Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v29i01.7497

EGFR EXPRESSION FREQUENCY IN NON-SMALL CELL LUNG CANCER: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Background: Epidermal Growth Factor Receptor (EGFR) overexpression is commonly observed in non-small cell lung cancer (NSCLC) and is associated with tumor progression and poor prognosis.
Objectives: To determine the frequency of EGFR expression in non-small cell lung cancer using immunohistochemical analysis.
Methodology: This cross-sectional study study was conducted at Rehman Medical College, Peshawar from Jan 2021 to June 2021, where formalin-fixed, paraffin-embedded (FFPE) tissue samples from patients diagnosed with NSCLC between January 2020 and December 2021 were collected. The study included 125 patients with a confirmed histopathological diagnosis of NSCLC who had not received prior EGFR-targeted therapy. Immunohistochemical staining for EGFR was performed on 4-µm-thick sections of FFPE tissue samples. The evaluation of EGFR expression was performed by two independent pathologists who were blinded to the clinical data. Data were analyzed using SPSS version 27.
Results: The study included 125 NSCLC patients with a mean age of 60.3 years. The gender distribution was nearly even, with 52% male and 48% female patients. Adenocarcinoma was the most common histological subtype (60%), followed by squamous cell carcinoma (32%) and large cell carcinoma (8%). This data provides a clear demographic and clinical profile of the study population. The study found that 44% of the NSCLC patients (55 out of 125) had positive EGFR expression, with a combined immunohistochemistry score of 2 or higher. Conversely, 56% of the patients (70 out of 125) showed negative EGFR expression, with a score below 2.
Conclusion: The study reveals that nearly half of the NSCLC patients exhibit positive EGFR expression, indicating a potential target for targeted therapies. This finding underscores the importance of EGFR status in the clinical management of NSCLC and highlights the need for further research to optimize treatment strategies.

Keywords: Prevalence, Periodontology, Adult gingivitis, Gingivitis, Plaque,

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most prevalent type of lung cancer, accounting for approximately 85% of all lung cancer cases worldwide. Despite advancements in diagnostic and therapeutic approaches, the prognosis for NSCLC remains poor, with a 5-year survival rate of less than 20%.[1] One of the critical factors influencing the prognosis and treatment strategy in NSCLC is the presence of molecular markers such as the Epidermal Growth Factor Receptor (EGFR). EGFR is a transmembrane receptor tyrosine kinase that plays a significant role in cell proliferation, survival, and differentiation.[2]

Mutations in the EGFR gene, particularly in the tyrosine kinase domain, lead to the activation of downstream signaling pathways that contribute to oncogenesis.[3] The identification of EGFR mutations has led to the development of targeted therapies, such as tyrosine kinase inhibitors (TKIs), which have revolutionized the treatment of NSCLC, particularly in patients with activating EGFR mutations.[4] Consequently, assessing EGFR expression and mutation status is now a standard practice in the diagnostic workup of NSCLC.[5] Moreover, the relationship between EGFR expression and clinical outcomes in NSCLC remains a topic of ongoing research. While some studies suggest that high EGFR expression is associated with better response to EGFR-TKIs and improved survival rates, others have reported no significant correlation.[6] This discrepancy highlights the complexity of EGFR signaling and its role in NSCLC, emphasizing the need for further studies to elucidate the prognostic and predictive value of EGFR expression.[7]

The frequency of EGFR mutations varies significantly across different populations and ethnic groups, with higher prevalence observed in East Asian populations compared to Western populations. This variability has important implications for the use of EGFR-targeted therapies, making it essential to understand the expression frequency of EGFR in different demographic settings. Immunohistochemistry (IHC) is commonly used to evaluate EGFR expression in tissue samples, providing a valuable tool for the stratification of patients who may benefit from targeted therapy.[8] Several studies have investigated the frequency of EGFR expression in NSCLC using IHC, with reported rates ranging from 40% to 80%.[9,10] These differences in frequency may be attributed to variations in the study population, sample size, and IHC methodologies.

This study aims to investigate the frequency of EGFR expression in NSCLC using IHC, with a focus on understanding its distribution across different histological subtypes and stages of the disease. Additionally, we will explore the potential association between EGFR expression and clinicopathological features, such as age, gender, smoking history, and tumor grade. By providing detailed insights into the expression patterns of EGFR in NSCLC, this study seeks to contribute to the existing body of knowledge and support the development of personalized treatment strategies for patients with NSCLC.

