Original Article

Epidermal Growth Factor Receptor Mutation Status in Non Small Cell Lung Carcinomas in Lung Biopsies

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ABSTRACT

Background: With an estimation of approximately 1.6 million deaths and 1.8 million new cases per year, lung cancer is the leading cause of cancer-related death and represents the most common cancer type worldwide

Aim : The aim of the present study was to evaluate EGFR mutation status in NSCLC in lung biopsies for successful targeted therapies.

Materials and Methods: Lung biopsies of patients received in the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences during a period of December 2019 to May 2021 will be studied prospectively. All lung biopsies are fixed in 10% neutral buffered formalin and routinely processed and stained with hematoxylin and eosin (H & E).

Results: Out of 30 cases of NSCLC cases, 24 cases (80%) were diagnosed as adenocarcinoma and 6 cases (20%) as squamous cell carcinoma on histopathological examination. The age and sex distribution, correlation with presenting complaints, histopathological and IHC findings with EGFR, Exon mutation status were studied separately for NSCLC.

Conclusion: We observed that Adenocarcinoma in female patients with exon 19 mutation have a favourable response to oral Tyrosine Kinase Inhibitors.

Keywords: Epidermal growth factor receptor, adenocarcinoma, exon mutation status, lung biopsies

INTRODUCTION

With an estimation of approximately 1.6 million deaths and 1.8 million new cases per year, lung cancer is the leading cause of cancer-related death and represents the most common cancer type worldwide. [1] Historically, the practical utility of non-small cell lung cancer(NSCLC) as a clinical entity reflected the lack of distinct treatment regimens for the different histological types encompassed by this term. The advent of successful molecular targeted therapies, directed at specific cell types and sub types, has increased the need for a more specific sub typing of these cancers. [2,3,4] Over the past two decades, adenocarcinoma has replaced squamous cell carcinomas as the most common sub type of NSCLC. [5,6,7]

Recent studies have shown that somatic mutations in the EGFR tyrosine kinase domain in patients with lung adenocarcinoma are associated with sensitivity to EGFR tyrosine kinase inhibitors (TKI) Gefitinib and Erlotinib. Screening for these mutations in patients with NSCLC can be used to predict the patients responding to TKIs. [8]

Hence, the present study is being conducted to study the histopathological spectrum of NSCLCs and correlation of EGFR mutation status by PCR-gene sequencing method.

MATERIALS AND METHODS

Lung biopsies of patients received in the department of pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar during a period of December 2019 to May 2021 will be studied prospectively. All lung biopsies are fixed in 10% neutral buffered formalin and routinely processed and stained with hematoxylin and eosin (H & E).

Immunohistochemistry (IHC) was performed for confirmation and subtyping of Non small cell lung carcinomas by using TTF-1, Napsin-A (Adenocarcinoma) and P40, P63 (Squamous cell carcinoma) and CK7&CK20 (Metastatic carcinoma).

Paraffin embedded tissue blocks diagnosed as Non small cell lung carcinoma (Adenocarcinoma and Squamous cell carcinoma) with maximum amount of tumor after correlating histopathologically will be sent for detection of EGFR mutation status. Statistical proportions will be used to analyse the collected data.

Inclusion Criteria

- Histologically proven primary NSCLC patients with metastasizing NSCLC to other sites
- >18 years of age
- Both males and Females

Exclusion Criteria

- Small cell lung carcinomas
- Inadequate biopsies (Biopsies with extensive necrosis and scanty or no viable tumor)
- Lobectomy specimens

During the period of December 2019 to May 2021 the patients visiting Pulmonology, General Medicine Department and Cardiothoracic surgery OPD at Chalmeda Anand Rao Hospital in whom the clinical features were recorded in case proforma and lung biopsies were taken from mass lesions diagnosed by imaging modalities.

Methods of Immunohistochemistry : Two-step indirect technique.

Principles of the Procedure

The technique is based on the detection of antigens in the cells and tissues with the help of two-step process:

1. Specific epitopes are used to bind the primary antibody.
2. Followed by calorimetric reaction, which detects the antigen antibody binding.

EGFR Mutational Analysis-PCR followed by gene sequencing for detection of EGFR mutation status, paraffin embedded tissue blocks diagnosed as NSCLC with maximum amount of tumor after correlating with H & E and IHC were sent to Oncquest Laboratories Limited (Hyderabad).

RESULTS

A sample size of 30 cases were studied. The samples included lung biopsy specimens obtained by USG, CT and bronchoscopic guidance. The study period was conducted from December 2019 to May 2021.

