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Expression of epidermal growth factor receptor in oral and esophageal squamous-cell carcinoma

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ABSTRACT

Background: Squamous-cell carcinoma (SCC) represents the most common type of malignancies in the oral cavity (O) and esophagus (E). Epidermal growth factor receptor (EGFR) plays a key role in numerous processes that affects tumor growth, progression, differentiation, invasion, metastasis, and inhibition of apoptosis. In this study, we wanted to investigate the EGFR expression in OSCC and ESCC cases. As well, another purpose was to observe if there exists any relation between its expression and clinicopathologic factors. To the best of our knowledge, this is the first study which compares the EGFR protein expression between OSCC and ESCC.

Materials and Methods: This cross-sectional study was performed on 46 paraffin blocks (23 OSCC and 23 ESCC). The expression of EGFR was evaluated with immunohistochemical technique. Data analyses were done using SPSS software by Fisher's exact test. Significance was assigned at $P < 0.05$.

Results: Out of 46 patients, 25 cases (54.3%) were male and 21 (45.7%) were female. Seventy-eight percent of OSCCs and 73.9% of ESCCs showed high expression of EGFR. No statistically significant difference was observed between the two groups ($P = 0.73$). There were no statistically significant correlations between EGFR expression and clinicopathologic factors (age, gender, grade, and stage) of OSCCs ($P > 0.05$). A statistically significant correlation was found between EGFR expression and stage in ESCCs group ($P = 0.006$).

Conclusion: No significant correlation was found between the expression of EGFR protein in OSCCs and ESCCs. High expression of EGFR was observed in ESCCs with Stages II, III.

Key Words: Epidermal growth factor receptor, esophageal cancer, oral squamous-cell carcinoma

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INTRODUCTION

Oral squamous-cell carcinoma (OSCC) represents the most frequent form of oral malignancies. This carcinoma is considered the 12th most common cancer worldwide.[1] The mean 5-year survival rate of patients affected by this cancer is about 50%.[2] Moreover, the most common type of malignancy in the esophagus is squamous-cell carcinoma (SCC) which resulted in a great rate of mortality in China.[3] Therefore, increasing the survival rate and improving the prognosis of these patients is a continuing challenge for the clinicians.[4]

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In recent years, many biological markers have been described in tumors of the head and neck. In recent studies, however, prognostic markers that have been suggested for these tumors are as follows: mutations of p53, overexpression of the epidermal growth factor receptor (EGFR), overexpression of Cyclin D1, and transforming growth factor-α.[5]

As well, some prognostic markers such as vascular endothelial growth factor, cyclin D1 and some other factors have been identified for ESCCs.[6,7] Yu *et al*. demonstrated that EGFR overexpression plays an important role in the progression of ESCC, and it can be used as an unfavorable prognostic biomarker in ESCC cases.[8]

In our previous studies, we have compared the expression of Ki-67, p27, and CD34 proteins between ESCC and OSCC cases.[9-11] The results showed that Ki-67 expression in ESCC group was significantly higher than the OSCC group which can predict the progressive biologic behavior of this malignancy.[9] On the contrary, the expression of p27 and CD34 showed no significant difference.[10,11] Since the results of our previous studies showed controversy, the remaining question is whether poor prognosis of ESCCs comes from biological differences of ESCC and OSCC or other factors like their anatomy and delay diagnosis.

EGFR is a superficial tyrosine kinase receptor from ErbB family. This protein plays a key role in numerous processes that affect tumor growth, progression, differentiation, invasion, metastasis, and inhibition of apoptosis.[5] Since most cancers are epithelial in origin, the probability of EGFR expression is high.[12] Furthermore, overexpression of EGFR has been demonstrated in 80% of SCCs.[12] Several studies showed that overexpression of EGFR in SCC is related to the stage of the tumor, invasion, metastasis to the lymph nodes, distant metastasis, and differentiation of the tumor.[4,13] In addition, overexpression of EGFR in malignant tumors, including head-and-neck tumors, is associated with poor prognosis of the disease. However, there is controversy about the prognostic role of EGFR in oral carcinomas.[14]

According to the current studies on EGFR as a therapeutic target in human malignancies, determining the expression of this protein in OSCC and ESCC cases is required.[15] Hence, in this study, we wanted to investigate the EGFR expression in OSCC and ESCC cases and compare it between two groups as well as to observe if there exists any relation between its expression and age, the gender of the patients, stage, and grade of the tumor. To the best of our knowledge, this is the first study which compares the EGFR protein expression between OSCC and ESCC.

