Original Article

EGFR Expression in Patients with Esophageal Squamous Cell Carcinoma and its Association with Pathologic Response to Preoperative Chemoradiotherapy: A Study in Northeastern Iran

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Abstract

Introduction: Esophageal squamous cell carcinoma (ESCC) accounts for 80% of all esophageal cancers worldwide. It is the most common histological type of esophageal carcinoma in low-resource countries. ESCC is prevalent in Asian countries, accounting for more than 95% of esophageal cancers. The epidermal growth factor receptor (EGFR) is involved in cancer development, as its gene is often mutated and/or amplified in cancer cells. According to recent statistics, esophageal cancer is the eighth most common cancer in Iran.

Methods: In this retrospective study, we assessed EGFR overexpression, using immunohistochemistry (IHC) in 68 patients with ESCC, undergoing neoadjuvant chemoradiotherapy and esophagectomy in 2011-2014. The treatment protocol included external beam radiotherapy (40 Gy), concomitant with cisplatin 20mg/m^2 and 5- fluorouracil (5-FU) 1000 mg/m^2 for 4 consecutive days during the first and fourth weeks of treatment. To compare the two groups (EGFR positive and negative) in terms of complete pathologic response, Chi-square test was performed using SPSS version 16.

Results: The median age of the patients was 59 years (range: 27–70 years), with a female-to-male ratio of 1.06. Overall, 70% of the subjects showed EGFR overexpression. Complete pathologic response to neoadjuvant treatment was significantly higher in EGFR-positive patients (40% vs. 15.8%, P = 0.05). In all cases, 1- and 3-year overall survival rates were 86.6% ± 4.1 and 48% ± 6.9, respectively. The 1- and 3-year disease free survival rates were calculated as 71.8% ± 5.4 and 44.3% ± 6.5, respectively. The overall survival rate was relatively higher in cases with EGFR overexpression, although the difference was not statistically significant (5-year survival rate: 47.9 ± 8.2 vs. 30.9 ± 13, P = 0.23).

Conclusion: EGFR overexpression was reported in the majority of patients with ESCC in northeastern Iran. Moreover, EGFR overexpression was significantly associated with complete pathologic response.

Keywords: EGFR, esophageal squamous cell carcinoma, neoadjuvant chemoradiotherapy,

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Introduction

Esophageal squamous cell carcinoma (ESCC) accounts for 80% of all esophageal cancers worldwide. It is the most common histological type of esophageal carcinoma in low-resource countries.[1,2] ESCC is prevalent in Asian countries, accounting for more than 95% of esophageal cancers.[3]

Poor patient prognosis is related to the small number of cases diagnosed in early stages.[4,5] Despite the advances in modern chemotherapy regimens and new radiotherapy techniques, therapeutic methods have not been successful in management of ESCC patients. Hence, the necessity of defining new methods is strongly felt for treatment of these patients.[6] For instance, EGFR-KIT had a prognostic effect on outcome in esophageal or gastric cancers and also antineoplastic agents for targeting receptor tyrosine kinase could affect the outcome of some cancers.[7–9]

The overexpression of epidermal growth factor receptor (EGFR), a member of the ErbB receptor family, has been observed in different types of cancer, including esophageal cancer. Components of extracellular transmembrane and intracellular tyrosine kinase domains cause cell proliferation, cellular differentiation, angiogenesis, and metastasis, and activate the anti-apoptotic pathways.[10]

EGFR overexpression is detected in a variety of malignancies including head and neck, colorectal, breast, lung, and bladder tumors. The relationship between protein overexpression and gene amplification may demonstrate the potential therapeutic efficacy of EGFR in several types of carcinomas. EGFR is considered to be involved in cancer development as its gene is often mutated and/or amplified in cancer cells.[11,12]

There are several reports indicating a negative association between EGFR expression and survival rate in patients showing resistance to chemoradiotherapy and lymph node metastasis with

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high levels of EGFR in tumors. Conversely, many studies have suggested an association between EGFR overexpression and response to chemotherapy.[13–15]

Considering the great attention paid to EGFR as a target for cancer therapy and the paucity of studies addressing this issue in esophageal cancers, we investigated the frequency of EGFR expression in patients with ESCC. In addition, we assessed the patients' survival rate and the association between EGFR expression and tumor response to chemoradiotherapy.

