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Expression of Epidermal Growth Factor Receptor and the association with Demographic and Prognostic Factors in Patients with Non-small Cell Lung Cancer

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ABSTRACT

Introduction: Growth, proliferation, survival, and differentiation are the prominent characteristics of cells, which are affected by cancer. Epidermal growth factor receptor (EGFR) plays a pivotal role in the effective control of these features. Given the significance of EGFR signaling pathway in non-small cell lung cancer (NSCLC), EGFR expression is influential on these cell characteristics. In this paper, we studied EGFR expression and its association with demographic factors, clinicopathological features, and prognosis of NSCLC patients.

Materials and Methods: In this retrospective cohort study which was done during 2009-12 at Ghaem Hospital, Mashhad, Iran. EGFR expression was evaluated in 96 patients with formalin-fixed, paraffin-embedded NSCLC tissues (43 adenocarcinomas, 48 squamous-cell carcinomas, and 5 large-cell carcinomas) using immunohistochemistry (IHC). Data analysis was performed by SPSS version 20.0.

Results: Out of 96 specimens, approximately 53% were classified as positive for EGFR expression. The study group consisted of 68% (N=65) male and 32% (N=31) female subjects, with the mean age of 61.1±9.03 years. There was no difference between EGFR-positive and EGFR-negative patients in terms of the overall survival rate (P=0.49). In addition, no association was observed between tumor histology and EGFR expression (P=0.08), while EGFR-positive adenocarcinoma (N=28, 29%) was more prevalent compared to other subtypes of NSCLC. Moreover, there were no differences between tumor subtypes and the overall survival rate of the patients (P=0.21), and no association was found between EGFR expression and the patients' demographic factors (e.g. age and gender).

Conclusion: The results of this study indicated that EGFR expression could not be a prognostic marker in NSCLC patients; however, it seems that using standardized IHC scoring is likely to yield more reliable data in this regard.

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Introduction

Lung cancer is the leading cause of cancer related mortality worldwide, and non-small cell lung cancer (NSCLC) is responsible for

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approximately 80% of all lung cancer cases (1-3). The role of epidermal growth factor receptor (EGFR) and downstream proteins in cancer treatment has been widely investigated for over four decades (4).

EGFR signaling pathway plays a pivotal role in the survival and proliferation of NSCLC cells (5, 6). EGFR is a receptor tyrosine kinase (RTK) and a member of the ErbB family, which consists of an extracellular ligand-binding region, transmembrane region, and intracellular region containing the tyrosine-kinase domain (4, 5).

EGFR is abnormally activated in different human cancers, such as colorectal cancer and NSCLC (7-10). During the past decade, drugs that target EGFR have been commonly used for human cancer types. For instance, EGFR tyrosine-kinase inhibitors (EGFR-TKIs) are considered as the first- and second-line therapy for management of NSCLC, based on the presence of driver mutations in the tyrosine kinase domain (11-14).

The prognostic significance of EGFR expression in NSCLC is a controversial issue. Several reports are indicative of the association between EGFR expression and the prognosis of NSCLC patients (15-17); however, a number of more recent studies do not confirm such association (18-20). The present study aimed to evaluate EGFR expression and its association with the prognosis of NSCLC patients using immunohistochemistry (IHC) analysis.

Materials and Methods

This retrospective cohort study was conducted on 96 NSCLC patients undergoing surgery or biopsy at Ghaem Hospital, Mashhad, Iran from March 2009 to March 2012. The histological characteristics of different tumor subtypes were determined according to the World Health Organization (WHO) classification (21).

In addition, demographic and clinicopathological features including age, gender, tumor subtype, and history of previous chemotherapy or surgery were obtained from the medical records of the patients. However, some of the clinically important demographic data such as history of smoking, performance status, and tumor-node-metastasis (TNM) staging were not available.

Neither chemotherapy nor radiotherapy had been administered before surgery or biopsy. Patients with metastases or disease recurrence received standard platinum-based chemotherapy. The present study was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUMS), and informed consent was obtained from the subjects for specimen analysis.

IHC analysis

A 5-µm section was cut from the formalin-fixed, paraffin-embedded (FFPE) tissue blocks of the subjects, and then Hematoxylin and Eosin (H & E) was used to stain these sections in order to be examined by a pathologist for confirming the tumor areas. Following that, FFPE tissue blocks were sectioned at a thickness of 3-5µm and were placed on pretreated slides. After fixation at 80 ^{o}C for 8-10 minutes, the slides were deparaffinized in Xylene and rehydrated in graded alcohol series.