MATERIALS AND METHODS

This cross-sectional study was performed on paraffin blocks of patients with histopathologic diagnosis of SCC. We examined 23 cases of OSCC and 23 cases of ESCC which were obtained from the archive of Department of Oral and Maxillofacial Pathology, Dental School of Islamic Azad University of medical sciences, Tehran, and Department of General Pathology of Imam Khomeini hospital, respectively. Cases with incomplete or unavailable clinical records, or inadequate tissue for histopathologic evaluation, and samples of incisional biopsies with the diagnosis of SCC were not included in the study. As well, cases with extensive hemorrhage or necrosis were excluded from the study.

Then, the demographic data of patients, including gender and age at diagnosis and tumor information, including stage and grade of the tumor, were collected from the clinical and pathologic records. The pathologists were blind to these data. Sections with 5-μm thickness were cut and stained with hematoxylin and eosin. Afterward, all slides were verified by an oral and maxillofacial pathologist to confirm the diagnosis.

Immunohistochemical evaluation of EGFR expression (avidin-biotin technique) was performed on sections with 3-μm thickness which was mounted on silicone coated glass slides. Sections were dewaxed in xylene, rehydrated, and incubated in 10 mM citrate HCL buffer for 10 min. After cooling down in room temperature, sections were rinsed in phosphate-buffered saline (PBS) solution. To detect EGFR, mouse anti-human EGFR mAb (Dako, Cytomation, Glostrup, Denmark) was used. The sections were incubated with the antibody (diluted 1:10) for 1 h. After washing with PBS, the sections were incubated with the secondary antibody (biotinylated anti-mouse antibody) for 30 min. Then, the sections were washed again with PBS after which they were reacted with peroxidase-conjugated streptavidin for 30 min. After this, the sections were rinsed again in PBS. Sections were visualized with DAB

(3, 3'-diaminobenzidine). Then, the sections were counterstained with ethyl-green, dehydrated, and coverslipped. A section of cervical epithelium served as positive control and as negative control slides were stained with the omission of the primary antibody.

EGFR immunoexpression on the tumor cell membranes was evaluated by two pathologists separately who were unaware of the clinical data ($\kappa = 0.82$). Then, the protein expression has been scored as follows:[16] 0 (no labeling or labeling in <10% of tumor cells); +1 (weak labeling, homogeneous or patchy in >10% of the tumor cells); +2 (moderate labeling, homogeneous or patchy in >10% of the tumor cells); and +3 (intense labeling, homogeneous or patchy in >10% of the tumor cells). For data analysis, these scores were classified into following two greater categories: no staining/weak staining (Score 0/1) was labeled as "Low expression" and moderate/intense staining (Score 2/3) was categorized as "High expression".

Statistical analysis was performed using SPSS version 16 software (IBM, Chicago, United States). Fisher's exact test was used to analyze the correlation among the expression of EGFR protein and the clinicopathological findings.

RESULTS

Out of 46 patients studied, 25 cases (54.3%) were male and 21 (45.7%) were female. The mean age of patients was 58.63 ± 10.65 years. The mean age of patients with OSCC and ESCC was 57.95 ± 11.38 and 59.30 ± 10.07, respectively.