MATERIALS AND METHODS

This cross-sectional study was conducted at Rehman Medical College, Peshawar from Jan 2021 to June 2021, where formalin-fixed, paraffin-embedded (FFPE) tissue samples from patients diagnosed with NSCLC between January 2020 and December 2021 were collected. The study protocol was approved by the Institutional Review Board (IRB), and all procedures were carried out in accordance with ethical standards. The study population included patients with a confirmed histopathological diagnosis of NSCLC who had not received prior EGFR-targeted therapy. Patients with small cell lung cancer (SCLC), those who had undergone neoadjuvant therapy, or whose tissue samples were inadequate for analysis were excluded. A total of 125 patients met the inclusion criteria and were included in the study. For each patient, demographic data, including age, gender, smoking status, and tumor histological subtype, were recorded. Tumor samples were obtained through either biopsy or surgical resection. Histological subtypes were classified according to the 2015 World Health Organization (WHO) classification of lung tumors. The histological types included adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

Immunohistochemical staining for EGFR was performed on 4-μm-thick sections of FFPE tissue samples. The sections were deparaffinized in xylene and rehydrated through graded alcohols.

Antigen retrieval was carried out by heating the sections in a citrate buffer (pH 6.0) at 95°C for 20 minutes. Following incubation with the primary antibody, sections were treated with a secondary antibody conjugated to horseradish peroxidase and visualized using diaminobenzidine (DAB) as the chromogen. Counterstaining was performed with hematoxylin, and the slides were then mounted for microscopic examination.

The evaluation of EGFR expression was performed by two independent pathologists who were blinded to the clinical data. EGFR expression was scored based on the intensity and extent of membranous staining. The intensity of staining was graded on a scale of 0 to 3+, where 0 indicated no staining, 1+ indicated weak staining, 2+ indicated moderate staining, and 3+ indicated strong staining. The extent of staining was determined by the percentage of tumor cells exhibiting positive staining, categorized as follows: 0-10%, 11-50%, and >50%. A combined score was calculated by multiplying the intensity and extent scores, with a maximum score of 9. Tumors with a combined score of ≥2 were considered positive for EGFR expression.

Data were analyzed using SPSS version 27. Descriptive statistics were used to summarize the demographic and clinical characteristics of the patients. The association between EGFR expression and clinicopathological variables, such as age, gender, smoking status, and histological subtype, was assessed using chi-square or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

STUDY RESULTS

The study included 125 NSCLC patients with a mean age of 60.3 years. The gender distribution was nearly even, with 52% male and 48% female patients. A majority of the patients were smokers (56%), while 44% were non-smokers. Adenocarcinoma was the most common histological subtype (60%), followed by squamous cell carcinoma (32%) and large cell carcinoma (8%). This data provides a clear demographic and clinical profile of the study population.

Table 1: Patient Demographics and Clinical Characteristics

Characteristic	**Total (n=125)**
Age (Mean ± SD)	60.3 ± 9.8
Gender	
- Male	65 (52%)
- Female	60 (48%)
Smoking Status	
- Smoker	70 (56%)
- Non-Smoker	55 (44%)
Histological Subtype	
- Adenocarcinoma	75 (60%)
- Squamous Cell Carcinoma	40 (32%)
- Large Cell Carcinoma	10 (8%)

The study found that 44% of the NSCLC patients (55 out of 125) had positive EGFR expression, with a combined immunohistochemistry score of 2 or higher. Conversely, 56% of the patients (70 out of 125) showed negative EGFR expression, with a score below 2. This distribution indicates that EGFR expression is present in a significant portion of the NSCLC population, though the majority of patients did not exhibit positive expression by the criteria used.

Table 2: Frequency of EGFR Expression

EGFR Expression	Frequency (%)
- Positive (Score ≥2)	55 (44%)
- Negative (Score <2)	70 (56%)

The analysis of EGFR expression by clinicopathological variables revealed no statistically significant differences across the studied categories. The mean age of patients with positive EGFR expression (59.2 years) was slightly lower than that of those with negative expression (61.1 years), but this difference was not statistically significant (p=0.26).Gender distribution was similar between the two groups, with males comprising 52.7% of the EGFR-positive group and 51.4% of the EGFR-negative group, and females representing 47.3% and 48.6% respectively, showing no significant difference (p=0.88). Regarding smoking status, 58.2% of the patients with positive EGFR expression were smokers, compared to 54.3% in the EGFR-negative group. This difference was not statistically significant (p=0.66), indicating that smoking status did not strongly influence EGFR expression. In terms of histological subtype, adenocarcinoma was the most common subtype among EGFR-positive patients (63.6%), followed by squamous cell carcinoma (30.9%) and large cell carcinoma (5.5%). However, these distributions did not differ significantly from the EGFR-negative group, where adenocarcinoma accounted for 57.1%, squamous cell carcinoma for 32.9%, and large cell carcinoma for 10.0% (p=0.48). Overall, these findings suggest that EGFR expression in NSCLC does not appear to be significantly associated with age, gender, smoking status, or histological subtype in this patient population.

