



Occurrence of Epidermal Growth Factor Receptor Expression in Squamous Cell Carcinoma of Oral Cavity and Adjacent Sites: A Retrospective Observational Study

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Abstract

Introduction Oral cavity squamous cell carcinomas (OCSCCs) are one of the most common malignancies encountered. They have diverse clinicopathologic profiles. Epidermal growth factor receptor (EGFR) is an important marker of OCSCC for diagnosis, prognosis, and therapy.

Objective The study aimed to evaluate the distribution of clinicopathological parameters in cases of squamous cell carcinoma (SCC) of the oral cavity and adjacent sites and their EGFR expression. An attempt was made to correlate EGFR scores with different parameters.

Materials and Methods An observational retrospective study was undertaken for 1 year. Patients with histologically proven SCC of the oral cavity and adjacent sites were included. Clinicopathologic and immunohistochemical features of each case were analyzed.

Results A total of 59 cases of SCC were included of which 41 (69%) were males. The mean age of the patients was 56.4 ± 10.3 years. The most common location of the tumors was oral cavity (31, 53%) followed by larynx (10, 16%). Most cases were moderately differentiated tumors (31, 53%). Immunohistochemical analysis showed low EGFR score in 7 (11.9%) cases, intermediate in 6 (10.1%), and high in 46 (78%) cases. Association between gender of patients and EGFR score was statistically significant ($p = 0.000159$). Most lesions of the oral cavity (31 cases, 53%) had a high EGFR score (23 cases, 74.2%). A statistically significant correlation was found between tumor grade and EGFR score ($p = 0.03338$).

Conclusion This study from eastern region of India reveals unique characteristics of EGFR expression in SCC of the oral cavity and adjacent sites.

Keywords

- ▶ epidermal growth factor receptor
- ▶ histopathological grade
- ▶ oral cavity squamous cell carcinoma

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Introduction

Squamous cell carcinoma (SCC) of the oral cavity and adjacent sites constitute head–neck SCC (HNSCC). These tumors are aggressive malignancies and account for approximately 600,000 new cases annually. It is the sixth most common carcinoma in the world.¹ The age-adjusted rates of HNSCC are highest in countries such as India, France, and Brazil. Worldwide, the age-adjusted rate of HNSCC is found to be the highest among Indian females.^{2,3} HNSCC accounts for 30 to 40% of all the cancers in India.⁴

The clinicopathological profile of HNSCC varies from one part of the world to another, due to variation in the predominant etiological factors in different geographic locations. The site of HNSCC is influenced by the risk factors responsible. Oral cavity is the most common site affected in the Indian subcontinent. The major predisposing factors include tobacco smoking, alcohol intake, and ultraviolet radiation exposure (particularly in carcinoma of lip). Infectious agents, such as human papilloma virus and *Candida species* have also been implicated along with some genetic factors and nutritional deficiencies.⁵

The border of the tongue is the most common site affected in American and European countries.^{6,7} In Southeast Asia, the most common site is reported to be buccal mucosa.⁸ Studies in Hungary have reported floor of mouth, lips, and tongue to be the commonly affected sites.⁹ These variations are interesting, as they corroborate the role of different etiologic factors prevalent in a particular area.

Most oral cavity SCCs (OCSCCs) are reported as well-differentiated (WD) or moderately differentiated (MD) tumors. The site of the tumor has a significant relation with the histological grade of the lesion. Tumors of the buccal mucosa and lips are mostly of lower grade (WD) whereas those of the tongue and gingiva are of a higher grade poorly differentiated (PD).⁵

Epidermal growth factor receptor (*EGFR*) gene is located on chromosome 7p14–12. The protein has a molecular weight of 170 kDa. EGFR is expressed by all adult tissues, except hematopoietic elements. It plays an important role in tumorigenesis in many organs including prostate, bladder, ovary, breast, bladder, pancreas, and brain. EGFR is also implicated in OCSCC.¹⁰ In the recent years, EGFR has emerged as one of the most promising markers for OCSCC not only for diagnosis but also for therapy and prognostication.¹ Monoclonal anti-EGFR antibodies are commonly used for the treatment of HNSCC. One of the most common drugs that belong to this group is cetuximab which binds with EGFR and inhibits activation of the downstream signaling pathways.¹¹

The present article depicts an exploratory study which was undertaken to evaluate the distribution of various clinicopathological parameters in SCC cases of the oral cavity and adjacent sites and their EGFR expression. The study also attempted to find out if there is a correlation between EGFR score with grade of the tumor. Since data in this regard are scarce from the eastern region of India, this study hopes to contribute to literature at a time when targeted therapy is one of the well-established goals of molecular pathology.