All 46 cases showed EGFR immunoreactivity with a membranous pattern. Relative frequency distribution revealed that 78.3% of the OSCCs and 73.9% of ESCCs showed high intensity [Figures 1 and 2]. The remaining cases of OSCC and ESCC showed low intensity [Figure 3]. No statistically significant difference was observed between the two groups ($P = 0.73$) [Table 1]. No statistically significant correlations were found between EGFR expression and clinicopathologic criteria, including age, gender, grade and stage of OSCCs ($P > 0.05$) [Table 2]. A statistically significant correlation was observed just between EGFR and stage ($P = 0.006$) in ESCCs group, but no significant difference was seen between EGFR and gender, age, and grade of the tumor [Table 3].

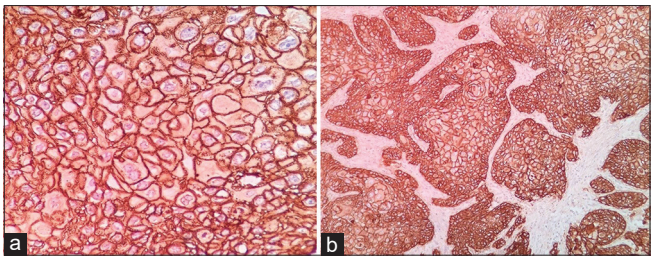

Figure 1: Severe (+3) epidermal growth factor receptor staining observed in oral squamous-cell carcinoma cases (a: 100X, b: 400X).

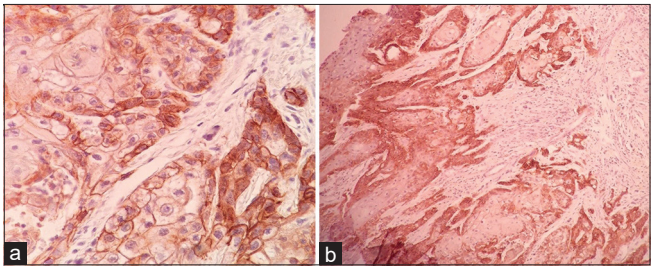

Figure 2: Moderate (+2) epidermal growth factor receptor staining observed in esophageal squamous-cell carcinoma cases (a: 400X, b: 40X).

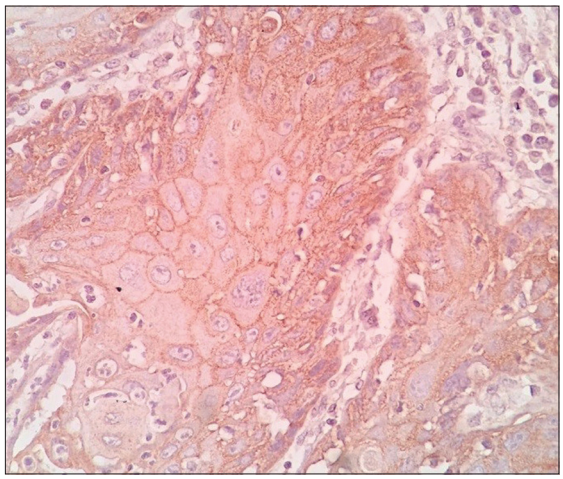

Figure 3: Weak (+1) epidermal growth factor receptor staining observed in oral squamous-cell carcinoma cases (400X).

DISCUSSION

In the present study which was performed on 23 cases of OSCC and 23 cases of ESCC, all the examined cases showed positive EGFR immunostaining. However, high EGFR expression was observed in 76% of all specimens (78.3% of OSCCs and 73.9% of ESCCs). In previous studies, Li *et al*., Yu *et al*., Hanabata *et al*., and Jahanbani *et al*. have reported high EGFR expression in 73.3% of OSCC cases, 61.8% of ESCCs, 50% of ESCCs, and 45% of OSCCs, respectively.[4,8,13,17]

In this series, no significant difference was found between the intensity of EGFR expression in two groups of OSCC and ESCC, which represents that the biologic behavior of the mentioned tumors with due attention to EGFR expression is the same. However, since various factors control the biologic behavior of tumors, it seems that the different behavior of OSCC and ESCC is not dependent to the role of EGFR. Therefore, more studies with greater number of cases are required to confirm this theory.

