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EGFR Expression Patterns in Non-Small Cell Lung Cancer: A Case Series via Immunohistochemistry

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Abstract

Lung cancer ranks among the leading causes of death for both men and women, representing 5.9% of all cancer cases and accounting for 8.1% of cancer-related fatalities. The most prevalent form of lung cancer is non-small-cell lung cancer (NSCLC), which encompasses various histological tumor types, such as adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. The uncontrolled proliferation and progression of cancer can arise from mutations or overexpression of EGFR, a transmembrane receptor tyrosine kinase protein. Consequently, comprehending the role of epidermal growth factor receptor (EGFR) in NSCLC is crucial, as this protein plays a significant role in regulating cell growth and division.

The objective of this study is to assess the expression of the EGFR in NSCLC through the application of immunohistochemistry. A collection of lung biopsy specimens was obtained from the Institute of ACS Medical College and Hospital. The tissue samples were preserved in formalin and subsequently subjected to standard histopathological and immunohistochemical analyses.In our investigation, we found that EGFR expression is predominantly associated with adenocarcinoma, exhibiting grades of 3+, 2+, and 1+. Conversely, other forms of NSCLC primarily demonstrate EGFR expression in grades of 2+ and 1+. Our results suggest that approximately two-thirds of NSCLC patients (exceeding 60%) possess an EGFR mutation. Therefore, the identification of EGFR expression is crucial for improving patient management, as it represents a significant negative prognostic indicator.

Keywords: Epidermal growth factor receptor (EGFR), non-small cell lung carcinoma (NSCLC), tyrosine kinase inhibitor (TKI), adenosquamous cell carcinoma (ASCC).

INTRODUCTION

Lung cancer represents the most prevalent form of malignancy, accounting for 5.9% of all cancer diagnoses and 8.1% of cancer-related mortalities. It is primarily categorized into two main classifications: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Among these, NSCLC constitutes the most common variant, encompassing various histological tumor types such as adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma (1,2,3). The EGFR is a transmembrane protein that belongs to the tyrosine kinase family and is present in normal epithelial and mesenchymal tissues. It exhibits widespread expression in various types of carcinomas, including those of the lung, breast, and ovary. EGFR mutations are prevalent in certain solid epithelial cancers, such as ovarian, breast, and colorectal malignancies. In NSCLC, EGFR was the first identified oncogenic target (3,4).

In these cancers, two primary mechanisms are observed: the overexpression of EGFR protein and the mutation of EGFR's tyrosine kinase domain. Research has indicated that EGFR expression in NSCLC correlates with diminished patient survival rate(5,6). Therefore, EGFR expression levels in NSCLC tumors are not only important for predicting the response to targeted therapies, but also for prognostic assessment and treatment decision-making.High levels of EGFR expression may indicate a more aggressive tumor phenotype and poorer prognosis, whereas EGFR-negative tumors may have different biological characteristics and treatment implications (5,6). Various testing modalities are available for identifying EGFR mutations, including immunohistochemistry (IHC), next-generation sequencing (NGS), polymerase chain reaction (PCR), and fluorescence in situ hybridization (FISH). Each method presents distinct advantages and limitations, and the selection of a testing approach is frequently determined by factors such as sample availability, cost, turnaround time, and the specific mutation of interest(3,5,6).

In this study, the researchers aimed to ascertain the EGFR mutation status in patients with NSCLC utilizing immunohistochemistry (IHC), a widely recognized and relatively cost-effective method for assessing protein expression

levels in tissue specimens. IHC facilitates the visualization of EGFR protein expression on the surface of tumor cells, thereby providing critical insights into the presence or absence of EGFR mutations. Through the analysis of EGFR mutation status via IHC, the researchers intended to identify patients who may benefit from EGFR-targeted therapies, including tyrosine kinase inhibitors (TKIs). The detection of EGFR mutations, indicated by positive EGFR expression observed through IHC, could inform treatment decisions and potentially enhance patient outcomes(7,8,10). This study highlights the essential importance of EGFR mutation testing in patients diagnosed with NSCLC. It also emphasizes the efficacy of immunohistochemistry (IHC) as a dependable technique for evaluating EGFR mutation status in clinical environments. Accurately identifying EGFR mutations through IHC enables the development of personalized treatment strategies, leading to enhanced response rates and better overall survival outcomes for NSCLC patients(8,9,10). As a result, EGFR mutations are prevalent in roughly 10-30% of NSCLC cases. These mutations can result in constitutive activation of the EGFR tyrosine kinase domain, which promotes cancer cell pwroliferation and survival. EGFR expression patterns can be used to predict a patient's response to EGFR tyrosine kinase inhibitors. Patients with EGFR mutations or amplification have a higher chance of responding to EGFR TKI. The EGFR expression patterns have important clinical implications in NSCLC. EGFR expression can be used as a predictive and prognostic biomarker, and it is a therapeutic target in NSCLC. Understanding EGFR expression patterns can help to guide therapy decisions and enhance patient outcomes.

