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Human Epidermal Growth Factor Receptor 2 Over-Expression in Patients with Esophageal Squamous Cell Carcinoma; Correlation with Response to Neo-Adjuvant Chemoradiation and Survival

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) is a common malignancy in gastrointestinal tract.
Objectives: This study was conducted to investigate the frequency of human epidermal growth factor receptor 2 (HER2), over-expression in patients with ESCC, and its correlation with pathologic response in cases undergoing neo-adjuvant, chemoradiation, and survival.
Methods: In this cross sectional study, 68 patients with non-metastatic esophageal SCC, who had undergone neo-adjuvant chemotherapy containing cisplatin and 5FU in conjunction with radiotherapy between 2007 and 2014 were evaluated. HER2 expression assessed by Immunohistochemistry and HER2 score was also calculated for each specimen. Tumor response to neoadjuvant chemoradiotherapy was evaluated in surgical blocks according to tumor regression grading (TRG) system. Patients were followed up every 3 months in first 2 years and every 6 months afterwards.
Results: The result of Hercep test was positive in 42.8% of cases, among whom 33.8% were 2+ and 8.8% were 3+. Her2 score was above 100 in 38.3%. Complete pathologic response was observed in 32.3%. There was no significant difference in the rate of complete response between patients with positive and negative HER-2 over-expression (P = 0.71). There was also no significant correlation between Her2 score among groups with favorable and unfavorable response to chemoradiation (P = 0.796 and 0.743). There was no difference in overall survival in Her2 positive and negative groups (3 years survival was 45 and 54 months, P = 0.32). Overall survival significantly reduced in patients with Her2 score above 100 (P = 0.045).
Conclusions: Her2 positive in ESCC had no effect on tumors biologic behaviors and its response to chemoradiation. Although no correlation was observed between Her2 expression and survival; Her2 score above 100 was associated with shorter survival.

Keywords: Her2, Chemo radiotherapy, Esophageal Squamous Cell Carcinoma, Survival

1. Background

Esophageal cancer is the eighth most common type of cancer worldwide (1). Esophageal squamous cell carcinoma (ESCC) is more prevalent in Asian countries like Iran than the other regions of the world (2). Esophageal cancer is usually diagnosed in advanced stages with early lymph node spread causing poor prognosis in most cases (3). Survival rates with surgery alone were not satisfactory. Neo-adjuvant chemoradiotherapy has been introduced to improve treatment outcome in cases undergoing esophagectomy (3, 4). Despite new chemotherapy drugs and modern techniques in radiotherapy, response rate to treatment and survival of these patients are still low (4).

Human epidermal growth factor receptor 2(Her2) is a proto-oncogene located on chromosome 17 (17q12-q21.23), which codes a trans-membrane glycoprotein, acting as an epidermal growth factor in relation with tyrosine kinase activity (5). High expression of Her2 gene is shown in colon, bladder, fallopian, head and neck, breast, esophageal, and gastric cancers. Her2 overexpression based on IHC has been demonstrated in 9% to 60% of ESCCs, which varies depending on the stage of the disease, tumor histology, and method of measurement (6). Multiple studies have shown a relation between Her2 expression, tumor stage, lymph node metastasis, and prognosis in breast cancer (5). Main treatment strategies in ESCC are surgery, radiotherapy, or chemotherapy, and the role of target therapy in ESCC is not well established yet (4). The over-

expression of HER2 in ESCC and its impact on response to treatment and survival is not well understood (7).

2. Objectives

In this study, we evaluated Her2 expression in patients with ESCC and its correlation with tumor response to neoadjuvant chemoradiotherapy and patients' survival. If we find a correlation, future studies could evaluate the use of monoclonal antibody against HER2 in treatment of ESCC.