Out of 30 cases of NSCLC cases, 24 cases (80%) were diagnosed as adenocarcinoma and 6 cases (20%) as squamous cell carcinoma on histopathological examination. The age and sex distribution, correlation with presenting complaints, histopathological and IHC findings with EGFR, Exon mutation status were studied separately for NSCLC.

Table 1: Age distribution of cases

Age	Frequency	Percentage%
<40 years	2	6.7
40-49 years	6	20
50-59 years	8	27
60-69 years	9	30
70-79 years	4	13.3
>80 years	1	3
Total	30	100.0

In age wise distribution of cases, highest number of cases-9(30%) were seen in the age group of 60-69 years followed by 50-59 years of age group -8 (27%) (Table 1).

Maximum number of mutations were seen in females in exon 19-5(16.70%) followed by males 2(6.70%). Maximum number of mutations were seen in males in exon 21-3(10.0%) followed by female -1(3.30%) and there were no mutations in exon18 (Table 2). Maximum number of mutations were seen in adenocarcinoma in exon 19-6 (85.7%) followed by exon 21-1(14.3%).

From above logistic regression it was observed that, mutation was likely to occur 0.178 times higher in females as compared to males, similarly nonsmokers had 0.755 times higher risk than smokers and Adenocarcinoma was 0.556 times higher risk than squamous cell carcinoma. (Table 3)

Table 2: Comparison of Gender with Exon mutated

Gender	Exon			Total	Chi-Square	P-value
	Exon 18	Exon 19	Exon 21			
Male	0	2	3	17		
	0.00%	6.70%	10.00%	56.70%		
Female	0	5	1	13	2.493	0.309
	0.00%	16.70%	3.30%	43.30%		
Total	0	7	4	30		
	0.00%	23.30%	13.30%	100.00%		

Table 3: Comparison of histopathological type with Exon mutated

Gender	Exon			Total	Fisher Exact	P-value
	Exon 18	Exon 19	Exon 21			
Adeno CA	0	6	1	7		
	0.00%	85.7%	14.3%	100%		
Squamous cell CA	0	1	1	2	3.286	0.202
	0.00%	50%	50%	100%		
Total	0	7	2	9		
	0.00%	77.8%	22.2%	100%		

Table 4: Logistic regression analysis for association of EGFR mutational status as an independent variable with gender, smoking habits and adenocarcinoma histology.

Parameters	B	Odds Ratio	95% Confidence interval		P-value
			Lower	Upper	
Female	-1.215	0.297	0.051	1.739	0.178
Non-Smokers	-0.404	0.668	0.053	8.388	0.755
Adeno	0.587	1.799	0.148	21.815	0.556

DISCUSSION

In the present study the mean age of adenocarcinoma patients with EGFR mutation was 54 years with a non significant p-value which correlates with study done by Antonio Marchetti et al. [9] 46.15% of females and 17.6% of males with adenocarcinoma had EGFR mutations which correlates with study done by Hisayuki et al [10] and Takayuki Kosaka et al. [11] The low frequency and non significant p-value may be attributed to small study population in the present study (Table 4).

In the present study 30% of cases had EGFR mutation which is close to the study done by Tarigopula et al [12] (Table 6). In the present study most common exon mutated was exon 19 followed by exon 21 which was in concordance with other studies (Table 5).

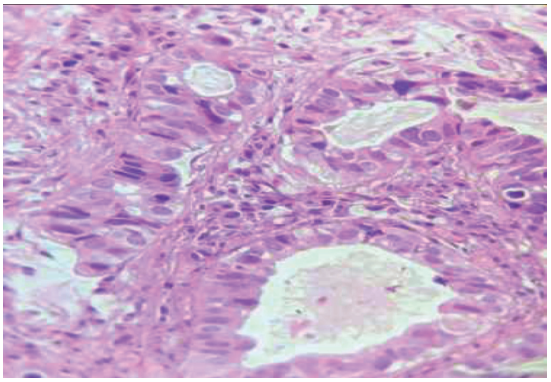

Figure 1: Section shows Acinar Adenocarcinoma (40X)