MATERIALS AND METHODS

The study was conducted in thepathology departmentin our institute.Fifteen lung biopsy cases were obtained from the Pathology files retrospectively.The tissues were fixed in formalin and used for routine histopathological and IHC studies.

The inclusion criterion was Non-small cell lung carcinoma.

The exclusion criteria were Benign Lung lesions and inadequate tissues.

The specimens were examined grossly, and representative sections were obtained and processed using the routine procedures of dehydration, clearing, and embedding.Paraffin-embedded sections were cut to a thickness of 4 μm and stained with hematoxylin and eosin.Furthermore, 3 μm thick sections of the paraffin-embedded blocks were obtained on poly L lysine-stained slides for immunohistochemical (IHC) techniques.IHC staining was performed for EGFR mutations using an EP22 Rabbit monoclonal antibody and grading was performed.Based on the positive tumor cells and intensity of the staining area, tumor samples were graded from 0 to 3+ and divided into four categories as follows: no staining, 0; weak staining, 1+ (light-brown membrane staining); intermediate staining, 2+; and strong staining, 3+ (dark-brown linear membrane staining) (6,8).

Statistical analysis:

Data analysis was performed using version 25.0 of the Statistical Package for Social Sciences (SPSS, IBM Company, Chicago, IL, USA). The data were given as the mean, standard deviation, and range. Categorized data was displayed using frequencies and percentages. We used the two-tailed independent t-test to compare continuous variables. Furthermore, Pearson's correlation test (r) was employed to assess the relationship between continuous variables. P-values < 0.05 were considered statistically significant.

RESULTS

Our study contains 15 cases based on the inclusion and exclusion criteria. Out of 15 cases, 11 were between the ages of 60 and 75, with the rest lying between the ages of 50 and 60. Out of the 15 cases, 13 were male and 2 were female(Ref Table1). Out of 15 cases examined, 8 were adenocarcinoma, 3 were squamous cell carcinoma, 1 was adeno squamous carcinoma, and 3 were not otherwise defined (NOS). Our study's mean and standard deviation for age are 64.33 and 5.948, respectively(Table 5).

Out of the 15 samples analyzed, 10 exhibited positive EGFR expression, while the remaining five were negative. Among the 10 cases with positive EGFR expression, there were 4 cases of adenocarcinoma with scores of 3+ and 2+, 2 cases of lepidic type adenocarcinoma with scores of 2+ and 1+, 2 cases classified as not otherwise specified (NOS), and 1 case of squamous cell carcinoma with a score of 1+. Additionally, there was one case of adenosquamous carcinoma with a score of 1+. Among the 5 cases that tested negative, 2 were adenocarcinoma, 2 were squamous cell carcinoma, and 1 was NOS type(Ref Table 2&3).In our study, 66.7% of the cases demonstrated positivity for the EGFR mutation-specific antibody, while 33.3% showed negativity. The findings indicate a sensitivity of 75% and specificity of 66.7% for EGFR-specific antibodies in immunohistochemistry. The positive predictive value (PPV) and negative predictive value (NPV) for our study were found to be 90% and 40%, respectively (Ref Table4).

Our study shows Asymptotic Significance 0.171 based on Pearson Chi-Square test (Table 6).

The test result variable(s): EGFR score has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased(Table 7).

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

The sensitivity and specificity of EGFR expression were demonstrated using the ROC curve (Figure 1). When comparing EGFR expression, our data suggest that adenocarcinoma has higher expression than squamous cell carcinoma (Figure 2).

TABLES &FIGURES

Table 1: Frequency table based on gender.

		Frequency	Per cent	Valid Percent	Cumulative Percent
Valid	Male	11	73.3	73.3	73.3
	Female	4	26.7	26.7	100.0
	Total	15	100.0	100.0	

Table 2: Immunohistochemistry results of EGFR

		Frequency	Per cent	Valid Percent	Cumulative Percent
Valid	Positive	10	66.7	66.7	66.7
	Negative	5	33.3	33.3	100.0
	Total	15	100.0	100.0	

Table:3 Frequency table based on scoring of EGFR

		Frequency	Per cent	Valid Percent	Cumulative Percent
Valid	0	5	33.3	33.3	33.3
	1+	5	33.3	33.3	66.7
	2+	3	20.0	20.0	86.7
	3+	2	13.3	13.3	100.0
	Total	15	100.0	100.0	

Table 4: Crosstabulation between EGFR diagnosis and scoring.

