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status via IHC, the researchers sought to identify patients who may benefit from EGFR-targeted therapies such as tyrosine kinase inhibitors (TKIs). The presence of EGFR mutations could be indicated by positive EGFR expression detected by IHC, which could guide treatment decision and potentially improve patient outcomes [7,8,10].

Overall, this study underscores the importance of EGFR mutation testing in NSCLC patients and highlights the utility of IHC as a viable method for determining EGFR mutation status in clinical practice. Accurate identification of EGFR mutations through IHC enables personalized treatment approaches, leading to enhanced response rates and overall survival in NSCLC patients [8,9,10].

II. MATERIALS AND METHODS

The study was conducted in the pathology department of ACS medical college and hospital. A total of 15 lung biopsy cases were obtained from the Department of Respiratory Medicine from September 2022 to August 2023. The tissues were fixed in formalin and taken up for routine histopathological studies and IHC studies.

Inclusion criteria: Non-small cell lung carcinoma cases.
Exclusion criteria: Benign Lung lesions, Inadequate tissue.
Histopathology technique: The specimens were examined grossly, and their representative sections were taken and then processed using the routine procedures of dehydration, clearing, and embedding. The paraffin-embedded sections were cut to 4 μm thickness, and all were stained with H&E. Furthermore, 3 μm thick sections of the paraffin-embedded blocks were taken on Poly L Lysine stained slides for immunohistochemical (IHC) techniques. IHC stain was performed for EGFR mutation using EP22 Rabbit monoclonal antibody and grading was done. Based on the positive tumor cells and intensity of the staining area, tumor samples were graded from 0 to 3+ and divided into 4 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].

III. RESULTS

A total of 15 cases were included in our study as per inclusion and exclusion criteria. Out of 15 cases, 11 cases were in a range of 60 to 75 years, and other cases were in a range of 50 to 60 years. Out of 15 cases, 13 cases were male and 2 cases were female. Out of 15 numbers of study cases, 8 were Adenocarcinoma, 3 were Squamous cell carcinoma, 1 was Adeno squamous carcinoma, and 3 were Not Otherwise Specified (NOS) typed. Out of 15 cases EGFR expression was seen in 10 cases and 5 cases showed negative results.

Out of 10 cases of EGFR positive expression,4 cases were adenocarcinoma with a score of 3+ and 2+, 2 cases were lepidic type adenocarcinoma with a score of 2+ and 1+, 2 cases of NOS and 1 case of squamous cell carcinoma was with a score of 1+ and other one case was Adenosquamous type with score1+. Out of 5 cases showing negative scores 2 cases were adenocarcinoma, 2 cases were squamous cell carcinoma and 1 case was NOS type. In our study, 66.7% of cases show positivity and 33.3% of cases shows negativity for EGFR mutation specific antibody (Table 2). The present study shows 75% sensitivity and 66.7% specificity for EGFR-specific antibodies for immunohistochemistry. The Positive predictive value (PPV) and Negative predictive value (NPV) for our study are 90% and 40%.

IV. DISCUSSION

Lung cancer is one of the leading causes of death worldwide and 85% of lung cancers are non-small cell cancers. Adenocarcinoma is the most common type of lung carcinoma when compared with other types of lung carcinoma. Based on the predominant histological pattern it is subclassified into lepidic, acinar, papillary, colloid, solid-predominant, and invasive mucinous [2,3,4]. The classification of non-small cell carcinoma is essential not only for diagnosis but also for identifying the patients who should be subjected to molecular testing to select targeted therapy against biomarkers and for chemotherapy [12]. Some of the studies proved that chemotherapy is more effective in adenocarcinoma than squamous cell carcinoma and proved antiangiogenic agents are contraindicated in squamous cell carcinoma. Therefore, it is necessary to classify non-small cell lung carcinoma [10,11]. Molecular diagnostic plays a crucial role in the evaluation of adenocarcinoma lung. Adenocarcinoma with EGFR mutation will respond well to EGFR inhibitor therapy. 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 by Nir Peled et al [4] found, EGFR mutation has improved survival of the patient and predicts better response when treated with target tyrosine inhibitor therapy. In the current study, EGFR mutation is seen in more than 60% of cases. Rui Jin et al [13] found, that EGFR-mutated lung squamous cell carcinoma shows poor prognosis than adenocarcinoma lung. In our study, squamous cell carcinoma shows negativity for EGFR expression. In a study by Deepali Jain et al [9], concluded that IHC is a potential tool for diagnostic purpose of lung adenocarcinoma as well as to guide the clinicians for patient management. The present study was also performed in small biopsies of NSCLC and we found that IHC is cost-effective and EGFR mutated antibodies have more sensitivity and specificity in the diagnostic purpose of adenocarcinoma of lungs than other types of non-small cell lung carcinoma.