During the next stage, antigen retrieval was performed by placing the slides in Tris-HCL and Ethylenediaminetetraacetic acid (EDTA) solution in a water bath of 94-98 ^{o}C for 20-30 minutes. Afterwards, the slides were treated with 3-5% hydrogen peroxidase for 10 minutes at room temperature (RT) for inhibition of endogenous peroxidase.

Then, the slides were incubated overnight with EGFR mouse monoclonal antibody (1.3 mg/ml, Cedarlane, Canada) at RT. After that, incubation with horseradish peroxidase-conjugated secondary antibody (Biogenex, USA) was performed for 15 minutes and followed by the application of diaminobenzidine (DAB) chromogen for 10 minutes at RT. Finally, the slides were counterstained in hematoxylin.

The assessment of EGFR expression was performed based on the cytoplasmic staining intensity of the tumor cells, and IHC staining was evaluated by an expert pathologist (A.J). Eventually, the tumors were classified into the four grades of negative (0), 1+ (weak and focal), 2+ (moderate and focal), and 3+ (intense and diffuse) (Figure 1) (22).

Statistical analysis

For the statistical analysis of the data, we used SPSS version 20.0, and tables and diagrams were drawn by Microsoft Office Excel. For the purpose of performing statistical analysis, the patients were classified in two groups (i.e. EGFR-positive and EGFR-negative). To assess the association between EGFR expression and clinical features, chi-square test was performed.

Furthermore, univariate analysis was performed via Kaplan-Meier survival curves in order to identify the prognostic factors, and the difference between the curves was compared using the log-rank test. In all the statistical analysis, the *P-value* of less than 0.05 was considered significant, and all the tests were two-tailed.

Overall survival (OS) was defined as the time between the patient's diagnosis and the time of death. Patients who were not reported dead or had died from other causes than cancer were excluded from data analysis.

Results

Out of 96 NSCLC patients, 68% (N=65) were male and 32% (N=31) were female, with the