			DIAGNOSIS		Total
			Positive	Negative	
EGFR SCORE	Positive	Count	9	1	10
		% within EGFR SCORE	90.0%	10.0%	100.0%
		% within DIAGNOSIS	75.0%	33.3%	66.7%
	Negative	Count	3	2	5
		% within EGFR SCORE	60.0%	40.0%	100.0%
		% within DIAGNOSIS	25.0%	66.7%	33.3%
Total		Count	12	3	15
		% within EGFR SCORE	80.0%	20.0%	100.0%
		% within DIAGNOSIS	100.0%	100.0%	100.0%

Table5: Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
AGE	15	54	74	64.33	5.948
Valid N (listwise)	15				

Table 6: Chi-Square Tests

	Value	Df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.875[a]	1	.171		
Continuity Correction[b]	.469	1	.494		
Likelihood Ratio	1.780	1	.182		
Fisher's Exact Test				.242	.242
Linear-by-Linear Association	1.750	1	.186		
N of Valid Cases	15				

Table 7 (Area under the curve): EGFR score

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.708	.179	.279	.357	1.000

The test result variable(s): EGFR SCORE has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

FIGURE LEGENDS

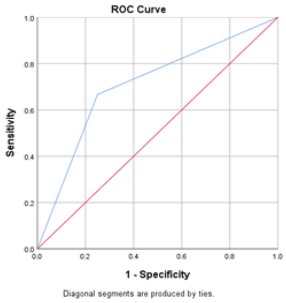

Fig1: ROC curve based on sensitivity and specificity of EGFR expression

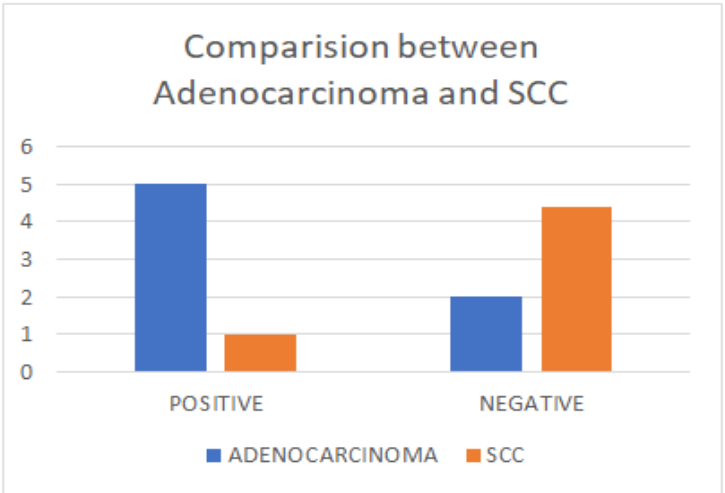

Fig2: EGFR expression in ADC and SCC

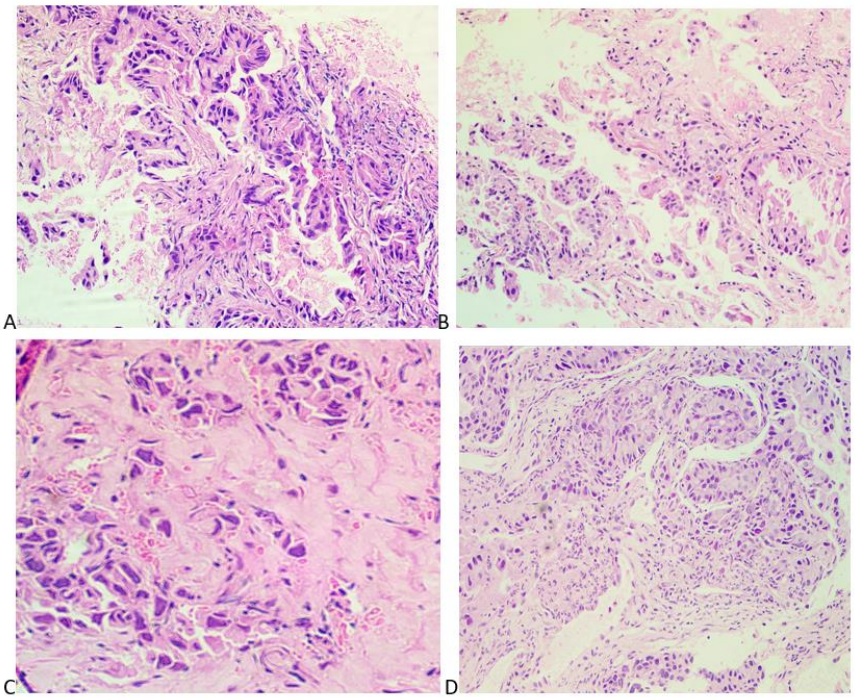

Fig 3:Representativeexample of NSCLC histopathology. (A)Adenocarcinoma at a magnification of 100X;(B)Lepidic type of adenocarcinoma at a magnification of 100X;(C)Squamous cell carcinoma at a magnification of 400X;(D)Not otherwise specific type at a magnification of 400X;

