Correlation of EGFR Expression with Survival Rate in Patients with Oral Squamous Cell Carcinoma

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Abstract

Background and Aim: This study aimed to indicate the role of epidermal growth factor (EGF) in proliferation and growth of epithelial tissue and to determine the correlation between the frequency of expression of EGF receptor (EGFR) by means of immunohistochemistry with survival rate in oral squamous cell carcinoma (OSCC).

Materials and Methods: Thirty-eight cases of OSCC diagnosed by hematoxylin and eosin (H&E) staining were included in this prospective study, and immunohistochemistry for EGFR was applied using antibodies against EGFR. The total score of stained cells and the correlation between the total score and survival rate of patients were recorded. The data were analyzed by Kaplan-Meier, Spearman and the log rank tests using SPSS software version 20.

Results: The mean age of patients was 71.8±1.09 years. The follow up range was 25-86 months. The mean survival time for EGFR score 3 (51±9.32 months) was significantly higher than that for score 4, (28.64±4.1 months) (P=0.002) and overexpression of EGFR was correlated with poor prognosis. A significant correlation was found between the grade of tumor and EGFR scale (P=0.049, R=0.318). There was no significant correlation between EGFR overexpression and OSCC stage (P>0.05).

Conclusion: EGFR is probably an independent prognostic factor for assessment of survival rate. A correlation also exists between the grade of tumor and expression of EGFR.

Key Words: Squamous Cell Carcinoma, Epidermal Growth Factor Receptor, Epidermal Growth Factor, Survival Rate

Introduction

Squamous cell carcinoma (SCC) is by far the most common malignant neoplasm of the oral cavity, and oral SCC (OSCC) is among the top 10 most common malignancies worldwide [1]. Despite recent advances in diagnostic and therapeutic options, the 5-year overall survival rate for OSCC has been stagnated at 40-50% over the last 40 years [2]. Early diagnosis can increase the 5-year survival rate by up to 80%; whereas, diagnosis in...
advanced stages can decrease this rate to 19% [3]. The carcinogenesis of OSCC is a multistep process in which multiple genetic events occur that alter the normal function of oncogenes and tumor suppressor genes. As with normal cells, cancer cells also need stimulation signals for growth, differentiation and proliferation, and growth factors play a role in this respect. Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor important for epithelial cell proliferation and survival [4]. Overexpression of EGFR may lead to invasion, cellular migration, enhanced angiogenesis, decreased cell apoptosis, and increased metastatic potential [5-8]. EGFR is detected in up to 90% of the head and neck SCCs and its over expression has been reported in 80% of SCCs [9].

There is a correlation between overexpression of EGFR and poor prognostic indices (i.e. size of the tumor and ulcers) in lip SCC [10]. Also, lower rates of EGFR expression have been accompanied by improvement of disease-specific survival in oropharyngeal SCC, which indicates that EGFR expression can be related to prognosis of surgically treated cases [11]. Overexpression of EGFR in the head and neck SCC has been shown to correlate with poor prognosis [12,13]; whereas, there are no definite data regarding its prognostic role in OSCC. This study aimed to assess the relationship of EGFR with the stage and grade of OSCC and its survival and recurrence rate.

Materials and Methods

Patient and specimen selection:

Thirty-eight patients selected from the Pathology Department of Shohada-E-Tajrish Hospital were enrolled in this prospective study. The inclusion criteria were definite diagnosis of SCC, complete medical record including surgical procedure and stage of disease, availability of sufficient biopsy sample and possibility of survival rate determination. The exclusion criteria were history of smoking and alcohol consumption after treatment and unclear treatment procedure or recurrence time. The clinicopathological variables that were considered were sex, age, tumor location, pathological stage and pathological grade. The pathological stage was determined according to the TNM (tumor, node, and metastasis) staging system at the onset of study and was retrieved from the medical records. After determining the degree of marker expression, the patients were recalled and their existing condition was examined. The time between the end of treatment and the recurrence of disease, death from disease or the time of last follow-up was used for evaluation of disease-free survival rate.

Avidin-biotin immunohistochemistry staining of the samples was performed. A cutaneous tissue specimen from a healthy individual was used as control. The specimens were fixed in 10% buffered formalin and embedded in paraffin wax.

Immunohistochemistry procedure:

Five micrometer sections of the paraffin embedded blocks were cut and mounted on special silanized slides. The DAKO EGFR pharmDx test (DAKO Cytomation, Glostrup, Denmark) EGFR detection system was used to assess EGFR expression. Slides were kept at 37ºC overnight and then at 60ºC for one hour and were deparaffinized and dehydrated sequentially. Specimens were placed in xylene for five times and each time for four minutes and immersed in 100, 96 and 70% graded ethanol and then rinsed with phosphate buffered saline (PBS) and water. Slides were immersed in 100% graded methanol for 2-3 minutes and in 3% hydrogen peroxide –containing methanol for 30 minutes afterwards; then they were incubated with proteinase in plastic dishes containing Tris buffer with a pH of 7.5 for 20 minutes. Block protein was shredded over the samples and after five minutes, primary monoclonal antibodies (EGFR/DAKO, clone 2-18C9) diluted 1/40 were decanted over the slides. Slides were incubated in a cold room for one hour and then at room temperature for another hour. Next, they were rinsed with PBS solution and secondary antibody was added for 10 minutes and rinsed again.