Patients and Methods

Patients

The current Historical-Cohort study was performed on 68 patients with ESCC, undergoing preoperative chemoradiotherapy.

The inclusion criteria were as follows: 1) primary ESCC; 2) lack of distant metastasis 3) < 70 years of age; 4) Karnofsky performance score ≥ 70 (cares for self, unable to carry on normal activity or to do active work); 5) normal hematological status; 6) normal liver and renal function test results (white blood cell count ≥ 4000, absolute neutrophil count ≥ 1500, platelet count ≥ 100.000, and *hemoglobin* ≥ 10); 7) patient's consent for treatment; and 8) no serious comorbidities preventing esophagectomy.

Patients who met the following criteria were excluded from the study: 1) other concurrent malignancies (except non-melanoma skin cancer), 2) non-thoracic esophageal (cervical) disease, 3) tracheoesophageal fistula, and 4) lack of appropriate samples during the initial endoscopic biopsy for immunohistochemistry (IHC) review.

Methods

Based on the inclusion and exclusion criteria, 68 patients with ESCC were enrolled in the study. Demographic and clinical data were extracted from the checklists. Following confirmation of the tumor and the availability of adequate percentage of tumor cells for IHC evaluation by an expert pathologist, EGFR expression was determined semi-quantitatively using a monoclonal antibody against EGFR (Mouse Anti-Human, Clone H11, Dako, Denmark).[1]

IHC findings were categorized into 4 groups based on staining intensity: a) faint (±): faint staining compared *to* normal esophageal epithelium, b) low (+): staining identical to *that of* normal esophageal epithelium, c) medium (++): moderately stronger staining compared *to* normal esophageal epithelium, and d) strong (+ + +): markedly stronger staining compared *to* normal esophageal epithelium. EGFR expression at levels (+) and (±) was regarded as negative; strong (++) and medium (+++) levels were considered as positive EGFR expression.

Treatment

Chemotherapy consisted of cisplatin 20 mg/m^2, concomitant with 5-fluorouracil (5-FU) 700 mg/m^2 for 4 consecutive days during the first and fourth weeks of radiotherapy. The patients received the second course of chemotherapy in case they had neutrophil count ≥ 1500 cells/mL, platelet count ≥ 100,000/mL, mucosal toxicity ≤ grade 2, and creatinine clearance ≥ 60 mL/min. In the external beam radiotherapy, the treatment volume was defined by conventional simulation; forty GY in 20 fractions was delivered by anteroposterior (AP) fields.

Three to four weeks after the completion of chemoradiotherapy, the patients underwent esophagectomy, and the samples were examined by a pathologist, considering the response rate to the neoadjuvant treatment, existence of living tumoral cells, and the extent of necrosis. The adjuvant chemotherapy was performed after surgery with 25mg/m^2 cisplatin and 5-FU 425 mg/m^2 for 3 days every 3 weeks (for 3–4 cycles). The patients were followed up every 3 months during the first year, with longer intervals afterwards.

Pathologic response assessment

Patients with complete pathologic response were those without living tumoral cells. Furthermore, the response after the chemoradiotherapy of primary tumor was classified according to Tumor Regression Grade (TRG), also defined by Mandard and colleagues.[16] TRG ranges from 1, defined as complete regression, to 5 indicating no regressive changes. TRG 1 and 2 were considered as major response to chemoradiotherapy.

Data analysis

Data were analyzed using SPSS version 16. We used chi-square test to compare major pathological responses between groups. Overall survival rates (OS) was calculated using Kaplan-Meier method from the time of diagnosis to the time of death for any reason or the last visit. Disease free survival rates (DFS) were calculated from the time of diagnosis to the time of death/recurrence or the last visit without evidence of the disease. Log-rank test was utilized to compare survival curves between groups in univariate analysis and Cox-regression test for multivariate analysis.

