present study, 42% of the patients gave history of quid chewing, which was the most common addiction followed by smoking and alcohol. 5 subjects had no history of any addiction (Table 1). P value for the addiction and EGFR was 0.1044 (Table 2), which was not significant.

Oral cavity (52%) was the most prevalent tumor location in our study, followed by maxilla (12%), tongue (8%), tonsil (6%), gingivobuccal sulcus (6%), nasal cavity and paranasal sinus (6%), nasopharynx (4%), oropharynx (4%), and oesophagus (2%) (Table 1). There was no relationship between site of the tumor and EGFR scoring, for which p value was 0.1044 (Table 2).

Based on the histological grade, most (76%) of the patients were diagnosed as well differentiated SCC while moderately differentiated cases were 24%. We did not diagnose any case of poorly differentiated SCC (Table1).

The representative sections were selected for IHC. EGFR expression was evaluated on the basis of extent and intensity of immunelabeling in tumor cell membranes and classified on a four-point scale. According to that four scale scoring system, 24(48%) cases were scored as +2, 16(32%) cases as +3, 8(16%) cases as +1 and 2(4%) cases were scored as 0(Table 1).

Among the 38 patients of well differentiated HNSCCs, 60.5% patients were EGFR score +2 and among 12 patients of moderately differentiated HNSCC, 91.7% patients were EGFR +3. (p value=<0.001), which is statistically significant (Table 3, Graph1).

Original Research Article

Table 1: Patients and disease characteristics.

Number of patients	50
Male/ Female	29/21
Mean age	58 years
Addiction/ no addiction	45/5
Sites	
Oral cavity	26
Maxilla	6
Tongue	4
Others	14
Grading	
Well differentiated	38
Moderately differentiated	12
Poorly differentiated	0
EGFR scoring	
0	2
+1	8
+2	24
+3	16

Table 2: Correlation of EGFR with clinicopathological variables.

Clinicopathological variables	p value
Age	0.0532
Sex	0.6415
Addiction	0.1044
Site of the tumor	0.7301
Grading	0.001

Table 3: Comparison of Histopathological Diagnosis with EGFR Scoring In HNSCC Patients.