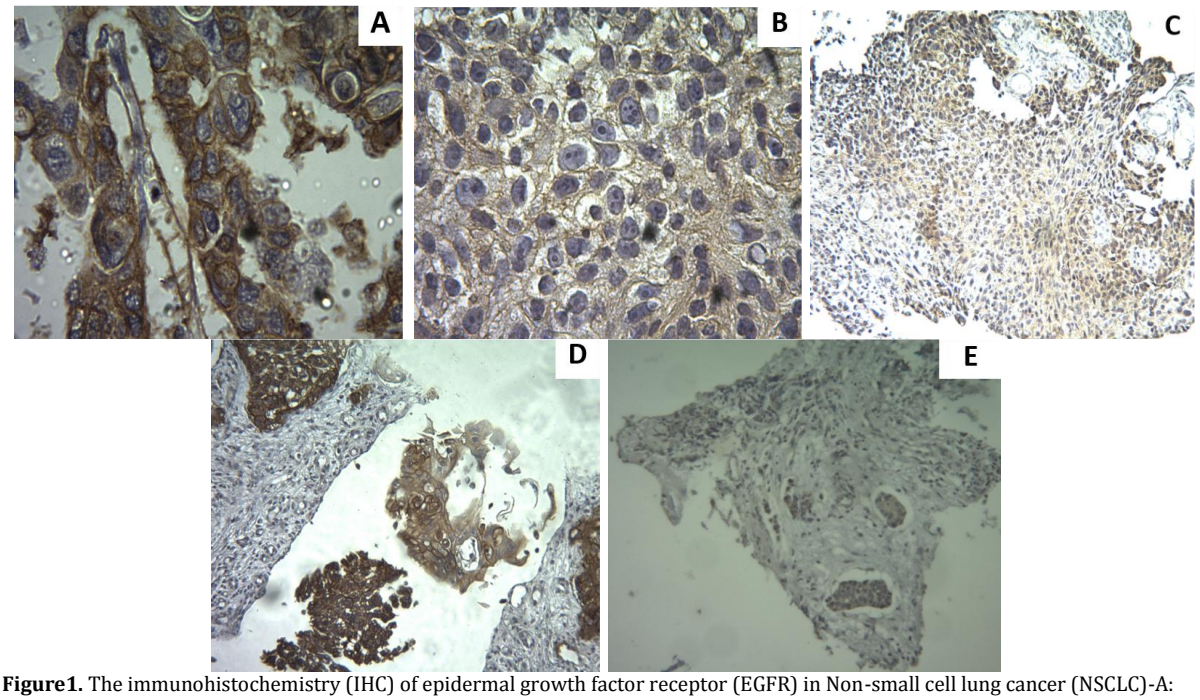

Figure1. The immunohistochemistry (IHC) of epidermal growth factor receptor (EGFR) in Non-small cell lung cancer (NSCLC)-A: +++ (intense and diffuse), adenocarcinoma ×40, B: ++ (moderate and focal), squamous cell carcinoma ×100, C: + (weak and focal), squamous cell carcinoma ×40, D: +++ (intense and diffuse), squamous cell carcinoma ×40, E: negative (0) ×40

mean age of 61.1±9.03 years (mean ± SD). The majority of the subjects were diagnosed with squamous cell carcinoma (SCC) (50%, N=48), while EGFR IHC results were negative in more than 45% (N=47) of the patients (Table 1). In addition, 58% (N=60) of patients had received chemotherapy. The mean OS was estimated as 9.6±6.5 months (mean ± SD).

According to the findings of this study, there was no statistically significant association between age (*P* =0.168), gender (*P*=0.521) and tumor resectability (*P*=0.709) in terms of EGFR expression. However, EGFR expression was found to be more prevalent in adenocarcinoma compared to other tumor subtypes, which was not statistically significant (*P-value*=0.104) (Figure 2).

According to Kaplan-Meier survival analysis, there was no statistically significant difference between the two IHC groups in terms of survival rate (Figure 3).

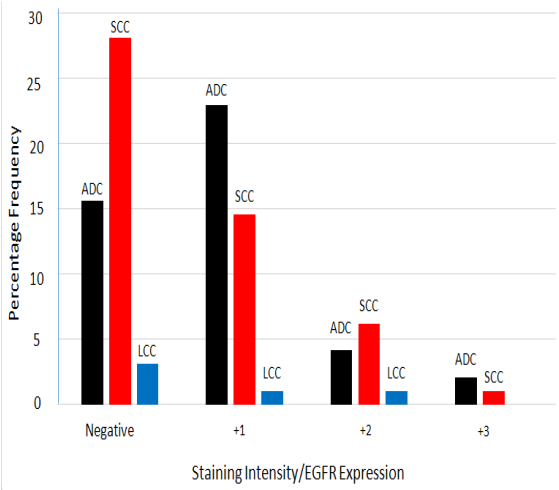

Figure2. Staining intensity/EGFR expression pattern in patients with non-small cell lung cancer: This chart shows the staining intensity/EGFR expression in different subtype of NSCLC. ADC: Adenocarcinoma SCC:Squamous Cell Carcinoma LCC: Large Cell Carcinoma

Discussion

Growth, proliferation, differentiation, angiogenesis, and apoptosis are the most

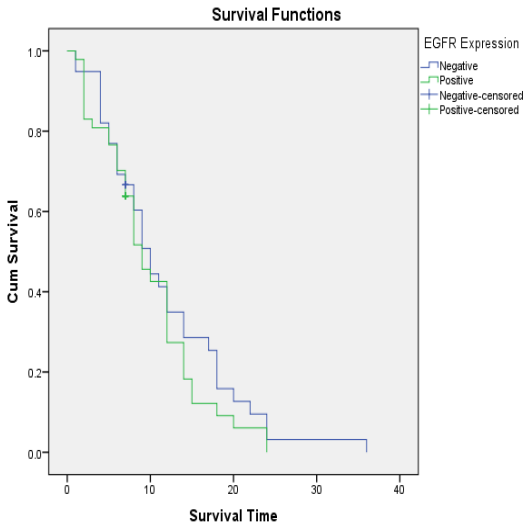

Figure3. Kaplan-Meier Survival Curve Showing Overall Survival (OS) According to Epidermal Growth Factor Receptor (EGFR) Expression in patients with Non-Small Cell Lung Cancer (NSCLC). (Log-rank Test Sig: 0.368, CI: 95%= 9.16-12.35)

Table1. Characteristics of NSCLC* patients in Ghaem Hospital, Mashhad, Iran (during 2009-2012)