Results

Demographic data

Out of 68 examined patients, 35 were female (51.5%) and 33 were male (48.5%). The median age of the patients was 59 years (range: 27–77 years). As mentioned earlier, all patients underwent preoperative chemoradiotherapy, according to the mentioned treatment protocols.

Treatments and outcomes

Out of 68 patients in this study, 35 subjects (51.5 %) received complete preoperative treatment, and 33 patients underwent only one course of chemotherapy before surgery.

In 63 patients, post-operative specimens were available for pathological response evaluation. According to TRG classification, TRG 1 was reported in 21 patients (33.3%) and TRG 2 in 18 subjects (28.6%). Therefore, 39 patients (61.9%) achieved major responses (Table 1).

After surgery, 8 patients (11.8%) died due to perioperative complications. The remaining 60 patients were followed up for 9 to 86 months (median follow-up duration: 27 months). The follow-up results indicated 33 cases of death (48.5%), 2 cases of active disease (2.9%), and 33 disease-free cases (48.5%). The follow-up examination of patients indicated 16 cases of l locoregional recurrence (35. 3%) and 16 cases of metastasis (35. 3%); simultaneous locoregional recurrence and distant metastasis were reported in 5 subjects (7.4%). The most common sites of recurrence were cervical lymph nodes (7 cases, 10.3%), followed by anastomotic and supraclavicular nodes (6 cases, 8.8%), respectively. The most common sites of distant metastases were

Table 1. Frequency of pathological response to neoadjuvant therapy in 63 patients with available post-operative specimen.

		No.(%)
TRG classification	1	21(33.3)
	2	18(28.6)
	3	4(6.3)
	4	11(17.5)
	5	9(14.3)
Response to chemoradiotherapy	Good	39(61.9)
	Poor	24(38.1)
Complete pathological response	Yes	21(33.3)
	No	42(66.7)

Table 2. Effect of EGFR on response rate to pre-operative chemoradiotherapy.

EGFR expression	Complete response		Incomplete response	
	No.	(%)	No.	(%)
Positive	18	40.9	26	59.1
Negative	3	15.8	16	84.2
Total	**21**	**33.3**	**42**	**66.7**
P-Value		0.05		

lungs (7 cases) and liver (6 cases).

In all cases, 1- and 3-year overall survival rates were 86.6% ± 4.1 and 48% ± 6.9, respectively. The 1- and 3-year disease free survival rates were calculated as 71.8% ± 5.4 and 44.3% ± 6.5, respectively.

EGFR expression and its relationship with tumor-associated factors

In this study, staining scores 0 and 1 were considered negative for EGFR expression, and scores 2 and 3 were regarded as positive. Among the 68 enrolled patients, EGFR expression was positive in 48 cases (70.6%). I. Histological H-scores of low (0–100), medium (101–200), and high (201–300), were observed in 23 (33.8%), 20 (29.4%), and 25 (36.8%) patients, respectively.

Among women (n = 35), and men (n = 33), EGFR was positive in 24 (68.5%) and 23 (69.6%) cases (p value = 0.9). Among patients under 60 (36 cases) and over 60 (32 cases), EGFR was positive in 27 (75%) and 20 (62.5%) patients, respectively (*P* value = 0.26) .

EGFR expression was evaluated based on complete or incomplete pathological response to neoadjuvant treatment in 63 patients with available postoperative samples. Among 44 patients with positive EGFR expression, complete response was achieved in 18 patients (40.9%); meanwhile, in 19 EGFR negative cases, there were only 3 (15.8%) cases of complete pathological response (*P* value = 0.05) (Table 2).

We also examined the association between response rate and EGFR expression. Although complete response rate was higher in the group with H-score of 100 (38.1 vs. 23.8), the difference was not statistically significant (*P* = 0.25).

