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Expression of EGFR in non small cell lung carcinoma

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Background: The incidence and mortality associated with lung cancers are increasing at an alarming rate. Studies have shown that activation of Epidermal Growth Factor Receptor (EGFR) triggers the tumorigenesis in these cancers. The potential role of analyzing EGFR expression in these carcinomas could go a long way in devising screening tools for early detection of lung carcinoma. This study was carried out to evaluate the prevalence and factors associated with EGFR expression. **Methods:** This cross sectional study was carried out among 75 paraffin block specimens of lung carcinoma received in our tertiary care center for a period of five years. Three micron thick paraffin sections were cut and Hemotoxylin & Eosin staining was done. All the adenocarcinoma cases were immunostained with EGFR antibody and the results were analyzed. **Results:** Majority of the tumors were moderately differentiated (46%) and were negative for lymph node metastasis (69%). With regards to EGFR positivity, majority of the tumors showed EGFR expression 3+ (57.3%) flowed by 2+ (16%). Among the EGFR negative cases, ALK expression was positive in 5% of the cases. **Conclusion:** The present study has reinforced the fact that Indian patients have high expression of EGFR and therefore can be benefitted by targeted therapy. IHC coupled with molecular analysis would be of maximum benefit to patients with EGFR & ALK mutations.

Keywords: EGFR, Immunohistochemistry, Lung cancer, Tyrosine kinase

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Introduction

Lung carcinoma is the leading cause of cancer deaths in the world [1]. It is the most common carcinoma among males and is associated with a high mortality rate.The five year survival rate for male patients with lung carcinoma is around 6% to 14% and for female patients with lung carcinoma it ranges between 7% and 18%.

The number of estimated deaths due to lung carcinoma is 1.38 million per year [2]. The carcinoma of lung is broadly classified into Non-Small Cell Lung Carcinoma (NSCLC) which accounts for 85% and Small Cell Lung Carcinomas (SCLC) which accounts for about 15% [3].

Extensive research on the pathophysiology of lung carcinoma has suggested the existence of two distinct molecular pathways, one associated with smoking and activation of the KRAS oncogene and the other not associated with smoking but with activation of epidermal growth factor receptor (EGFR). Introduction of targeted therapy with epidermal growth factor receptors, the tyrosine kinase inhibitors, has revolutionized the treatment of adenocarcinoma. Patients with these tumors survive significantly longer with EGFR tyrosine kinase inhibitor therapy than with conventional chemotherapy [4-8].

Somatic mutations within the tyrosine kinase catalytic domain of EGFR leads to conformational changes that promote permanent active status and are found in approximately 20% of lung adenocarcinomas [9-10]. They are considered to be the most reliable predictors of response to EGFR tyrosine kinase inhibitors [11].

EGFR inhibitors can be mainly categorized into two classes: monoclonal antibodies against the extracellular domain of EGFR, such as cetuximab; and small-molecule tyrosine kinase inhibitors that target the kinase domain, such as erlotinib and gefitinib [12-18]. Sahoo et al observed that 51.8% of the NSCLC population had EGFR mutations in their DNA[19]. This suggested that high prevalence of EGFR positivity is seen in NSCLC in the Indian subpopulation therefore providing opportunities for targeted therapy.

Methodology

Study setting and study participants: This cross-sectional study was carried out in the Department of Pathology of our tertiary teaching institution for a period of four years. All the lung carcinomas diagnosed during the study period constituted the study population. A total of 75 specimens were taken up for the study.

Inclusion criteria: All the histopathologically confirmed new cases of lung adenocarcinoma included.

Exclusion criteria

01. Other histological subtypes of lung carcinomas were not included in the study group.
02. Metastatic carcinomas to lung were not included in the study.

Sampling technique: The specimens were taken up for the study by convenient sampling

Ethical approval: Approval was obtained from the Institutional Ethics Committee prior to the commencement of the study.