Sadri *et al*. found that Ki-67 expression in ESCC group were significantly higher than OSCC group which can predict the progressive biologic behavior of this malignancy.[9] Shahsavari *et al*. evaluated the expression of p27 on 20 cases of OSCC and 20 cases of ESCC and showed no significant difference between the two groups. They reported the weak expression of p27 in both groups which reflects the proliferative activity of the cases.[10] These two studies revealed no significant correlation between the expression of Ki-67 and p27 protein with age and gender of the patient, size and grade of the tumor. None of these studies evaluated the stage of the tumor.[9,10] Shahsavari *et al*. in another study evaluated the expression of CD34 on 37 cases of OSCC and ESCC and found severe expression of this protein in both groups. Nevertheless, they presented no significant difference between the two groups. In their study, higher expression of CD34 in OSCCs in older patients and tumors <2 cm in diameter was observed.[11]

Some researchers found a positive relationship between EGFR expression and advanced stages of tumor, lymph node metastasis, distant metastasis, grade, and invasion of tumor,[3,4,15,18] however, the others did not confirm this finding.[8,13] In the present study, a significant correlation was found between high EGFR protein levels and advanced clinical stages of ESCCs ($P < 0.05$). All the cases of ESCC with clinical Stage II and III showed high EGFR expression. This means that EGFR could be used as a prognostic marker. Li *et al*. evaluated the expression of EGFR signaling pathway-related proteins in 60 cases of ESCC and revealed that positive EGFR immunostaining was significantly correlated with the stage of the tumor ($P = 0.007$).[4] Similar results have also been achieved by Moghbeli *et al*. ($P < 0.001$) and Liu *et al*. ($P < 0.001$) on ESCC.[3,15] Conversely, Yu *et al*. in 2011 found no relationship between EGFR expression and stage of ESCC.[8] Nevertheless, in the present study, no significant correlation between

Table 1: Frequency of epidermal growth factor receptor expression in oral squamous-cell carcinoma and esophageal squamous-cell carcinoma cases according to intensity

Intensity	Frequency		
	OSCC, *n* (%)	ESCC, *n* (%)	*P*
Low expression	5 (21.7)	6 (26.1)	0.73
High expression	18 (78.3)	17 (73.9)	
Total	23 (100)	23 (100)	

OSCC: Oral squamous-cell carcinoma; ESCC: Esophageal squamous-cell carcinoma

Table 2: Epidermal growth factor receptor expression and its association with clinicopathologic parameters in oral squamous-cell carcinoma

Clinicopathologic factors	Intensity			*P*
	High expression	Low expression	Total	
Age				
>58	9 (50)	3 (60)	23 (100)	0.69
≤58	9 (50)	2 (40)		
Total	18 (100)	5 (100)		
Sex				
Male	11 (61.1)	1 (20)	23 (100)	0.1
Female	7 (38.9)	4 (80)		
Total	18 (100)	5 (100)		
Grade				
Well-differentiated	9 (50)	1 (20)	23 (100)	0.23
Poorly-moderately differentiated	9 (50)	4 (80)		
Total	18 (100)	5 (100)		
Stage				
I	9 (50)	4 (80)	23 (100)	0.23
II, III	9 (50)	1 (20)		
Total	18 (100)	5 (100)		

Table 3: Epidermal growth factor receptor expression and its association with clinicopathologic parameters in esophageal squamous-cell carcinoma

Clinicopathologic factors	Intensity			
	Low expression	High expression	Total	*P*
Age				
>58	2 (33.3)	10 (58.8)	23 (100)	0.28
≤58	4 (66.7)	7 (41.2)		
Total	6 (100)	17 (100)		
Sex				
Male	3 (50)	10 (58.8)	23 (100)	0.7
Female	3 (50)	7 (41.2)		
Total	6 (100)	17 (100)		
Grade				
Well-differentiated	1 (16.7)	2 (11.8)	23 (100)	0.75
Poorly-moderately differentiated	5 (83.3)	15 (88.2)		
Total	6 (100)	17 (100)		
Stage				
I	6 (100)	6 (35.3)	23 (100)	0.006
II, III	0	11 (64.7)		
Total	6 (100)	17 (100)		

EGFR expression and stage of OSCC was observed. Hanabata *et al*. in 2012 have also reported similar findings.[13] We conclude that EGFR could be used as a prognostic factor just in ESCCs.