EGFR SCORING	Histopathological diagnosis		TOTAL	p value 0.001
	Well differentiated	Moderately differentiated		
0	2(5.3)	0(0)	2(4)	
1	8(21.1)	0(0)	8(16)	
2	23(60.5)	1(8.3)	24(48)	
3	5(13.2)	11(91.7)	16(32)	
TOTAL	38	12	50	
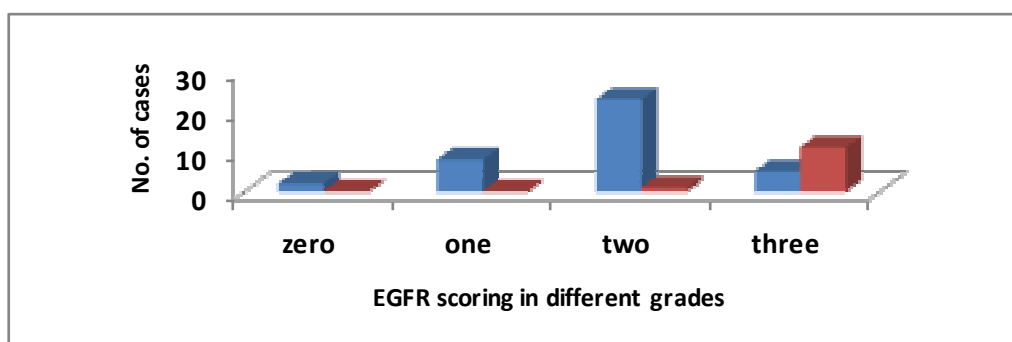
Graph-1: Comparison of Histopathological Diagnosis with EGFR Scoring In HNSCC Patients.
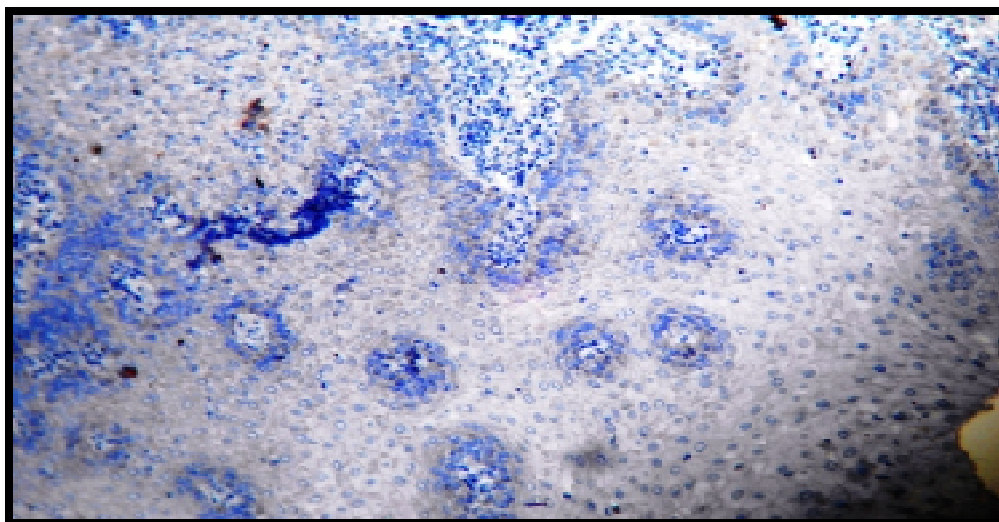
Figure 1: Membrane immunoreactivity of EGFR in well differentiated squamous cell carcinoma showing no positive cells: EGFR scoring: 0. (40x)
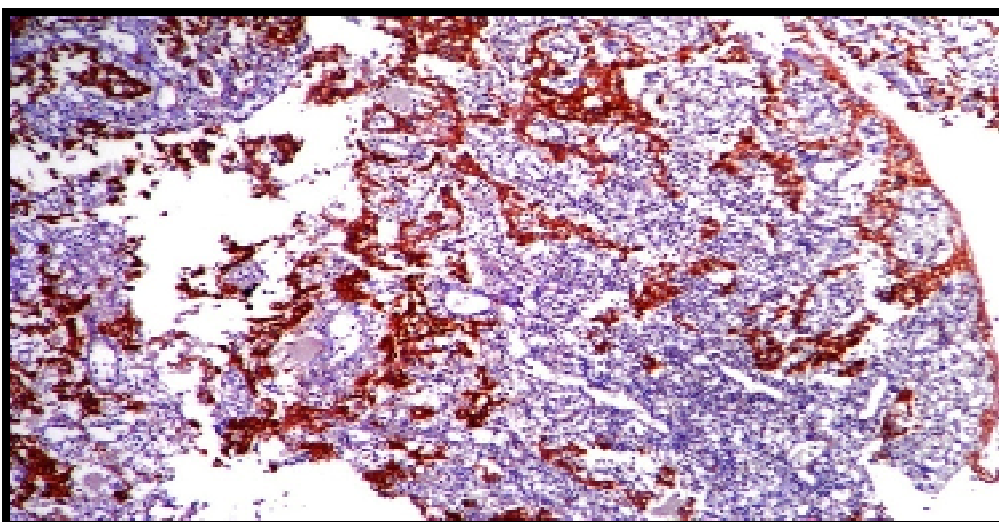