The study was approved by the Institutional Review Board of Sri Ramachandra University (IRB No. CSP-MED/13/JUN/07/35) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Data collection: Paraffin blocks of all the cases of NSCLC were retrieved from the archives. Clinical data of the patients including demographic characteristics, metastatic status and prognosis was noted from the medical records. Threemicronthick paraffin sections were cut and Hemotoxylin & Eosin staining was done. The NSCLC casesin for which sub typing was difficult were further immunostained for TTF1 and p63 and the tumors were subtyped. Immuno-histochemical staining for TTF1 was performed using the Biogenex monoclonal TTF1 mouse antibody; clone (BGX397A) diluted in phosphate buffered saline (PBS). Staining for p63 was performed using Biogenex monoclonal p63 mouse antibody; clone (4A4) diluted in PBS. Subtyping of NSCLC into adenocarcinoma and squamous cell carcinoma was done based on the morphology and IHC.

The algorithm followed by the International Association for the Study of Lung Cancer/ American Thoracic Society/ European Respiratory Society (IATC/ATS/ ERS) International Multidisciplinary Team was used.All the adenocarcinoma cases were immunostained with EGFR antibody and the results were analyzed.

Immunohistochemical staining for total EGFR protein was performed using the Biogenex monoclonal EGFR rabbit antibody; clone (Tyr p1068) prediluted in PBS on both control and test sections according to the 0 manufacturer's instructions.

Assessment of Immunohistochemical staining: Initially, slides were scanned at 10xmagnification to obtain a general impression of the overall staining pattern of the tumor cells.H score was performed for which 10 fields were chosen randomly at 400 x magnification and the intensity of staining of EGFR was scored on a scale of 0 to 3 based on the staining of the cell membrane of the tumor cells. (Table 1)

Table-1: H score for intensity of staining of EGFR[20].

S. No	Score	Description
1	0	No staining or Faint staining in <10% of tumor cells
2	1+	Faint staining in >10% of tumor cells
3	2+	Moderate staining
4	3+	Strong staining

The total number of 100 cells in each field was counted and the number of cells stained for each level of intensity was counted. A H-score between 0 and 300 was obtained where 300 was equal to 100% of tumour cells stained strongly (3 +). A cutoff Hscore of above 100 was used to categorize tumors as positive,and tumors with scores of 100 and less were categorized as negative [20]. Squamous cell carcinoma of cervix was used as a positive control.

Data analysis: Data was entered and analyzed using SPSS ver 20 software. Chi square test was used to analyze the statistical significance between EGFR expression and clinicopathological parameters. A p value <0.05 was considered statistically significant.

Results

A total of 111 specimens of lung cancer were received during the study period. Among them, majority constituted non small cell carcinoma (95%). Among the non small cell carcinoma, majority of the cases were adenocarcinoma (75) followed by squamous cell carcinoma (27) (Figure 1).

Majority of the participants were > 60years of age in this study. Males were predominant (72%) compared to females. Most tumors were present in the upper lobe (57.3%) followed by lower lobe (32%). Majority of the cases were smokers (64%) (Table 2).

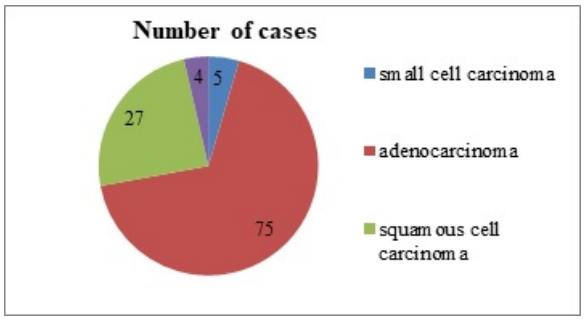

Figure-1: Distribution of cases according to the type of carcinoma:

Table-2: Background characteristics of the study participants.

S. No	Characteristics	Frequency N = 75	Percentage (%)
1	Age (in years)		
	0-30	0	0.0
	31-40	3	4.0
	41-50	8	10.7
	51-60	19	25.3
	61-70	32	42.7
	71-80	9	12.0
	81-90	4	5.3
2	Gender		
	Males	54	72.0
	Females	21	28.0
3	Location of the tumor		
	Upper lobe	43	57.3
	Middle lobe	5	6.7
	Lower lobe	24	32.0
	Multiple	3	4.0
4	Smoking history		
	Smoker	8	11.0
	Non smoker	48	64.0
	Not known	19	25.0

Table-3: Histological features of adenocarcinoma:

S. No	Characteristics	Frequency N = 75	Percentage (%)
1	Grading of adenocarcinoma		
	Differentiated	20	27.0
	Moderately differentiated	35	46.0
	Poorly differentiated	20	27.0
2	Presence of lymph node metastasis		
	Positive	23	31.0
	Negative	52	69.0
3	Presence of distant metastasis		
	Metastasis	11	15.0

	No metastasis	64	85.0
4	Correlation between scores of EGFR positivity		
	0+	9	12.0
	1+	11	14.7
	2+	12	16.0
	3+	43	57.3
5	Correlation of ALK in EGFR negative cases (n=55)		
	Positive	3	5.0
	Negative	19	35.0
	Not done	33	60.0

Table-4: Association between EGFTable 4: R expression and distant metastasis:

S. No	Characteristics		Number of cases	EGFR IHC		P value
				(+)	(-)	
1	Distant metastasis	Present	10	10	0	0.041*
		Absent	65	45	20	

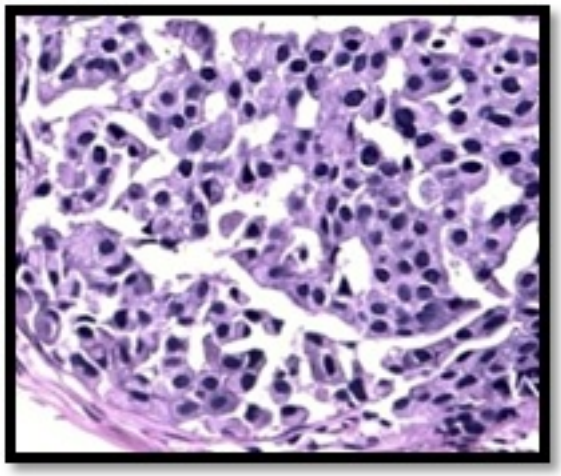

Figure-2: Moderately Differentiated adeno carcinoma 200X

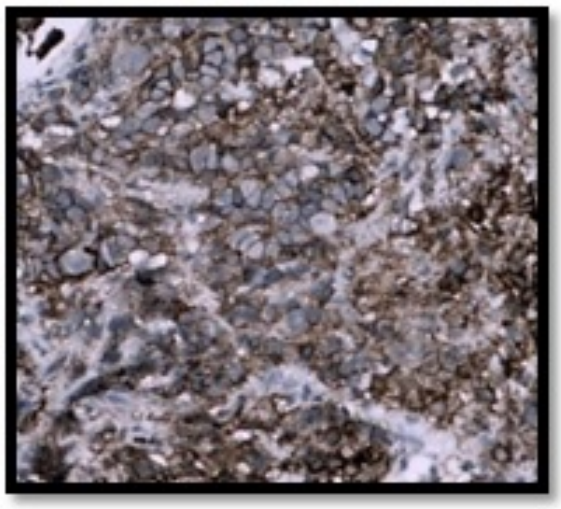

Figure-3: EGFR IHC Staining showing 1+ Positivity 200x

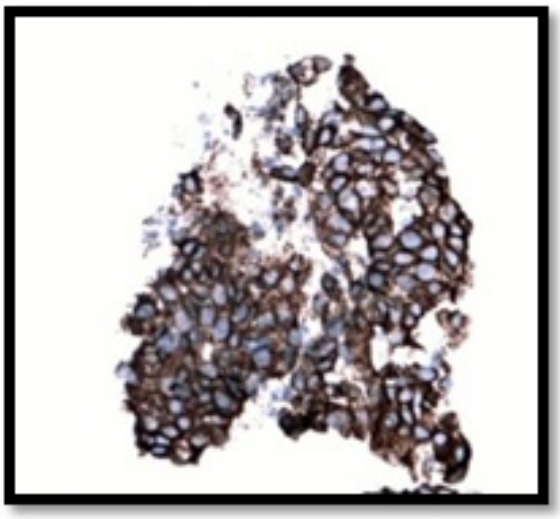

Figure-4: EGFR IHC Staining showing 2+ Positivity 200x

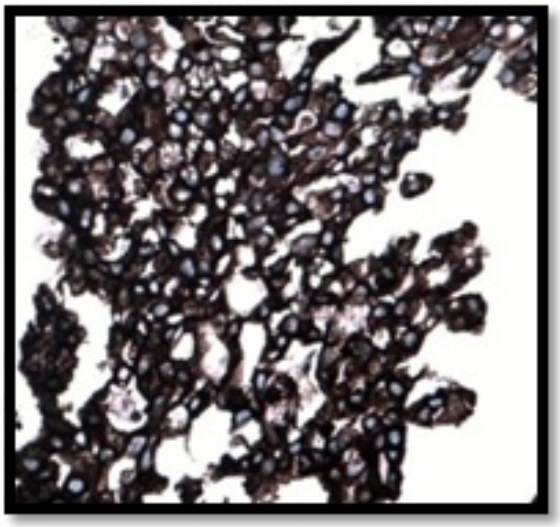

Figure-5: EGFR IHC Staining showing 3+ Positivity 200x

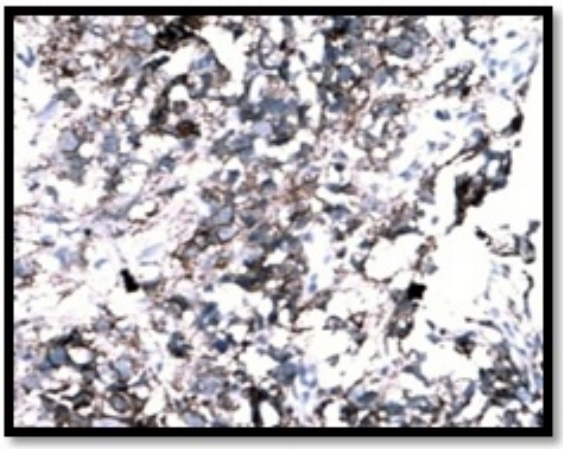

Figure-6: ALK IHC showing 1+ Positivity 200x

The histological features of the adenocarcinoma are depicted in table 3. Majority of the tumors were moderately differentiated (46%) and were negative for lymph node metastasis (69%). With regards to EGFR positivity, majority of the tumors showed EGFR expression 3+ (57.3%) followed by 2+ (16%). Among the EGFR negative cases, ALK expression was positive in 5% of the cases. (Table 3)

This study showed a statistically significant association between EGFR positivity and presence of distant metastasis ($p<0.05$) (Table 4) The immunohistochemical staining demonstrating EGFR expression are depicted in Figures 2-6.

Discussion