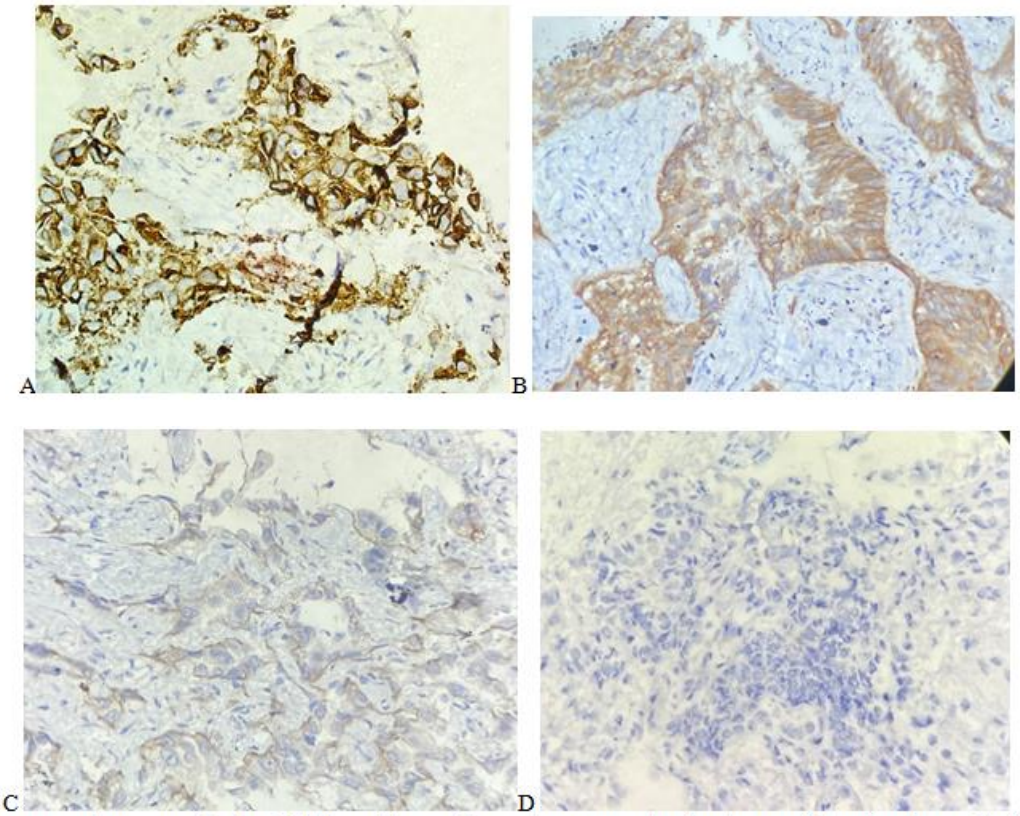

Fig4:Representative example for EGFR positive and negative expression by immunohistochemistry. Grading of membrane staining based on intensity as follows (A)3+ for dark brown linear membrane staining (B) 2+ for intermediate staining(IHC,100X). (C)1+ for weak light brown staining of the linear membrane. (D) 0 for no staining (IHC,400X).

DISCUSSION

Lung cancer is a leading cause of global mortality, with non-small cell lung cancers representing 85% of all cases. Among the different forms of lung carcinoma, adenocarcinoma is the most common subtype. This subtype is further classified into several categories based on its predominant histological patterns, which include lepidic, acinar, papillary, colloid, solid-predominant, and invasive mucinous types(2,3,4). The classification of non-small cell carcinoma is essential for accurate diagnosis and for guiding decisions regarding molecular testing for targeted therapies based on specific biomarkers, as well as for chemotherapy options. Research indicates that chemotherapy demonstrates greater efficacy in treating adenocarcinoma relative to squamous cell carcinoma. Furthermore, it has been established that the use of antiangiogenic agents is contraindicated in the management of squamous cell carcinoma.It is essential to categorize non-small cell lung carcinoma for accurate diagnosis and treatment planning(10,11).Molecular diagnostic plays a crucial role in the evaluation of adenocarcinoma lung. Adenocarcinoma with EGFR mutation will respond well to EGFR inhibitortherapy. EGFR mutation is reported in 15%to 20% of lung carcinoma and is more commonly seen in women and non-smokers(13,14).