In a study by Alvin Ho-Kwan Cheung et al [15], they found that squamous cell carcinoma lacks EGFR- mutation shows poor response to tyrosine inhibitor therapy. The present study revealed a 66% negative result for EGFR mutation in squamous cell carcinoma. In a study by Chi

Hong Kim et al [8], showed high sensitivity (76.6%) and specificity (94.5%) for EGFR mutation antibodies using the IHC method. In our study, EGFR mutant specific antibody shows 75% sensitivity and 66.7% specificity. Hsiang-Ling Ho et al [11] found, 46.2% of EGFR mutation in NSCLC favors squamous cell carcinoma from small lung biopsies and found to have a high probability of being Adenosquamous cell carcinoma. The present study shows 20% of squamous cell carcinoma cases and around 6.6% of adenosquamous cell carcinoma from small biopsies histopathologically. The present study shows 6.6% EGFR mutation in SCC and 6.6% in ASCC by the IHC method. Therefore, we conclude that IHC is necessary for small biopsies in diagnostic and therapeutic options for EGFR-mutated NSCLC.

In a study by Tomasz Powrozek et al [16], mutation of EGFR was observed in 28.6% of Adenosquamous cell carcinoma of the lung. In a study by Kouhi Ohtsuka et al [12], they identified an EGFR mutation in NSCLC with an adenocarcinoma element, but no mutation was identified in pure Squamous cell carcinoma. The present study shows EGFR mutation in 6.6% of squamous cell carcinoma which has a possibility of being Adenosquamous cell carcinoma.

V. 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.

CONFLICT OF INTEREST: None.

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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 Positive	Negative	Total
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

