ORIGINAL RESEARCH PAPER

Pathology

MUTATIONS IN PULMONARY ADENOCARCINOMA. FREQUENCY STUDY FROM A ONCOLOGY CENTRE IN CENTRAL KERALA.

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ABSTRACT

Background: Non small cell carcinomas of lung, notably adenocarcinoma is associated with genetic mutations in EGFR (chromosome 7) , ALK (chromosome 2), ROS1(chromosome 6) . The patients harbouring these mutations are greatly benefited from tyrosine kinase inhibitor targeted chemotherapy. The prevalence of these mutations in central kerala has not been studied and documented.

Aim: This study aims to analyse the frequency of different mutations in lung adenocarcinomas presenting to a oncology centre in kerala.

Study Design: Descriptive study.

Materials And Methods: The study spanned over a period of two years from 2019-2021. A total of 169 consecutive lung adenocarcinomas were studied.
PCR for EGFR was done in 89 cases and were tested for the common mutations. ALK1 IHC using ALK-D5F3 clone and ROS with ROS-D4D6 clones were done in 40 cases.

Results: EGFR mutation was present in 15 cases (17.44%). The most common age range was 40-60yrs. Two most common patterns were solid and acinar. ALK and ROS 1 mutation was found in 3 cases (7.5%) and 2 cases (5%) respectively. 4 cases (16.66%) of EGFR mutant lung adenocarcinoma patients had metastasis.

INTRODUCTION

Lung cancer is considered to be one of the leading causes of cancer-related deaths worldwide with more than 1 million cases diagnosed every year [1].

Non small-cell lung cancer (NSCLC) includes 3 major cell types (adenocarcinoma, squamous cell carcinoma and large cell carcinoma), and these can be further divided into various subtypes or variants. NSCLC accounts for most (~85%) of the lung cancers, with lung adenocarcinoma being the most common subtype [1,2].

Major advances in understanding the pathogenesis of NSCLCs, have led to the identification of molecular pathways, which promotes tumor growth. One among them, is the discovery of epidermal growth factor receptor (EGFR). EGFR is a transmembrane receptor tyrosine kinase protein that is expressed in most of the normal epithelial, mesenchymal, and neural tissue. Mutations in EGFR has shown increased downstream signalling. Thereby promoting cell proliferation, differentiation and growth [3,4] .EGFR TKI targeted therapies have significantly changed the outlook on this subset of patients. [5]

EGFR mutations are commonly detected in adenocarcinomas. 90% of mutations have been deletions noted in exon 19 [3,4]. When these EGFR mutated patients are treated with tyrosine kinase inhibitors like gefitinib and erlotininb, nearly all respond compared to patients without mutation. This mutation has not been demonstrated in smokers [6].

Anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (ROS1) are the other mutations frequently found in NSCLC. Frequency of ALK rearrangement in earlier studies is around 3-7% of NSCLC [1,7] and ROS 1 being approximately around 1-2% of NSCLC [7,8]. Identifying the above mutations are very important as these patients are likely to benefit from targeted therapy. Those who are positive for ROS1 mutations are treated with crizotinib [8].

MATERIALS AND METHODS

Lung biopsies of 169 patients were retrieved from the archives of the department of pathology, at Amala institute of medical sciences, Thrissur from January 2019 to January 2021.

Study Inclusion Criteria
- Lung biopsies
- Biopsies from metastatic sites such as lymph nodes, bone, pleura etc. Which were proved to be of pulmonary origin by IHC markers.

Study Exclusion Criteria
- Primary Lung carcinomas other than adenocarcinoma.
- Carcinoma / sarcoma metastasis to lung from other sites.
- Cases where slides and paraffin blocks were not available for review.

Histological Examination

All specimens are fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with H&E. The sections were classified according to the 2015 WHO Classification of lung tumors by experienced pathologists. We also graded the tumors into well differentiated (>95% glands), moderately differentiated (50-95% glands) and poorly differentiated (< 50% glands) depending on the extent of glandular differentiation using the system in endometrial cancers [1]. IHC for lung adenocarcinoma panel was done in 25 cases to aid the H& E examination.