Materials and Methods

The duration of the present retrospective observational study was from January 2019 to December 2019. All patients who presented with histologically proven SCC of the oral cavity and adjacent sites were included in the study. Patients with other variants of malignancy, metastatic carcinoma, recurrence, and lesions diagnosed by cytological means were excluded from the study.

For each case, the following data were collected: age, sex, duration, presence of any risk factors, clinical presentation, location, and size of the lesion. The clinical presentation of lesions was grouped into three categories: ulcers (both plain ulcers and exophytic ulcerated lesions), leukoerythroplakias, and lesions having both ulcerated and leukoerythroplakic regions.⁵ Location of tumor in the oral cavity was classified into the following sites: buccal mucosa, borders of tongue, alveolar mucosa and gingiva, floor of mouth, soft palate, upper and lower lips, and tonsils. Histopathological slides were examined, and each case was classified as WD, MD, or PD tumors, in accordance with the WHO criteria.¹²

Immunohistochemical staining for EGFR was done in each case. Scoring was done after taking into account the intensity of staining and the percentage of positive cells. The intensity of staining was graded as 1, 2, or 3 for weak, moderate, or strong intensity, respectively. The percentage of positive cells was graded as follows: <1%: 0; 1 to 20%: 1; 20 to 50%: 2; 50 to 80%: 3; and >80%: 4. Each slide was scored by two observers independently and the scores were combined to render the final score of EGFR expression, that is, scores 0 to 2 were designated as low expression, 3 to 4 as intermediate expression, and 5 to 7 as high expression.^{1,13}

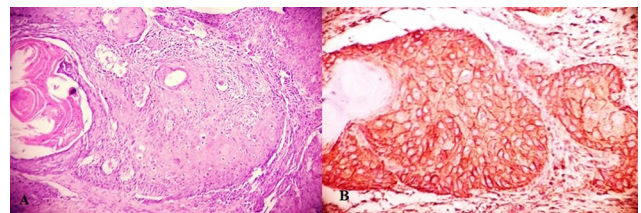


Fig. 1 (A) Well-differentiated squamous cell carcinoma of the oral cavity (H&E, $\times 100$); (B) high epidermal growth factor receptor score in a case of well-differentiated squamous cell carcinoma of oral cavity (H&E, $\times 100$). H&E, hematoxylin and eosin.

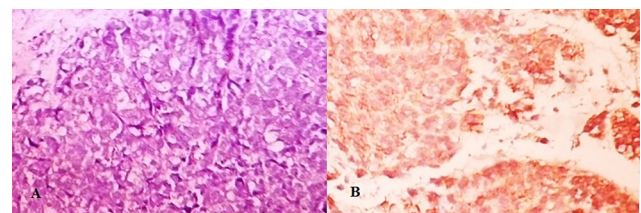


Fig. 2 (A) Poorly differentiated squamous cell carcinoma of the lip (H&E, $\times 100$); (B) low epidermal growth factor receptor score in a case of poorly differentiated squamous cell carcinoma of the lip (H&E, $\times 100$). H&E, hematoxylin and eosin.

Statistical Analysis

Standard statistical methods including Student's *t*-test were utilized to analyze the data collected.

Ethics

Approval for the study was obtained from the Institutional Ethical Committee (IEC), Employees State Insurance Postgraduate Institute of Medical Sciences and Research (ESI-PGIMSR), Maniktala (no. ESI-PGIMSR/MKT/IEC/02/2019, dated January 11, 2019). Due to the retrospective nature of the study, waiver of informed patient consent was obtained from the Ethics Committee. The procedures followed were in accordance with the ethical standards of the IEC and with the Helsinki Declaration of 1964, as revised in 2013.

Results

In the present study, a total of 59 cases of SCC of the oral cavity and adjacent sites were included. The age of the patients ranged from 21 to 79 years with a mean of 56.4 ± 10.3 years. Maximum number of patients (30, 50.8%) belonged to the age group of 41 to 60 years.

Out of the 59 patients, 41 (69%) were males and the rest (18, 31%) were females. The ratio of male-to-female patients was 2.3:1. In 23 cases (38.9%), history of oral tobacco or betel nut chewing was noted. History of smoking was present in 34 cases (57.6%) and that of alcohol consumption in 24 patients (40.7%).

The most common location of the tumors was found to be the oral cavity (31, 53%) followed by larynx (10, 16%). Most of the cases on histopathological examination were MD tumors (31, 53%). PD tumors constituted only 10% (6) of cases.