Figure 2: Membrane immunoreactivity of EGFR in well differentiated squamous cell carcinoma showing <20% positive cells: EGFR scoring: 1+(10x)
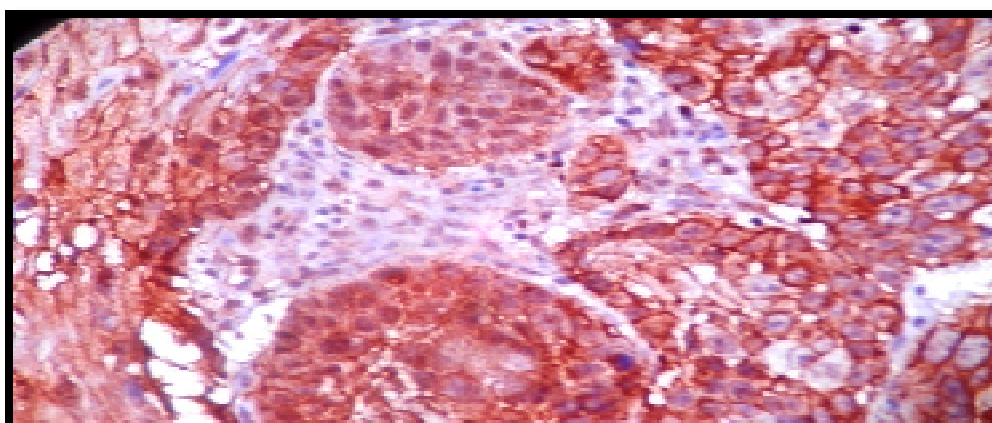

Figure 3: Membrane immunoreactivity of EGFR in well differentiated squamous cell carcinoma showing 20-49% positive cells: EGFR scoring: 2+, (400x)
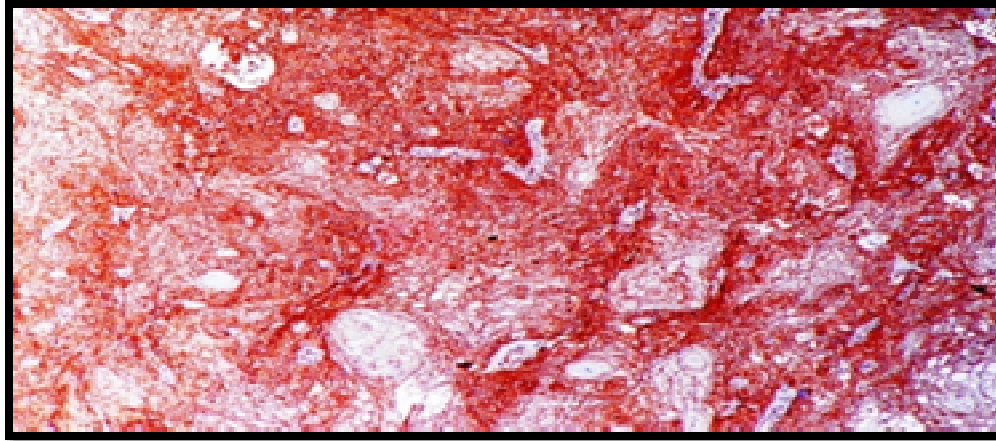
Figure 4: Membrane immunoreactivity of EGFR in well differentiated squamous cell carcinoma showing >50% positive cells: EGFR scoring: 3+. (10x)

Discussion

HNSCC is one of the leading cancers worldwide and is becoming a great health threat in Indian subcontinent. It has been reported that majority of HNSCC express EGFR, since most HNSCCs are epithelial in origin [8]. The family of EGFR (HER - 1, HER-2, HER-3 and HER-4) includes cell membrane receptors with intrinsic tyrosine kinase activity which can transduce a proliferation signal in response to the binding of different ligands. These receptors play a key role in malignant proliferation of cells in a variety of human tumors. After ligand receptor binding, EGFR undergoes dimerization and activation of tyrosine kinase occurs, with receptor autophosphorylation, downstream signal transduction through activation of RAS and MAP kinase occurs, and ultimately gene activation leading to cell proliferation. EGFR is a 170kDa membrane glycoprotein, with an extracellular ligand binding domain and intracellular domain with tyrosine kinase activity [9].

Xia et al. examined the expression of four EGFR family members in oral SCC and their relationship with TNM staging, patient survival, and other EGFR family members and concluded that a combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members [13]. Chen et al in 2003 studied in 59 patients, of which, EGFR was overexpressed in 2(3%) normal mucosal tissues and 34(58%) cancer tissues [5]. Sharafinski et al studied about EGFR expression in HNSCC and clinical responses to EGFR inhibitors [10].