3. Methods

This retrospective study was performed in Omid Hospital (oncology hospital of Mashhad University of Medical Sciences) between 2007 and 2014 on patients with non-metastatic esophageal SCC undergoing neoadjuvant chemoradiotherapy. The study was approved by the research Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.REC.1392.8). Inclusion criteria were primary ESCC without distant metastasis, karnofsky performance status $\geq$ 70, no hepatic or renal disease, and available paraffin samples for evaluation. Patients who had other malignancies, cardiopulmonary disease, cervical ESCC, or tracheoesophageal fistula were excluded.

Patients received neo-adjuvant chemoradiation with radiation dose of 40 Gy in 20 fractions over 4 weeks and chemotherapy with cisplatin20 mg/m^2 bolus for 4 days and 5Flu 700 mg/m^2 24-Hour infusion for 4 days in the first and the last 4 days of radiotherapy. Patients were planned for esophagectomy 3 weeks following radiation. Adjuvant chemotherapy was administered with cisplatin 25 mg/m^2 and bolus 5 Fu 425 mg/m^2 every 3 weeks for 3 courses. Patients were followed- up every 3 months in first 2 years and every 6 months afterwards.

3.1. Hercep Test

We used S-B-O-P technique for IHC reactions (Strept-ABC, DAKO, Denmark). Deparafinised tissue slices were put in a citrated buffer (pH = 9) in a pressure cooker for the purpose of antigen retrieval; then, internal peroxidase was blocked by Hydrogen provide 3%. Tissue slices were incubated by a polyclonal anti-body agent HER2; then, the samples were incubated by En Vision solution and streptavidin HRP and, finally, were counterstained by hemoxilin.

IHC analysis for HER2 evaluates the intensity and pattern of tumoral cells. This test divides the results into 4 classes. 0 and 1+ groups were considered negative for HER2 expression, whereas 2+ and 3+ groups were considered positive (8) (Table 1).

Table 1. Hercep Test Classification

Grade	Definition
0	Staining weaker than normal epithelium
1+	Staining equal to normal epithelium
2+	Staining moderately stronger than normal epithelium
3+	Staining markedly stronger than normal epithelium

3.2. Definition and Measurement of HER 2 Score

H score determines the IHC expression of a biomarkers based on both staining degree and percentage of stained tumor cell. Hscore is calculated by the following formula:

Therefore, the result is a continuous variable, ranging from 0 to 300 (9).

3.3. Evaluation of Response to Treatment

Tumor response to neoadjuvant chemoradiotherapy was classified according to Tumor Regression Grading TRG) system. Complete response, as no viable tumor in the resected specimen, was considered as TRG1. TRG 5 was considered in cases with no response. TRG scores of 1 to 2 were considered as favorable response (8) (Table 2).

3.4. Statistical Analysis

We used Chi-square test to compare proportions between groups. The overall survival rate was calculated from the time of surgery to the time of death or the last visit, using Kaplan- Meier method. Log-rank test was utilized to compare survival curves between groups. Survival analyses were performed only in cases who survived the surgery. P values less than 0.05 were considered significant. We used SPSS version 13 for statistical analysis.

4. Results

A total of 68 eligible patients with esophageal SCC, who had undergone neo-adjuvant chemoradiotherapy were included. The mean age was 57 years (27 - 77) with a male to female ratio of 33/35 (0.94).

IHC HER2 over-expression was negative in 39 cases (57.3%), 2+ in 23 cases (33.8%), and 3+ in 6 cases (8.8%). HER2 score was between 0 to 99 in 42 cases (61.8%) and between 100 to 199 in 21 cases (30.9%) and 5 cases (7.4%) were above 200.

Thirty-five patients had received 2 courses of concomitant chemotherapy and radiotherapy. The second course of chemotherapy was not administered in 33 patients due to inappropriate hematological condition. All patients

$$H\,score = (\text{percentage of } 1+ \text{ cells} \times 1) + (\text{percentage of } 2+ \text{ cells} \times 2) + (\text{percentage of } 3+ \text{ cells} \times 3) \quad (1)$$

Table 2. TRG Classification

TRG	Defination
1	Absence of residual cancer and fibrosis extending through the different layers of the esophageal wall
2	Presence of rare residual cancer cells scattered through the fibrosis
3	Increase in the number of residual cancer cells, but fibrosis still predominated
4	Residual cancer outgrowing fibrosis
5	Absence of regressive changes

completed intended radiotherapy course. All cases underwent esophagectomy. Postsurgical mortality was recorded in 8 cases (11.8%).