Evaluation the effects of factors on overall survival.

Table 3 presents the effects of multiple factors on overall survival rates. The 3-year survival rates in EGFR positive and negative patients were 47.9% ± 8.2% and 30.9% ± 13%, respectively (*P* value = 0.23) . The 3-year overall survival rate was almost significantly lower in cases with low H-score (36.4% ± 11.5%) than those with medium (69.4% ± 11.6%) or high H-score (54.2% ± 12.3%) (Table 4; Figures 1 – 4).

Discussion

In the current study, we examined patients' pathologic response to preoperative chemotherapy and EGFR expression as a potential biomarker for ESCC. Complete pathologic response was observed in 35.2% of patients, and the median length of follow-up was 29.5 months (range: 10–83 months). In addition, 3- and 5-year survival rates were 57.1 and 49.5, respectively.

Although these findings are considered favorable for esophageal carcinoma, there are still many deficiencies in the treatment process. To prevent the recurrence of carcinoma and metastasis, more comprehensive information is required about the associated prognosis and predictive factors.

In the majority of studies conducted so far, EGFR is considered positive when IHC intensity is greater than +1. Contrarily, Wang *et al.* (2007) considered EGFR positive when the staining level was greatly higher than 5%.[17] In the current article, EGFR was considered positive if the staining intensity was + 2 or +3; in addition, we used another assessment method, called H-score.

In several studies, the usual range of EGFR expression is claimed to be 40–70%.[1] Liu *et al.* (2011) reported an EGFR expression of 14%, while Yang's study showed a greater rate of expression in 86% of patients with ESCC.[18,19] According to our findings, EGFR expression was 70.6% positive and 29.4% negative.

In a similar study in Iran, Moghbeli *et al.* (2013) assessed the expression of EGFR in tumoral tissues and margins in patients with ESCC[20]; EGFR expression was reported in 38.2% of samples. The difference between Moghbeli's study and the current research lies in the methodology; in the mentioned study, real time Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was applied, while in the current study, IHC was used.

Although many studies have examined EGFR expression in esophagectomy, no examinations have been carried out

Table 3. Assessment of possible factors affecting overall survival rate.

Factor	1- Year survival % (CI)	3-Year survival % (CI)	Log rank *P*-Value
Sex			
Male	74%(69.97,78.03)	33.6%(28.14,39.06)	0.19
Female	76.3%(72.52,80.08)	50.9 %(46.16,55.64)	
Age			
>60	84.3%(72.15,96.45)	47.9 %(27.12.68.68)	0.55
<60	88.5%(79.68,97.32)	47.7 %(29.47,65.93)	
EGFR			
Pos	89.3% (80.48,98.12)	51.9 %(36.22,67.58)	0.23
Neg	80.6%(63.55,97.65)	41.2 %(16.9,65.5)	
H score			
0-100	82.4%(66.52,98.28)	36.4 %(13.86,58.94)	0.056
101-200	95 %(85.59,100)	69.4 %(46.66,92.14)	
201-300	84.1%(69.79,98.41)	54.2 %(30.09,78.31)	
Pathological Response			
Good	88.3%(78.7,97.9)	52.8 %(36.53,69.07)	0.58
Unfavorable	91.1%(79.34,100)	40.5 %(16.98,64.02)	
CI = confidence interval.			

Table 4. Assessment of possible factors affecting disease free survival rate (1-, 3-year)

Factor	1- Year survival % (CI)	3-Year survival % (CI)	Log rank *P*-Value
Sex			
Male	66.1%(49.83,82.37)	40.4%(20.21,60.59)	0.68
Female	68.2%(52.72,83.68)	42.6%(25.35,59.85)	
Age			
> 60	65.6%(49.14,82.06)	38.2%(19.38,57.02)	0.61
<60	71.6%(56.7,86.5)	45%(27.36,62.64)	
EGFR			
Positive	74.5%(61.96,87.04)	49.5%(34.02,64.98)	0.049
Negative	51.3%(29.15,73.45)	22.6%(1.63,43.57)	
H score			
0-100	59.8%(39.42,80.18)	19.9%(0.89,38.91)	0.022
101-200	66.7%(44.55,88.85)	57.2%(31.52,82.88)	
201-300	56%(36.6,75.4)	47%(27.01,66.99)	
Pathological Response			
Good	72.1%(58.77,85.43)	43.7%(28.22,59.18)	0.829
Unfavorable	63.8%(43.61,83.99)	41.8%(16.32,67.28)	
CI = confidence interval			