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FIGURE LEGENDS

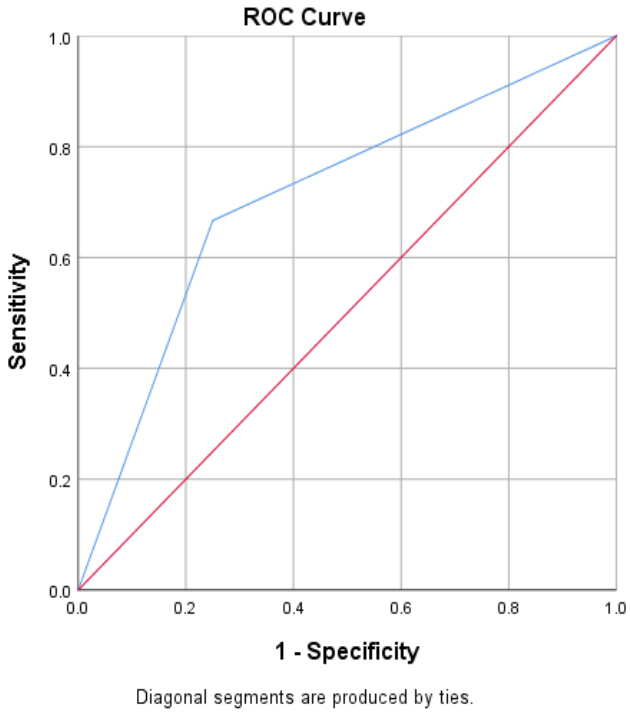

Fig1: ROC curve based on sensitivity and specificity of EGFR expression

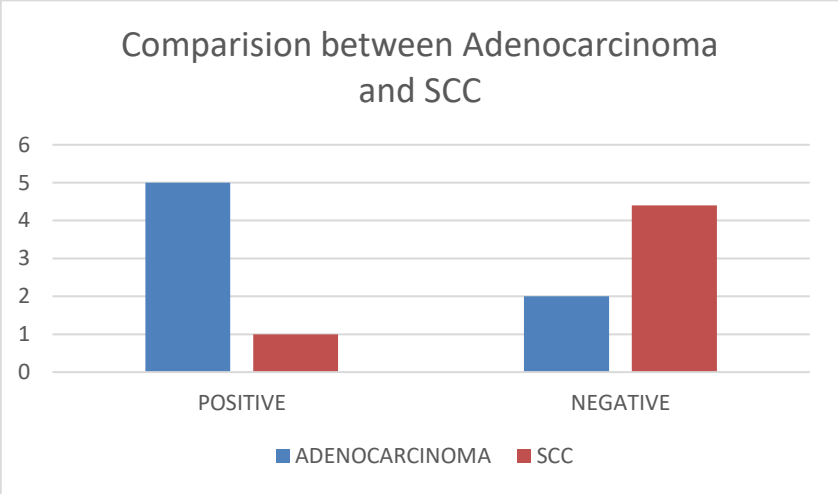

Fig2: EGFR expression in ADC and SCC

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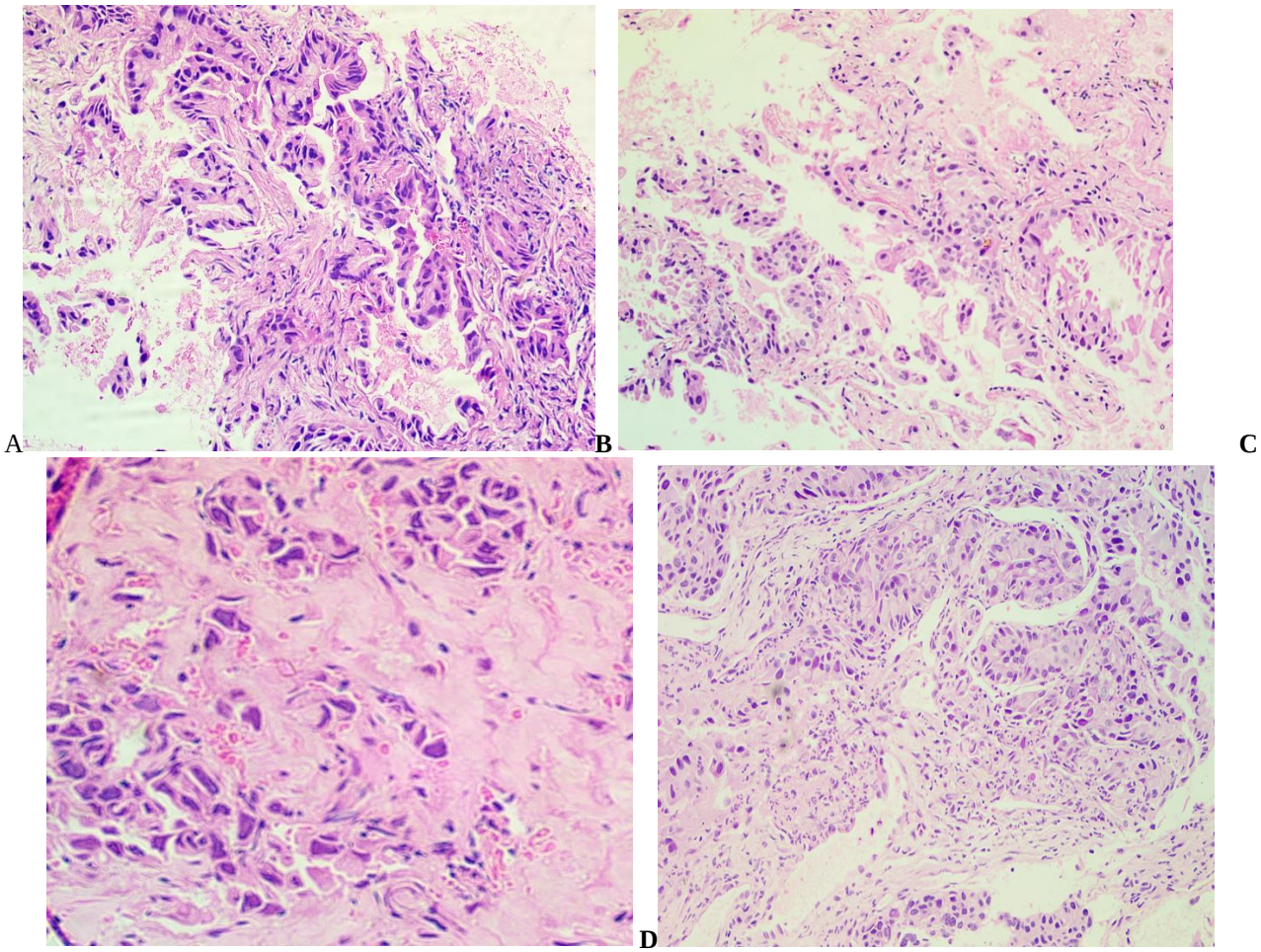
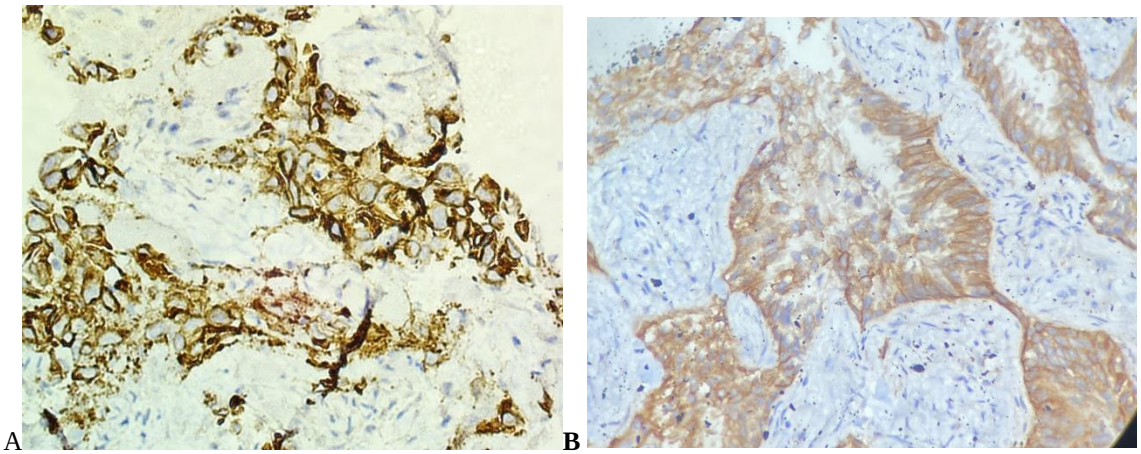