Immunohistochemical analysis revealed low EGFR score in 7 (11.9%) cases, intermediate in 6 (10.1%) cases, and high

in 46 (78%) cases. Analysis was done to find out if there is any correlation between EGFR score and age and gender of patients, risk factors, location of tumor, and tumor grade. In the age group between 61 and 80 years (22 cases), only 1 (5%) case showed low EGFR score, whereas 17 patients (77%) showed high score. Among males (41, 69%), 38 cases (93%) showed high EGFR score.

High EGFR score was found in 23 out of 31 cases (74.2%) located in the oral cavity. In larynx, 6 out of 10 cases (60%) showed high EGFR score. Most WD tumors (19 out of 22, 86.4%) showed high EGFR score. Low score was observed among five (16%) MD cases and one (16.7%) PD tumor.

Statistically significant correlation ($p = 0.002$) was found between gender of patients and EGFR score. The correlation between grade of tumor and EGFR score was also found to be statistically significant ($p = 0.03338$). However, there was no significant association between age, location of tumor, and EGFR score (►Tables 1–4). Further, the correlation between risk factors (smoking, oral tobacco/betel nut chewing, and alcohol consumption) and EGFR expression was not statistically significant. A comparison between the findings of the present study and previous works which evaluated EGFR score in cases of HNSCC is shown in ►Table 5.

Discussion

OCSCC is the most common malignancy of the head-neck region. There is wide variation in the site predilection of the tumors according to the geographic distribution of the patients. This has been attributed to the different etiologic factors prevalent in different regions. In Southeast Asia, the common habit of chewing tobacco and betel nut is responsible for buccal mucosa being the most common site affected in that region.⁵

Males are more commonly affected by OCSCC than females. This has been reported in various studies conducted

Table 1 Correlation between age groups and epidermal growth factor receptor score

Age groups (y)	Number of cases (<i>n</i> = 59) <i>n</i> (%)	EGFR score <i>n</i> (%)		
		Low (0–2)	Intermediate (3–4)	High (5–7)
21–40	7 (11.9)	2 (29)	1 (14)	4 (57)
41–60	30 (50.8)	4 (13.3)	2 (6.67)	24 (80)
61–80	22 (37.3)	1 (5)	4 (18)	17 (77)

Abbreviation: EGFR, epidermal growth factor receptor.

Note: Chi-square statistic is 4.5458. The $p = 0.337147$. The result is not significant at $p < 0.05$.

Table 2 Correlation between gender distribution and epidermal growth factor receptor score

Gender	Number of cases (<i>n</i> = 59) <i>n</i> (%)	EGFR score <i>n</i> (%)		
		Low (0–2)	Intermediate (3–4)	High (5–7)
Male	41 (69)	1 (2)	2 (5)	38 (93)
Female	18 (31)	6 (34)	4 (22)	8 (44)

Abbreviation: EGFR, epidermal growth factor receptor.

Note: Chi-square statistic is 17.496. The $p = 0.000159$. The result is significant at $p < 0.05$.

in different parts of the world.¹⁴ The present study recorded a ratio of male-to-female patients of 2.3:1.

OCSCC usually affects adults, probably reflecting the requirement of exposure of patient to the carcinogen for a prolonged period. The incidence of OCSCC in patients below 40 years of age is between 0.4 and 6%.^{5,15} In the present study, most of the patients (47%) belonged to the age group of 61 to 80 years.

The prognosis of OCSCC has remained bleak for the last few decades despite the advent of combined regimens consisting of surgery, chemotherapy, and radiotherapy. It has been reported that complete recovery could only be achieved in <50% of the patients. Researchers have therefore stressed on the need of molecular targets for OCSCC cases. The most promising target that has emerged over the years is EGFR.

Table 3 Correlation between location of tumor and epidermal growth factor receptor score

Location	EGFR score n (%)		
	Low (0–2)	Intermediate (3–4)	High (5–7)
Oral cavity (31, 53%)	5 (16.1)	3 (9.7)	23 (74.2)
Lip (8, 14%)	1 (12.5)	1 (12.5)	6 (75)
Pharynx (4, 7%)	1 (25)	1 (25)	2 (50)
Pyramidal sinus (6, 10%)	1 (16.7)	1 (16.7)	4 (66.6)
Larynx (10, 16%)	2 (20)	2 (20)	6 (60)

Abbreviation: EGFR, epidermal growth factor receptor.

Note: Chi-square statistic is 1.8886. The $p = 0.984239$. The result is not significant at $p < 0.05$.