Characteristics	N (%)
Sex	
Male	65 (68)
Female	31 (33)
Total	**96 (100)**
Age groups	
<40	1 (1)
40-60	41 (43)
>60	44 (46)
Unknown	10 (10)
Total	**96 (100)**
Smoking history	
Yes	27 (28)
No	20 (21)
Unknown	49 (51)
Total	**96 (100)**
Tumor subtype	
Adenocarcinoma	43 (45)
Squamous-cell carcinoma	48 (50)
Large-cell carcinoma	5 (5)
Total	**96 (100)**
EGFR IHC result	
Negative	45 (47)
+	37 (38)
++	11 (12)
+++	3 (3)
Total	**96 (100)**
Chemotherapy	
Yes	58 (60)
No	27 (28)
Unknown	11 (12)
Total	**96 (100)**
Tumor resectability	
Yes	26 (27)
No	60 (63)
Unknown	10 (10)
Total	**96 (100)**

* This table contains characteristics of NSCLC patients whose EGFR expression was evaluated using IHC. NSCLC: non-small cell lung cancer SD: Standard Deviation

prominent features of cells which are influenced by cancer, and EGFR plays a pivotal role in controlling of these features. Regarding the significance of EGFR in NSCLC, the behavior of the cancer cells could be affected by EGFR expression level (5, 6, 9).

Different clinical studies have been conducted worldwide on the importance of EGFR signaling pathway in NSCLC, in order to clarify the association between EGFR expression and several clinical features of the NSCLC patients, such as prognosis, tumor histology, metastasis, and demographic factors. In large number of these studies EGFR expression have been evaluated by IHC analysis and conflicting results have been presented.

In 1989, Dazzi et al. indicated that there was no significant association between EGFR expression level and survival rate (23), which is similar to the findings of another study conducted in 1997 (24). In the early 20th century, a systematic review on the same subject revealed that EGFR expression might be associated with poor prognosis of NSCLC patients (15), while subsequent studies did not confirm such association (18-20, 22, 25, 26). On the other hand, a couple of studies which were conducted in 2004 were indicative of a significant correlation between EGFR expression level and the survival rate of NSCLC patients (16, 17). However, we could not find any association between EGFR expression and the survival time of these patients in the current study.

Conflicting results exist on the association between EGFR expression level and factors such as tumor histology and demographic factors, while the majority of studies conducted on this subject could not find any association between EGFR expression and demographic factors (20, 25-27). Nevertheless, a study conducted in Poland in 2004 found an association between EGFR expression and the age of NSCLC patients (17). Our findings were indicative of no such association between EGFR expression level and demographic factors.

Another study performed in Denmark in the late 1990s indicated that EGFR expression level was more prevalent in SCC compared to other tumor subtypes (25). Similarly, most of the studies performed during 2003-2014 indicated that EGFR expression in SCC was more frequent than other tumor subtypes, in some of which a significant correlation was also observed between the presence of SCC and EGFR expression level (18-20, 26, 27). However, a number of studies performed in 2004 could not find this association (16, 22). In the current study, we found no association between EGFR expression and tumor histology, while it was observed that EGFR expression was more prevalent in adenocarcinoma compared to other tumor subtypes. In general, we attributed observed difference to limitation in subjects selection, ie, if all of the subjects during this period of time had been included; it might be led to another pattern.

Moreover, a few studies have evaluated EGFR expression level along with the expression of other related biomarkers such as insulin-like growth factor receptor-1 (IGFR-1) (18, 19). In such studies, it was demonstrated that EGFR and other biomarkers, such as IGF-1, could also be used as significant prognostic markers in NSCLC patients. It is also noteworthy that some of these studies also found a correlation between EGFR expression level and the survival time of the patients via different scoring methods of evaluating EGFR expression and staining intensity (17). One of the limitations of the present study was insufficient FFPE tissues for

EGFR evaluation in several cases, which were excluded from the study. Moreover, some of the clinicopathological factors of the studied patients such as performance status, TNM scores, and alcohol consumption were unavailable since the data were obtained from paper-based medical records. As a result, further statistical analysis and clinical interpretations could not be performed.

Conclusion

It seems that using standardized scoring methods, such as IHC analysis, for the evaluation of EGFR expression as well as using reference guidelines could probably yield more reliable results. And using another biomarkers in combination with EGFR could be promising and it can be suggested as a test panel for disease prognosis.

Conflict of Interest

The authors declare no conflict of interest.

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