Fig3: Representative example of NSCLC histopathology. (A)Adenocarcinoma at a magnification of 100X;(B)Lepidic type of adenocarcinoma at a magnification of 100X;(C)Squamous cell carcinoma at a magnification of 400X;(D)Not otherwise specific type at a magnification of 400X;



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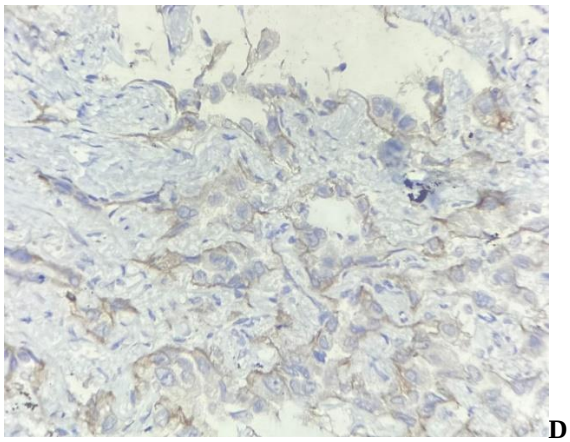
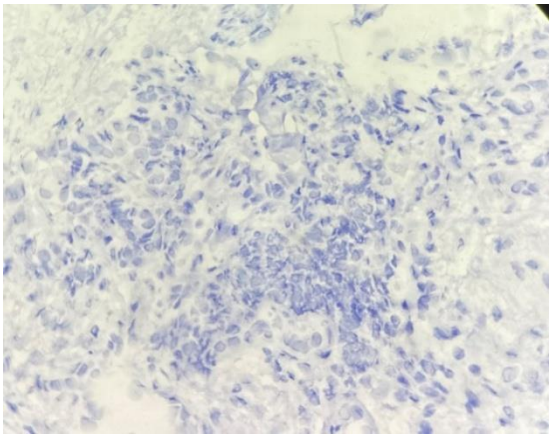
C  D 

Fig 4: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).