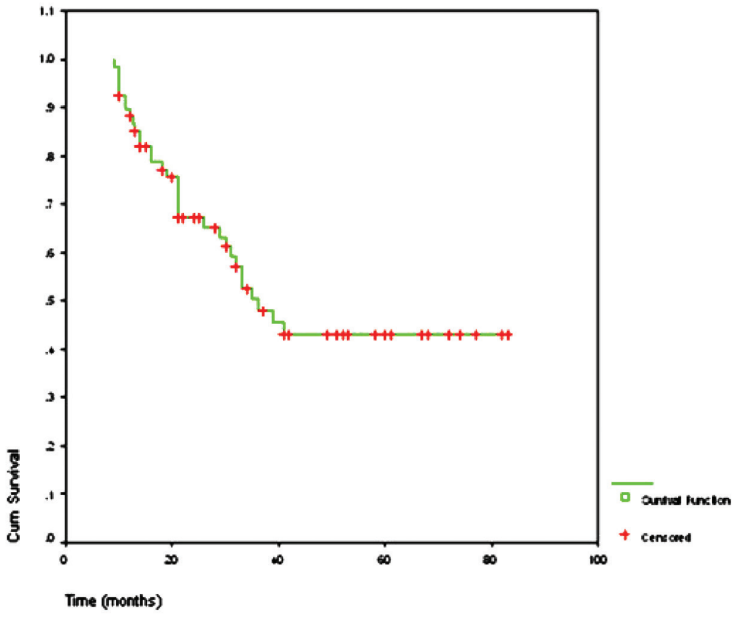

Figure 1. Main survival rate of the patients.

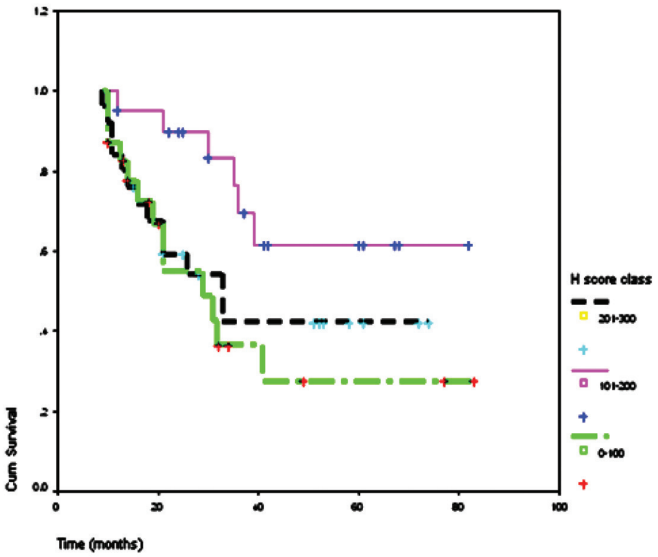

Figure 2. Patients' survival curves, based on the expression of EGFR (according to H-score).