Afterwards, one or two drops of 3, 3’ dianimobenzidine were added. Rinsing with water was performed and samples were stained with hematoxylin for 30 seconds. Specimens were then rinsed with water and immersed in graded alcohol and xylene for five minutes sequentially. Slides were then mounted.

Immunohistochemistry method:

To assess the expression of EGFR, the total score [which is the result of multiplying the intensity of the

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score (0 to 3) by the proportion score (0 to 4)] was taken into account. For calculation of the intensity score, each slide was scored on the basis of intensity of the marker staining in membrane staining of tumoral cells as follows: 0, no staining; 1, weak staining; 2, intermediate staining; and 3, intense staining [14]. The proportional staining score was the ratio of stained cells to unstained cells [15] and was scored as: 0, none of the cells were stained; 1, less than 10% were stained; 2, 10-50% were stained; 3, 50-80% were stained; and 4, more than 80% were stained. The total score was a number in the range of 0-12, which was classified as 0: no expression, score 1; 1-3: low expression, score 2; 4-7: intermediate expression, score 3; and 8-12: high expression, score 4 (Figures 1-3) [16].

**Statistical analysis:**
The obtained data were analyzed by Kaplan-Meier, Spearman and the log rank tests using SPSS software version 20 (SPSS Inc., IL, USA). To determine the mean survival time, the Kaplan Meyer analysis was used. The level of significance was set at P<0.05).

**Results**
Thirty-eight cases were enrolled in this study. The mean age of patients was 71.8±1.09 years. Of all, 53% (20 patients) were males and 60.5% of patients (23 patients) was smokers. Data of tumor location and grade for the 38 enrolled patients are summarized in Table 1. Twenty-six patients (68.4%) were in stage III and 12 patients (31.5%) were in stage IV. Table 2 shows EGFR expression with regard to location, smoking and grade of OSCC in selected cases. The follow up range was 25-86 months.

For assessment of analytical indices, we used the log rank test. In general, death or recurrence occurred in 50% (n=19) of 38 cases. The mean survival time was 32.75±3.9 and 32.8±9.39 months in the poor and moderate grade groups, respectively. The survival time for the well-grade group could not be calculated due to small sample size. There was no significant difference in the mean survival time between the moderate and poor grade groups (P=0.862). The mean survival time for EGFR score 3 (51±9.31 months) was significantly higher than that for score 4 (28.64±4.1 months) (P=0.002). There was no significant correlation between EGFR overexpression and OSCC stage (P>0.05). The Spearman’s correlation test showed a linear moderately significant correlation between the grade of tumor and EGFR scale (P=0.049, R=0.318). Only 10% of the changes in grade and EGFR were in the same direction, and the remaining was influenced by other factors. Diagrams 1 and 2 show the Kaplan-Meier survival analysis according to stage and grade determined by immunohistochemical staining in oral squamous cell carcinoma.
Table 1. Clinicopathological characteristics of 38 oral squamous cell carcinoma patients

| Tumor location     | N (%)   |
|--------------------|---------|
| Tongue             | 25 (65.7) |
| Gingiva            | 11 (28.9) |
| Floor of the mouth | 2 (5.2)   |

| Stage     | N (%)   |
|-----------|---------|
| III       | 26 (68.4) |
| IV        | 12 (31.6) |

| Grade    | N (%)   |
|----------|---------|
| Well     | 4 (10.5) |
| Moderate | 13 (34)  |
| Poor     | 21 (55.5) |

Table 2. EGRF expression in relation with location, smoking and grade of oral squamous cell carcinoma

| Location            | Score 2 | Score 3 | Score 4 |
|---------------------|---------|---------|---------|
| Tongue              | 3       | 15      | 7       |
| Floor of the mouth  | 0       | 4       | 7       |
| Gingiva             | 2       | 0       | 0       |

| Smoking  | N (%)   |
|----------|---------|
| Yes      | 1       | 12      | 10      |
| No       | 4       | 7       | 4       |

| Grade     | N (%)   |
|-----------|---------|
| Well      | 3       | 1       | 0       |
| Moderate  | 1       | 7       | 5       |
| Poor      | 1       | 11      | 9       |

| Survival time (months) | Mean ± SD | P value |
|------------------------|-----------|---------|
|                        | 48.93 ± 27.22 | 0.002   |