The purpose of this study is to better understand the role of EGFR expression in Head and Neck tumorigenesis and to compare EGFR scoring by IHC with different clinicopathological variables.

In this study we found that majority of the patients were males. However male to female ratio was low as compared to other studies [5,13,10]. HNSCC is more commonly seen in male because of the habits of smoking and alcohol. The lower proportion of tobacco related cancers among women is mostly explained by the fact that, tobacco use, especially smoking, which is more common among men than among women [14].

But in our study incidence in female subjects was almost comparable with males. In India, tobacco chewing is more common than smoking, which is also very common in females [6]. Betel nut and tobacco chewing is more common in Indian and southeast Asian women [15]. Pan (consisting of betel leaf, areca nut and lime with or without tobacco) chewing is a fairly common social habit particularly in the older population and the habit is relatively more frequently seen in women than men, as men more often smoke than chew tobacco [16].

Other reason may be Betel quid chewing without tobacco, which is common in South India. Chewing products without tobacco is also an independent risk factor for cancers of the oral cavity and oesophagus. Quid chewing without tobacco induced a higher risk factor of cancers than chewing with tobacco. This may be explained by

swallowing the liquid extract produced by chewing as opposed to spitting it out [17].

The present study has maximum cases of well differentiated SCC and was comparable with the study by Fong et al [18]. However, in the studies by Chen et al and Bernardes et al, moderately and poorly differentiated SCC were reported in higher incidence [5,15].

Some studies have found tumour grade to have prognostic significance, but determinations of grade using traditional histopathologic criteria often vary among different observers. Therefore, tumour grade is of limited prognostic value compared with clinical stage, as many or most HNSCCs are graded as moderately differentiated [19].

In the present study, of the 50 cases of HNSCC, EGFR +ve cases were 90% and EGFR –ve cases were 10%. A similar observation was made by Schuler et al, Sarkis et al [8,20]. In these studies, most of the cases were positive for EGFR [20]. Only few cases were scored as negative. Study done by Bernardes et al, Chen et al and Pectasides et al showed discrepant result from the present study [5,15,21]. In these studies EGFR positive and negative cases were almost the same [5,15,21].

In present study, there was strong correlation between histopathological diagnosis and EGFR scoring, as p value was 0.001, which is highly significant. EGFR positivity was seen in both, well and moderately differentiated SCC, but it was more in high grade of the tumors. Sheikh et al have also reported similar observation with p value 0.02 [22]. In the studies done by Chen et al and Pectasides et al, p value was not significant [5,21].

They did not found any significant correlation between EGFR scoring and grading of the tumor. In the present study no association was found between EGFR scoring with clinicopathologic variables like age, sex, site, addiction, etc. Similar observation is seen in the studies done by Sheikh et al and Temam et al [22,23].

Overall there are several aspects that can explain the differences among our study and the previous studies described above. The difference in study design, sample collection and EGFR expression

examination technique, interobserver variation in EGFR scoring can explain the discrepancy between various results.

Follow up of the subjects could not be done to find out relation between EGFR scoring and prognosis, which was one of the limitations of our study.

Conclusion

Based on our study we conclude that majority of the patients of HNSCC shows expression of EGFR. EGFR expression is also shown to be associated with high grade of tumor and poorer prognosis.

EGFR expression is independent of the clinicopathologic variables. EGFR has been identified as a target receptor for targeted chemotherapy. So testing for EGFR expression in patients of HNSCC can help us in identifying those patients who have high grade tumor and have poorer prognosis. At the same time it also identifies patients who are candidate for targeted therapy which is more effective than conventional therapy and has less adverse effects. So we recommend that in every patient with HNSCC testing for EGFR expression should be done by semi quantitative or quantitative method. It is also possible that patients with higher expression of EGFR may have better results with targeted therapy especially in this subgroup of patients which can be studied further.

Future studies should also focus on EGFR gene mutation and polymorphism and their effects on prognosis and therapy.

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