Postsurgical specimen was available in 63 patients. Pathological evaluations revealed distal margin involvement in one and proximal margin in 2 cases. Complete pathological response (TRG1) was achieved in 21 patients (32.3%); 18 patients were classified as TRG2 (28.6%). Therefore, favorable response to chemoradiation was found in 39 patients (61.9%).

For 60 patients who survived the surgery, the median follow up time was 28.5 months (range; 9 - 86 months). Twenty-five patients succumb to the disease during follow-up (48.5%), 2 had active disease at last visit (2.9%), and 33 patients were disease-free. For all 60 cases, the 3-year overall survival was calculated as 51.6 ± 6.6. Most common sites of local recurrence were anastomosis site in 7 patients (10.3%), cervical, and supra clavicular lymph nodes in 6 patients (8.8%). The most common organs, in which metastases were found, were lung (9 cases) and liver (7 cases).

Neither of the investigated factors including Hercep test and HER-2 H score were significant predictor of favorable pathological response.

According the results, age, the number of neo-adjuvant chemoradiation cycles, adjuvant chemotherapy, and HER2 positive test had no significant impact on the overall survival. The survival rates were relatively more favorable in HER2 negative patients; however, it did not reach statistically significant (P = 0.32). Meanwhile, patients with HER2 score of less than 100 had significantly higher overall survival compared to those with a score above 100 (Table 3).

5. Discussion

The present study revealed that HER2 was 2+ or 3+ based on IHC in 42.6% of cases and 0 or 1+ in 57.4%. HER2 score was above 100 in 38.2% of cases. The rate of HER2 over-expression in esophageal cancers has been various in previous trials. This rate was reported 36% in a study conducted by Hardwick et al. (10), 10% in Lam's study (11), 11% in Scheer's study (12), 30.3% in Mimura's study (5), and 36.8% in Sato-Kuwabara's study (13).

As compatible with this study, in the previous trials including studies conducted by Hardwick et al. (10), Lam et al. (11), and Friess et al. (14) HER-2 expression had no significant effect on survival in patients with esophageal cancer.

Miyazono et al. (15) evaluated quantitative c-erbB-2 (HER-2) and c-erbB-1 mRNA expression as a predictor of response to neo-adjuvant and radical surgical resection in patients with esophageal cancer. They showed that the high c-erbB-2 mRNA expression in pre-treatment samples was associated with minor histopathologic response to neo-adjuvant chemoradiation protocol containing cisplatin plus 5-FU.

Akamutso et al. (16) studied 34 patients with esophageal SCC, who had available pre-treatment biopsies. They divided the patients to 2 groups based on their response to total radiation dose of 40 Gy (13 were sensitive and 12 were resistance). Then, they studied multiple factors by IHC. In this study, although HER2 oncoprotein expression was significantly higher in the resistance group (like our study), it was not related to patient's survival. Also, no significant difference was observed in histopathologic response to chemoradiation between HER2+ OR - in groups. Although age, tumor histopathologic response to chemoradiation, cycles of chemotherapy after surgery, and the intensity of HER2 had no impact on 3 years survival, HER2 score above 100 was associated significantly with shorter survivals. The studies performed by Khan (17) and Scheer (12) failed to show a relation between clinocopathologic factors of ESCC like TNM, tumors grade, patient's survival, and their disease period.