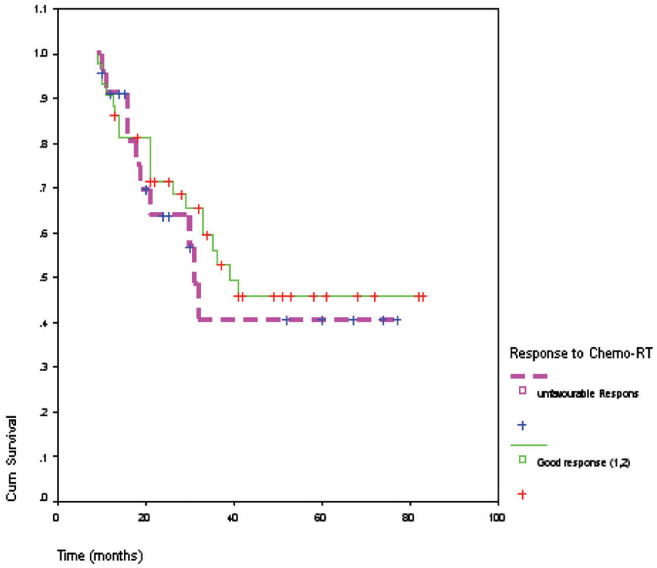

Figure 3. Association between patients' survival rate and response to chemoradiotherapy.

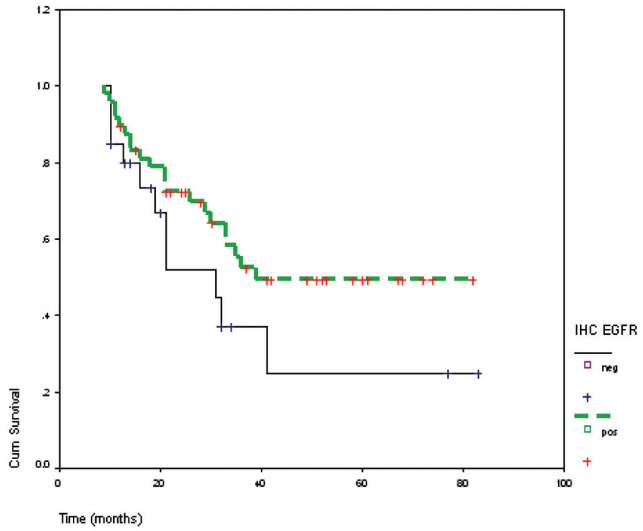

Figure 4. Relationship between patients' survival rate and EGFR expression (positive and negative).

to evaluate the association between response to neoadjuvant treatment and EGFR expression. Gotoh (2007) studied EGFR as a possible predictor of sensitivity to chemoradiotherapy in ESCC primary lesions.[2] Correspondingly, among all the assessed factors, a significant association was observed between patients' response and chemoradiotherapy in both positive- and negative-EGFR groups.

Yamamoto *et al.* (2011) showed that EGFR overexpression was associated with high recurrence and poor prognosis in patients, followed by a remarkably high sensitivity to chemotherapy. Similar to our findings, they showed that complete response rate was significantly associated with EGFR expression.[21]

In the current study, EGFR expression was not associated with age or gender. According to H-scores, survival rate was remarkably higher in those with medium H-score (101–200), compared to those with low or high H-scores (<100 or >200); the difference was statistically significant. Since we could not find similar studies assessing H-score, it is not possible to compare the obtained results with those of other studies.

Contrary to our findings, Sarbia (2007) and Hironaka (2002) showed that EGFR overexpression was not associated with improved overall survival rate.[22,23] These studies reported more responses to neoadjuvant treatment, despite the lower survival rate; this might be related to the nature of EGFR as an oncogene, which is composed of 28 exons. The activation of this marker is associated with an increased cell proliferation, angiogenesis, invasion, and metastasis.[24]

Moreover, Yu *et al.* (2011) evaluated the clinicopathological and prognostic significance of EGFR overexpression in ESCC in a meta-analysis.[25] Four out of five examinations in this study analyzed the relationship between EGFR expression and survival rate. These studies demonstrated that EGFR overexpression could result in lower survival rate. It seems that the expression of EGFR, HER2, and cyclooxygenase plays an important role in prediction of pathologic responses and probably targeted therapies in esophageal carcinomas.

In conclusion, EGFR overexpression was reported in the majority of patients with ESCC in northeastern Iran. Moreover, EGFR overexpression was significantly associated with complete pathologic response

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