Discussion
The expression of EGFR gene has been assessed in different types of neoplasms and researchers have found a significant correlation between the expression of this receptor and stimulation of migration and division of normal epithelial cells [17,18]. Similar studies have evaluated the role of EGFR expression in OSCC. EGFR gene copy number is an early sign of tumor progression and contributes to OSCC risk [19]. As EGFR overexpression is a key event in carcinogenesis of the head and neck carcinomas, its role is especially highlighted in premalignant lesions and early carcinomas, with a proportional increase that is
Diagram 1. Kaplan-Meier survival analysis according to stage determined by immunohistochemical staining in oral squamous cell carcinoma

Diagram 2. Kaplan-Meier survival analysis according to grade determined by immunohistochemical staining in oral squamous cell carcinoma

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directly related to advancement of the dysplastic/neoplastic process [20,21]. In the current study, our aim was to evaluate the correlation of EGFR expression with the grade and stage of the disease and survival rate of patients. In our study, there was no significant correlation between EGFR overexpression and OSCC stage (P>0.05). This finding was similar to that of Laimer et al. [22] Carren et al. [23] and Hiraishi et al [24]. Hiraishi et al. [24] reported that even though 92% of the samples showed EGFR expression and 63% of them showed high expression of EGFR, there was no correlation between this expression and tumor stage. Conversely, another study stated that EGFR expression was higher in advanced stages of OSCC and non-homogenous leukoplakia. Bagan et al. [25] also showed higher EGFR copy number in advanced stages than in early stages. Such discrepancies in the results may be justified by differences in sample size, stage of the disease and methods of EGFR expression evaluation. For example, in the study by Bagan et al. [25] EGFR expression was assayed by real-time polymerase chain reaction that was estimated with copy number and from 19 cases of OSCC, only 10 cases were at stage III and IV of disease.

In contrast to the results of Ulanovski et al. [26] that showed significant reduction in differentiation of OSCC cells with an increase in EGFR expression, in our study there was no statistically significant correlation between EGFR overexpression and grade of cell differentiation. The method of staining of samples in their study was similar to ours, but their biopsies were taken from the tongue only and the time passed from the onset of disease was different. Bernardes et al. [27] showed that salivary level of EGFR increased after removal of the tumor but no correlation was found between the receptor salivary level and clinicopathological features of the tumor. The main reason for different results compared to ours may be the nature of the specimens (saliva versus cancer cells).

In our study, the relationship of EGFR overexpression and survival rate was significant (P=0.0162), which means that patients with higher rate of EGFR expression had a poor prognosis. Our results were similar to those of Ang et al. [28] who showed that EGFR is an independent and strong prognostic factor for evaluation of the survival rate and disease relapse. Keren et al. [29] performed a meta-analysis on the prognostic role of EGFR in the head and neck SCC and concluded that EGFR overexpression was associated with shortened overall survival. Another meta-analysis highlighted the role of EGFR overexpression as a prognostic factor in nasopharyngeal carcinoma [30]. In addition, a study stated that overexpression of EGFR cannot be a prognostic factor per se [31]. This study had a sample size of 113 cases and its follow up period was nine years. They included oropharyngeal biopsies in their study, which may explain different outcomes.

Monteiro et al. [32] evaluated phosphorylated EGFR (pEGFR) expression in OSCC as a prognostic factor and concluded that the independent value of pEGFR expression in cause-specific survival of OSCC can be a reliable marker to determine the prognosis of different cases. The main difference of this study with ours was that they evaluated the rate of pEGFR rather than EGFR itself. Actually, the expression of this marker depends on many known and unknown factors. For example, in a study by Eriksen et al. [33] EGFR expression in the initial stage of tumor invasion was more than that in advanced stages, and after metastasis another rise in EGFR expression was seen.

Giralt et al. [34] showed that EGFR-positive expression before radiotherapy is an indicator for poor response to therapy and low disease-free survival rate in gastrointestinal tract malignancies [34]. In their study, the proportion of stained cells to non-stained cells was evaluated and the intensity of staining was not taken into account. This method is not useful for OSCC because of formation of the cells. In our study, this ratio was 1 or close to 1. In a study by Yamada et al. [35] only the intensity of the staining was examined and it was reported that staining intensity increased in higher grades. Also, there are many studies about EGFR expression changes during chemotherapy and radiotherapy periods [36]; to omit this confounding factor, we used the biopsies, which were taken prior to chemotherapy or radiotherapy in order to assess EGFR expression based on the primary characteristics of the tumor.
Finally, the strength of this study was the accurate method of assessment of EGFR expression and attention to the exact date of follow-up visits. The main limitation was related to the specimens which were not uniform in staining. Our study may have a high rate of censored data, which can be due to short follow-up time.

In conclusion, although we did not find any significant correlation between EGFR overexpression and stage and grade of the biopsies, we can use the data regarding the survival rate. However, more studies are needed to better elucidate this subject.

Conclusion

EGFR is probably an independent prognostic factor for assessment of survival rate. A correlation also exists between the grade of tumor and expression of EGFR.

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