The study carried out by Mimura et al. (5) in 2005 showed that HER2 gene expression evaluated by FISH (which is in correlation with HERCEP test) can predict patient's survival and tumor's clinical and pathological behavior . They evaluated HER2 by IHC and HER2 gene expression by FISH method in 66 patients with esophageal SCC in a 5 years follow-up. They showed HER2 positive tumor based on IHC in 30.3% of patients and HER2 gene expression by FISH method in 11% of patients. All patients with 3+ Hercep test and 50% of cases with 2+ Hercep test showed HER2 gene expression. Patient survival was shorter in HER2 positive patients. Also, patients with HER2 gene expression

Table 3. Comparison of 3 Years Overall Survival Among Patients

Factor	No.	Event, No.	Survival, ± 1 SE	Log Rank P Value
Age				0.9
< 60	33	13	53.9 ± 9.5	
> 60	27	11	64.8 ± 10	
Adjuvant chemotherapy				0.47
Performed	39	14	58.9 ± 9	
Not performed	21	10	57.7 ± 11	
Complete pathologic response				0.892
Yes	21	11	48.62 ± 6.8	
No	39	13	52.36 ± 6.4	
Intensity of Her2 in Hercep test				0.326
0, 1+	34	11	54.15 ± 6.2	
2+, 3+	26	13	45.66 ± 7.1	
Her-2 H score				0.045
0 - 99	37	11	56.78 ± 5.8	
> 100	23	13	40.29 ± 7.4	

had worse survival.

Zhan et al. (18) studied HER2 expression in 145 patients with esophageal SCC in 2012 .It showed HER2 2+ in 41.4% of patients. Association was seen between increased HER2 expression and carcinoma differentiation and also between gene expression and tumor grade. In the present study, HER2 2+ and her2 gene expression were associated with significantly increased mortality and could be referred to as an independent prognostic factor. Also, tumors with HER2 positive had shorter periods of median survival (38.3 months against 52.8 months).

The results of a study conducted by Schoppmann et al. (19) revealed that among 341 patients with esophageal cancers (152 cases of SCC, 189 cases of adenocarcinoma, 39 cases of barrett esophagus, and 11 cases of squamous cell dysplasia), HER2 was positive in 15.3% of adenocarcinoma and 3.9% of SCC cancers. In this study, no significant correlation was seen between HER2 condition and survival.

Sato-Kuwabara et al. (13) studied HER2 protein expression in 199 patients with esophageal SCC with FISH and IHC techniques. There was no significant correlation between gene expression, clinical situation, pathologic response, and overall survival. Among the patients who were evaluated by FISH method, gene expression was seen only in 19.1% of cases; this gene expression was only seen in patients with HER2 protein expression of 3+. HER2 gene expression had significant effect on survival (P = 0.003). They concluded that despite correlation between FISH and IHC results, only gene expression could be referred as a prognostic factor.

In a report published in 2006, Dreilich et al. (20) studied HER2 expression in esophageal cancers and its effect on survival .They used IHC and FISH methods for those purpose among 70 patients with esophageal cancers, 13% was HER2 3+ and among 27 patients with adenocarcinoma, 30% was HER2 3+. In patients with high expression of HER2 (3+), there was tendency toward worse prognosis (P = 0.057). Also, in SCC patients, HER2 3+ expression was associated with worse prognosis, which was not the case in patients with adenocarcinoma (P = 0.035).

For better understanding of the role of genetic and molecular factors on ESCC, a parallel study was performed on 68 cases for evaluating EGFR. Forty-eight patients had increase expression of EGFR and 20 patients were negative for EGFR. Complete pathologic response was seen in 40.9% of EGFR positive and 15.8% in EGFR negative patients (P = 0.051).Three and 5 years survival was higher in EGFR positive patients, but this difference was not statistically significant (P = 0.23).

Our rates of Her2 expression was higher than the mentioned studies, which could be due to some factors such as the existence of SCC in all patients, differences in molecular or genetic patterns, and detection methods.

By considering the above studies, it can be concluded that the result of the present study is mainly consistent with their results which is: although the grade of Hercep test is not related to tumors clinocopathologic behavior or survival, a more quantitive parameter of HER2 score could

be predictor of worse survival in patients.

In conclusion, the present study showed that HER2 positive is not associated with different tumor biological behaviors, but higher her2 scores in patients with esophageal SCC is associated with shorter survival.

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Footnotes

Authors' Contribution: None declared.

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