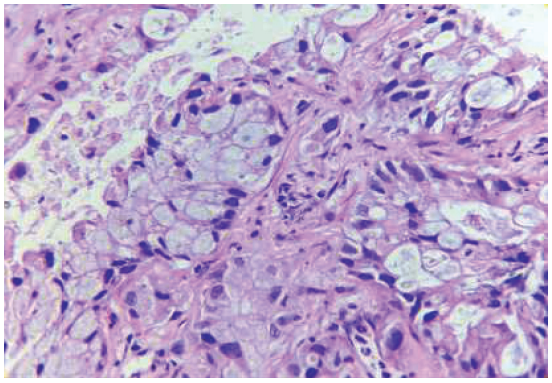

Figure 2: H&E, Section shows Invasive Mucinous Adenocarcinoma (40x,)

Table 5: Comparison of EGFR mutation detection rate

S.No	Studies	Detection Rate
1.	Tarigopula et al [12]	23%
2.	Chantharasamee et al [13]	47%
3.	Yuan Ch et al [14]	60.23%
4.	Present study	30%

Table 6: Comparison of Frequency of Exon mutated

S.No	Studies	Detection Rate
1.	Tarigopula et al [12]	Exon 18=0 Exon 19= 21.3% Exon 20=0 Exon 21=11%
2.	Chougule A et al [15]	Exon 18= 7% Exon 19= 50% Exon 20=0 Exon 21=42%
3.	Present study	Exon 18=0 Exon 19= 77.8% Exon 20=0 Exon 21=22.2%

In the present study 32% of non-smokers with adenocarcinoma had an EGFR mutation which correlates with other studies. The EGFR mutations were present more commonly in non-smokers (Table 7).

The association of EGFR mutations, as a dependent variable with tumor histology, gender and smoking habits was evaluated by logistic regression analysis. EGFR mutations were not found to be independently associated

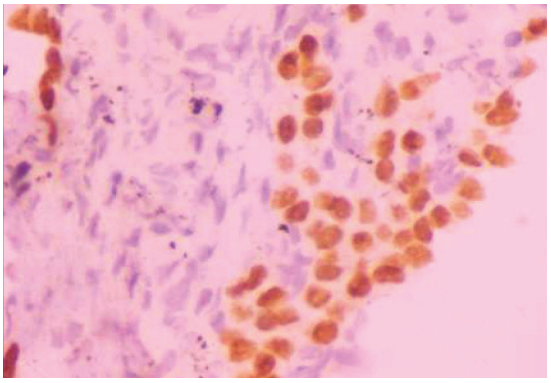

Figure 3: TTF-1 nuclear expression in adenocarcinoma (10X)

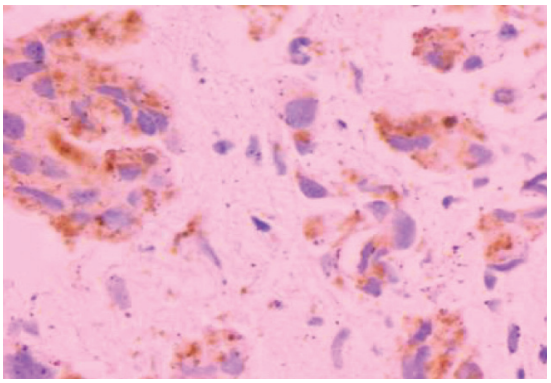

Figure 4: NAPSIN A cytoplasmic expression in adenocarcinoma (10X)

with any of the variables.

In the present study as compared to other studies by Takayuki et al.[10] Antonio M et al. [9] and Masaki Tokumo et al who concluded that EGFR mutations were independently associated with one, two or all of the variables (absence of smoking history, female gender and Adenocarcinoma histology).

CONCLUSION

Translation of the molecular biology underlying NSCLC into the clinical decision making process in treating NSCLC patients has advanced in the last few years. In the present study EGFR mutations were found in NSCLCs and were significantly expressed in adenocarcinoma in female patients and in non-smokers. EGFR mutations do constitute a distinct heterogeneous group with differential sensitivity and varied responses to treatment. We

Table 7: Comparison of Smoking habits with EGFR mutation in NSCLC

Studies	Mutated	Non-Mutated	P-value
Takayuki Kosaka et al [16]	Smokers=22% Nonsmokers=66%	Smokers=83% Nonsmokers=34%	<0.001
Antonio Marchetti et al [9]	Smokers=41% Nonsmokers=59%	Smokers=59% Nonsmokers=41%	
Hisayuki et al [17]	Smokers=10% Nonsmokers=51%	Smokers=10% Nonsmokers=51%	0.02
Present study	Smokers=20% Nonsmokers=32%	Smokers=80% Nonsmokers=68%	0.593

observed that Adenocarcinoma in female patients with exon 19 mutation have a favourable response to oral Tyrosine Kinase Inhibitors.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

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