Lung carcinoma is the leading cause of cancer related mortality.It accounts for approximately 1.4 million deaths per year worldwide.Increasing Knowledge about the pathogenesis of lung carcinomas especially adenocarcinoma has led to the development of newer therapeutic strategies.In the present study NSCLC accounted for 95% of the lung cancers and adenocarcinoma constituted the major histologic subtype. This was in concordance with worldwide incidence [2]. In the present study the mean age at diagnosis was 62 years.This was similar to the findings of Noronha V et al[2] In the present study,64% of the participants with NSCLS were non smokers. Similar findings were observed in the data reported by Samet JM et al[21]. In the present study the site,laterality and grading had no significance in lung carcinoma.

The assessmentof the EGFR status in adenocarcinomas with pTyr1068 phosphorylated EGFR which is a predictive biomarker for screening the NSCLC with wild type of EGFR was done, as they are responsive to tyrosine kinase inhibitors treatment [22]. The EGFR expression was also correlated with the clinico-pathologic parameters. EGFR positivity was seen in 73% of adenocarcinomas which is in concordance with the study done by Wang et al[22] The EGFR positivity was more common in the 6th decade and in males (74%).

EGFR was positive in 87% of smokers and 71% ofnon smokers. EGFR positivity was seen 100% in patients who had lesions in the middle lobe and in patients who had lesions in both the lungs.

Poorly differentiated adenocarcinoma expressed more EGFR positivity (85%) compared to the other grades.In patients who had lymph node metastasis, EGFR was positive in 87%. There was a statistically significant correlation between EGFR positivity and presence of distant metastasis ($p<0.005$) and this feature is in concordance with the study done by Welsh et al [23]. The possible hypothesis in support of this is related to the delayed presentation of EGFR which independently increases the risk for metastasis [23].