Liu *et al*. evaluated 50 cases of ESCC and stated no significant correlation between EGFR expression and histological grade of ESCC.[3] As well, Moghbeli *et al*. in 2013 found no significant relationship between EGFR expression and microscopic grade of the tumor.[15] Yu *et al*. in 2011 evaluated 802 cases in a meta-analysis of nine previous studies and claimed a significant relationship between EGFR expression and grade of the tumor. Moreover, tumors with lower histological grade demonstrated less EGFR expression. They also showed no significant correlation between EGFR expression and stage of the tumor and depth of invasion which are two important criteria in tumor progression.[8] Störkel *et al*. in 1993 assessed the prognostic significance of EGFR expression in 100 cases of OSCC. All cases showed EGFR immunolabeling. In addition, EGFR expression was related to grade and 5-year survival.[19] This study was different from our study from the point of relation between EGFR expression and grade of the tumor. This difference may be due to the greater number of cases that Störkel evaluated. In this study, no significant correlation between EGFR expression and grade of the tumor was seen in both OSCC and ESCC groups ($P \geq 0.05$). Jahanbani *et al*. also revealed no relationship between EGFR expression and grade of the OSCCs[17] which is consistent with our results.

Disagreements about the prognostic value of EGFR in OSCC and ESCC still remain.[20] Some researchers found a relationship between severe expression of EGFR and poor prognosis,[21] whereas the others correlate severe expression of EGFR with good prognosis.[22] This difference might be due to the different scoring techniques, different methods of quantifying EGFR expression, technique sensitivity, specific antibodies used, and the fixation time of the tissue.[23]

The results of this study showed no significant correlation between EGFR expression and age of the patients which is in line with the previous reports.[3,8,15,17] Furthermore, no significant correlation between EGFR expression and gender of the patients was observed ($P > 0.05$). Contradictory findings have been reported by Jahanbani *et al*. who have reported high EGFR expression in females.[17]

In spite of the prognostic significance of EGFR, the key role of this protein in carcinogenesis leads it to be a new focus of researches with the aim of finding specific inhibitors of EGFR.[23] One of the novel treatment modalities in tumors which express EGFR is targeted biologic therapy against this protein.[24] Several strategies for inhibiting EGFR have been proposed such as monoclonal antibodies and tyrosine kinase inhibitors. The optimal use of these potential therapies needs to assess the frequency of EGFR expression. We anticipate that these new treatment approaches for cancers are effective only in cases

with EGFR overexpression. Hence, several studies were performed to determine the frequency of EGFR expression in OSCC or head-and-neck SCC, but no standard method exists for evaluating this protein.[16]

Finally, we used a semi-quantitative scale and categorized the analyzed data into two groups, namely no expression/low expression and moderate/high expression. High EGFR expression in this study reveals that this type of tumors would be an appropriate candidate for new molecular therapies. Consequently, application of new therapeutic agents such as anti-EGFR monoclonal antibodies for target therapy of these malignancies in Iranian population requires further investigations with greater number of cases.

CONCLUSION

The findings of this study showed no statistically significant difference in EGFR expression between two groups of OSCC and ESCC. Besides, no significant correlations were found between EGFR expression and age, gender, grade, and stage of OSCCs. Although a significant correlation was observed between EGFR and stage in ESCCs, hereby overexpression of EGFR was observed in tumors with Stage II or more.

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Conflicts of interest text should be

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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