A study conducted by Nir Peled et al.(7) demonstrated that EGFR mutations are associated with improved patient survival and predict a favorable response to targeted tyrosine kinase inhibitor therapy. In the current investigation, EGFR mutations were identified in over 60% of the cases examined. Rui Jin et al(15) reported that lung squamous cell carcinoma with EGFR mutations exhibits a poorer prognosis compared to adenocarcinoma of the lung. Conversely, our study found that squamous cell carcinoma was negative for EGFR expression. Additionally, research by Deepali Jain et al. (11) concluded that immunohistochemistry (IHC) serves as a valuable diagnostic tool for lung adenocarcinoma and aids clinicians in patient management. The present study, which focused on small biopsies of NSCLC, revealed that IHC is a cost-effective method, with EGFR-mutated antibodies demonstrating greater sensitivity and specificity in the diagnosis of lung adenocarcinoma compared to other non-small cell lung carcinoma subtypes.

In a study conducted by Alvin Ho-Kwan Cheung et al. (19), it was found that squamous cell carcinoma lacking EGFR mutations demonstrates a poor response to tyrosine inhibitor therapy. The current study indicated a 66% negative rate for EGFR mutations in squamous cell carcinoma. Additionally, research by Chi Hong Kim et al(10) reported high sensitivity (76.6%) and specificity (94.5%) for EGFR mutation antibodies utilizing the IHC method. In our investigation, the EGFR mutant-specific antibody exhibited 75% sensitivity and 66.7% specificity. Hsiang-Ling Ho et al. (13) reported that 46.2% of EGFR mutations in NSCLC are associated with squamous cell carcinoma, particularly in small lung biopsies, and indicated a significant likelihood of adenocarcinoma differentiation. The current study reveals that 20% of squamous cell carcinoma cases and approximately 6.6% of adenosquamous cell carcinoma cases were identified through histopathological examination of small biopsies. Additionally, the study found a 6.6% prevalence of EGFR mutations in both squamous cell carcinoma and adenosquamous cell carcinoma using the immunohistochemistry (IHC) method. Consequently, we assert that IHC is essential for the evaluation of small biopsies in the diagnostic and therapeutic management of EGFR-mutated NSCLC.

In a study conducted by Tomasz Powrozek et al. (18), it was observed that EGFR mutations occurred in 28.6% of adenosquamous cell carcinoma cases of the lung. Similarly, Kouhi Ohtsuka et al. (14) reported the presence of an EGFR mutation in NSCLC characterized by an adenocarcinoma component, whereas no mutations were identified in pure squamous cell carcinoma. The current investigation indicates that EGFR mutations are present in 6.6% of squamous cell carcinoma cases, thereby suggesting a potential association with adenosquamous cell carcinoma.

CONCLUSION

The present study proves that there is prevalence of EGFR mutation in NSCLC and IHC has diagnostic and therapeutic role for EGFR mutated specific antibody in Adenocarcinoma. However, EGFR is negatively expressed in pure squamous cell carcinoma in almost 70% of cases, so it indicates downregulation of EGFR antibody in NSCLC without adenocarcinoma element. Therefore, we conclude that EGFR has a diagnostic role in distinguishing adenocarcinoma from squamous cell carcinoma and other types of NSCLC in small lung biopsies.Uncovering the patterns of Epidermal Growth Factor Receptor (EGFR) expression in non-small cell lung cancer (NSCLC) enhances our understanding of the disease.

EGFR expression patterns have significant clinical implications in NSCLC. EGFR expression can serve as a predictive and prognostic biomarker, and EGFR is a therapeutic target in NSCLC. Understanding EGFR expression patterns can help guide treatment decisions and improve patient outcomes.Continued study into EGFR IHC techniques and therapy outcomes is critical. Improving present approaches may result in better patient care. Healthcare providers should prioritize the use of IHC in NSCLC. By embracing precision oncology, we can greatly enhance patient outcomes. The discovery of new EGFR targets, such as EGFRvIII and EGFR exon 20 insertions, is a current research area for future studies.

Limitations of study: A small sample size may limit the statistical power of the study and make it difficult to detect significant differences between groups.Prospective studies with larger sample sizes and longer follow-up periods are needed to confirm the findings and establish the clinical utility of EGFR expression patterns in NSCLC.

Conflict of interest: No conflict of interest.

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