In the present study, the semi quantitative expression of EGFR positivity was also observed and it was found that 43 cases (57%) showed EGFR staining intensity corresponding to 3+,12 cases (16%) showed EGFR staining intensity of 2+,11 cases (15%) showed EGFR staining intensity of 1+, and the remaining 9 cases (12%) showed 0 staining.Comparison was made based on the present scoring with the study done by Liang et al,in which they found that 45.8% of the cases showed EGFR levels of 3+,22.6% cases showed 2+ positivity, 17.3% cases showed EGFR levels of 1+, the remaining 19 cases showed level 0 staining,and their findings were similar to the present study [20].

Rosell et alstudied EGFR mutations in 350 cases and they found that in women mutations constituted 69.7%, which was similar to the present study where EGFR mutations in women constituted 71.4%. In their study mutations in non smokers constituted 66.6%, which was similar to the present study.It was observed that non smokers expressed 71.4% of EGFR positivity.On the whole in their study EGFR positive adenocarcinomas constituted 80%,which is concordant with the present study were EGFR expression constituted73% of our cases [24]. In a study done by Fred R.Hirsch et al, the scores in adenocarcinoma was also in concordance with the present study [25].

The presence of KRAS, HER2, BRAF, PI3K, LKB1 and SHP2 mutations is supposed to be associated with a lack of response to EGFR tyrosine kinase inhibitors in the treatment of lung cancer.Hence additional mutational analysis of genes other than EGFR may be required to improve patient selection for EGFR targeted therapies. The EGFR mutation status is best determined by fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) and protein expression determined by IHC with mutationspecific antibodies.

Ruschoff et al evaluated the inter-observer reproducibility of the EGFR IHC scoring system based on both the tumor cell membrane staining intensity (graded 0 to 3+) and the percentage of cells staining at each level of intensity, as was done in the present study.This allowed a highly reproducible allocation of NSCLCs into clinically relevant high or low EGFR expression groups [26] Determination of H score and a cut off value as suggested by Liang et al was useful in determining clear cut positive and negative groups[20]. In the present study EGFR expression was high (73%), which emphasizes the need and importance for IHC screening of Indian patients with adenocarcinoma lung.

It paves way for targeted therapy when combined with subsequent molecular techniques for specific EGFR mutation analysis. This adds more value to the treatment of lung adenocarcinoma patients.The antibody used in the present study pTyr1068 has been proposed to be a predictive biomarker for screening the population for clinical outcomes of EGFR-TKIs treatment,especially for patients with wild type EGFR [27-30].

It was further studied that the ALK positivity in the EGFR negative patients as EML4-ALK fusion has been observed to be present in adenocarcinoma patients lacking EGFR mutation [31]. It was observed ALK positivity in 5% of the EGFR negative cases, which was similar to the literature published[32-34].The single case which showed ALK positivity had a low level of expression (1+), however was similar to the study done by P. Hofman et al[35].This expression pattern has been explained due to the fact that ALK rearrangements in lung carcinoma are usually associated with low levels of ALK expression in contrast to anaplastic lymphomas with ALK rearrangements where the protein is robustly detected by immunohistochemistry[36].

Limitations

- Treatment details of the study cases have not been analysed.
- Molecular studies have to be performed for specific EGFR mutation

Conclusion

Introduction of targeted therapy with EGFR and ALK TKI has revolutionized the treatment of adeno-carcinoma. Patients with these mutations survive significantly longer with TKI therapy than with conventional chemotherapy.

EGFR expression was seen in 73% of the cases studied; reinforcing the fact that our subset of patients have high expression of EGFR and therefore can be benefitted by targeted therapy. There was a significant correlation between EGFR positivity and adenocarcinoma patients who had distant metastasis, proving that EGFR expression is more in advanced cases. IHC coupled with molecular analysis would be of maximum benefit to patients with EGFR& ALK mutations.

What the study adds to the existing knowledge?

The present study has reinstated the need for molecular analysis and immunohistochemical evaluation of EGFR expression for effective management of adeno-carcinoma.

Authors' contribution

Dr. Prema Devi–Conceptualization, manuscript writing

Dr. Sai Shalini – Data collection and analysis

Dr. Prathiba D –Manuscript editing and literature review

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