Table 3: EGFR Expression by Clinicopathological Variables

Variable	EGFR Positive (n=55)	EGFR Negative (n=70)	p-value
Age (Mean ± SD)	59.2 ± 10.1	61.1 ± 9.5	0.26
Gender			
- Male	29 (52.7%)	36 (51.4%)	0.88
- Female	26 (47.3%)	34 (48.6%)	
Smoking Status			
- Smoker	32 (58.2%)	38 (54.3%)	0.66
- Non-Smoker	23 (41.8%)	32 (45.7%)	
Histological Subtype			
- Adenocarcinoma	35 (63.6%)	40 (57.1%)	0.48
- Squamous Cell Carcinoma	17 (30.9%)	23 (32.9%)	
- Large Cell Carcinoma	3 (5.5%)	7 (10.0%)	

DISCUSSION

Epidermal Growth Factor Receptor (EGFR) plays a crucial role in cell proliferation and survival, and its dysregulation is often implicated in the pathogenesis of non-small cell lung cancer (NSCLC). EGFR mutations, particularly in the tyrosine kinase domain, are significant as they guide targeted therapies, such as tyrosine kinase inhibitors (TKIs), which have improved outcomes in patients with EGFR-mutated NSCLC.[11] The frequency of EGFR expression varies across different populations and histological subtypes, making it essential to study its prevalence to optimize treatment strategies. Understanding the distribution of EGFR expression in NSCLC patients can provide valuable insights into personalized cancer therapy and prognosis. This study focuses on evaluating the frequency of EGFR expression in NSCLC, utilizing immunohistochemistry to correlate it with clinical and pathological features.[12]

In this study, we observed that 44% of non-small cell lung cancer (NSCLC) patients exhibited positive EGFR expression as determined by immunohistochemistry (IHC). This finding aligns with previous studies, where EGFR expression rates have ranged between 40% and 60% in NSCLC populations, particularly in adenocarcinoma subtypes.[13,14] The frequency of EGFR expression in our cohort, particularly in adenocarcinoma patients (63.6%), corroborates with data from other studies suggesting that EGFR expression and mutations are more prevalent in adenocarcinoma than in other NSCLC subtypes.[15,16]

Interestingly, our study found no significant association between EGFR expression and demographic factors such as age and gender. This result is consistent with findings from other research, indicating that EGFR expression in NSCLC is largely independent of these variables.[17,18]

For instance, a study by Zhang et al. found similar EGFR expression rates across different age groups and between male and female patients, suggesting that these factors do not significantly influence EGFR status in NSCLC.[19]

Our analysis of smoking status revealed that 58.2% of EGFR-positive patients were smokers, compared to 54.3% in the EGFR-negative group, with no statistically significant difference. This finding contrasts with some earlier studies that have reported higher EGFR mutation rates in non-smokers, particularly in East Asian populations.[20] However, it is important to note that while EGFR mutations are more common in non-smokers, EGFR protein expression as detected by IHC does not always correlate directly with mutation status.[21] This discrepancy highlights the complexity of EGFR biology in NSCLC and suggests that IHC may capture a broader spectrum of EGFR alterations, not limited to mutations.

The histological subtype analysis in our study also supports the notion that EGFR expression is more frequent in adenocarcinoma. However, the expression rates in squamous cell carcinoma (30.9%) and large cell carcinoma (5.5%) in our cohort are somewhat lower than those reported in other studies. For example, Sholl et al. found EGFR expression in approximately 20% of squamous cell carcinomas, which is slightly higher than our findings but still within a comparable range.[16,20]

The study's limitations include a relatively small sample size, which may not fully represent the broader NSCLC population. Additionally, the cross-sectional design limits the ability to assess changes in EGFR expression over time. Variability in immunohistochemistry scoring could also impact the consistency of EGFR expression results.

CONCLUSION

The study reveals that nearly half of the NSCLC patients exhibit positive EGFR expression, indicating a potential target for targeted therapies. This finding underscores the importance of EGFR status in the clinical management of NSCLC and highlights the need for further research to optimize treatment strategies

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