Table 4 Correlation between tumor grade and epidermal growth factor receptor score

Tumor grade	Number of cases (n = 59) n (%)	EGFR score n (%)	
		Low (0–2)	Intermediate (3–4) and high (5–7)
Well differentiated	22 (37)	2 (9)	20 (91)
Moderately and poorly differentiated	37 (63)	6 (16)	31 (84)

Abbreviation: EGFR, epidermal growth factor receptor.

Note: The $t = 3.67374$. The $p = 0.03338$. The result is significant at $p < 0.05$.

Table 5 Comparison of current and previous studies that evaluated epidermal growth factor receptor score in cases of head-neck squamous cell carcinoma

Study (year)	Number of cases	EGFR expression n (%)		Parameters which showed statistically significant correlation with EGFR score	Parameters which did not show statistically significant correlation with EGFR score
		Low and intermediate	High		
Hiraishi et al (2006) ¹⁹	52	19 (36.5)	33 (63.5)	Tumor invasion	<ul style="list-style-type: none"> Age Gender Grade of tumor Stage of tumor
Zafar et al (2017) ¹⁴	52	16 (30.8)	36 (69.2)	Age	<ul style="list-style-type: none"> Gender Grade of tumor
Hashmi et al (2018) ¹⁷	115	108 (93.9)	7 (6.1)	Tumor stage	<ul style="list-style-type: none"> Age Gender Risk factors Site of tumor Grade of tumor
Verma et al (2018) ¹	48	36 (75)	12 (25)	Tumor grade	<ul style="list-style-type: none"> Tumor stage Lymph node metastasis
Present study (2020)	59	13 (22)	46 (78)	Gender Tumor grade	<ul style="list-style-type: none"> Age Risk factors Site of tumor

Abbreviation: EGFR, epidermal growth factor receptor.

The EGFR antagonists that have been studied include cetuximab and nimotuzumab.¹⁶

The rationale for using the anti-EGFR antibodies is based on the high levels of EGFR expression by HNSCC tumor cells. However, it has also been found that constitutive EGFR activation may not always be accompanied by enhanced EGFR expression. Nevertheless, elevated EGFR score on immunohistochemical analysis holds promise of the efficacy of these targeted therapeutic agents.¹¹

In the present study, statistically significant association was found between gender of patients and EGFR score. Further, the correlation between grade of tumors and EGFR score was found to be statistically significant. However, the correlation of age of patients, risk factors, and location of tumors with EGFR score was not statistically significant. Zafar et al found significant association between age of patients and EGFR score, whereas the correlation between gender of patients, grade of tumor, and EGFR expression was statistically insignificant in their study.¹⁴ On the other hand, Hashmi et al found significant association of EGFR score with tumor stage and disease-free survival. They did not find any statistically significant correlation between age, risk factors, location of tumor, and EGFR score.¹⁷ This corroborates with the findings of the present study. Of note, Verma et al reported a significant association between EGFR score and tumor grade, but not with stage of tumor.¹

Many studies conducted in the past have reported a high level of expression of EGFR in SCC cases of the oral cavity and adjacent sites. Sarkis et al reported positive EGFR staining in 87.5% of their cases.¹⁸ Hiraishi et al found 92.3% of cases to be positive for EGFR.¹⁹ Laimer et al reported high EGFR expression in 73.42% of cases.²⁰ In the present study, 78% of cases showed high EGFR score. Further, out of the 31 cases in oral cavity, 23 (74.2%) showed high EGFR expression. Comparable results have been showed by Hashmi et al who reported high EGFR score in 68.7% of cases located in the oral cavity.¹⁷

Verma et al reported 62% of both grade-I and -II tumors to have intermediate EGFR score.¹ Most grade-I (86%) and -II (64.5%) cases in the present study, however, showed high scores of EGFR. In most instances, studies show that EGFR expression is higher in low-grade tumors (WD) than high-grade ones (PD).¹⁴ The exception to this lies in cases of larynx and nasopharynx. It has been reported that higher EGFR scores are associated with PD laryngeal SCC cases. These patients have poor prognosis with low relapse-free survival and also poor overall survival.²¹ The present study conforms to these observations. Six of 10 (60%) laryngeal tumors were PD. All these tumors were found to have high EGFR score (5–7).

Conclusion

The present work is a study from the eastern region of India evaluating the EGFR expression in SCC of the oral cavity and adjacent sites. A statistically significant association has been found between gender of patients and EGFR score ($p = 0.000159$). The association between grade of tumor

and EGFR score was also found to be statistically significant ($p = 0.03338$). Further studies from this part of the country are essential to understand the emerging characteristics of various SCCs of oral cavity and adjacent sites, especially with regard to EGFR expression. The importance of the present study cannot be overemphasized in this era of targeted therapy.

Funding

Nil.

Conflicts of Interest

None declared.

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