Reagents And Equipments

Immunochemistry with p40, TTF1, Napsin A was performed on unstained tissue sections of 4 microns each using poly-L-lysine coated slides on automated immunostainer(Ventana Benchmark GX).

PCR For Epidermal Growth Factor Receptor Mutation Detection

PCR for EGFR was done in 89 confirmed adenocarcinoma cases. Paraffin embedded tissue blocks were sent for

mutation analysis. Tissues were tested for the common mutations described in lung cancer for example exons 19, exon 20 and exon 21.The remaining cases it was not done due to insufficient tissue not available for testing.

Immunohistochemistry For ALK & ROS

IHC was performed for 40 cases of adenocarcinoma to detect expression of ALK and ROS. This was outsourced to a NABL accredited laboratory (MedGenome). Clones used were ALK-D5F3 (Ventana) and ROS-D4D6 (cell signalling technology).

RESULTS
Epidermal Growth Factor Receptor Mutation. (Table1)

A total of 86 cases were studied for EGFR mutation.

In our study incidence of EGFR mutation, was noted in 15 patients (17.44%). Amongst those with the mutation, females showed slight preponderance than males . Majority of the positive cases were noted in the age group between 40-60, which were 9(60%) and 6 (40%) of them were above 60years.

Correlating Histoilgical Grade And Patterns With Epidermal Growth Factor Receptor Mutation.(table 1)

In our study, 8 cases (53.3%) were histological grade II, 6 (40%) with grade III histology and 1 case showed grade I histology.

In EGFR positive cases, acinar being the most frequent pattern in 8 cases (53.3%) followed by solid in 4 cases(26.6%), lepidic in 2 cases (13.3%) and 1 case with papillary pattern (6.6%).

Among the different mutation analysis performed, our study group showed Exon 19 deletions to be the most frequent which was found in 10/15 cases (66.6%) followed by exon 21 mutation in 3/15 cases (20%) and 2 cases (13.3%) showed exon 20 deletions.

Table 1. Frequency Of EGFR Mutation

	EGFR PCR for Exon 19/exon 20/exon 21	
	Positive (%)	Negative(%)
Frequency	15 (17.44)	71 (82.5)
Gender		
Male	7 (46.6)	48 (67.6)
Female	8 (53.3)	24 (33.8)
Age		
<40	0	2 (2.8)
40-60	9 (60)	26 (36.6)
>60	6 (40)	44 (61.97)
Grade		
WD I	1 (6.6)	6 (8.4)
MD II	8 (53.3)	41 (57.7)
PD III	6 (40)	24 (33.8)
Patterns		
Lepidic	2 (13.3)	6 (8.4)
Acinar	8 (53.3)	39 (54.9)
Mixed	0	0
Papillary	1 (6.6)	2 (2.8)
Solid	4 (26.6)	22(30.9)
Mucinous/signet ring	0	1 (1.4)
Micropapillary	0	1 (1.4)

ALK and ROS

ALK and ROS immunohistochemistry was performed in 40 cases of adenocarcinoma. 3 out of 40 cases showed ALK positivity and 2 out of 40 cases showed ROS 1 positivity.One case showed equivocal staining for ROS1 IHC. The rest of the cases were negative.

DISCUSSION

In this two year study of EGFR, ALK , ROS1 mutations in lung adenocarcinoma, conducted in our institute showed a predominance of females. This is in concordance with other similar studies [4,5,9,10].(Table 2). EGFR Mutation was more frequently noted in the age group of 40-60 years. The study by Matsumura M et al [11] and Ho HL et al [12] showed a higher median age of 70 yrs than any other study conducted on lung carcinoma. The reason could be attributed to the higher life span of Japanese population.

Table 2. Comparison Of Results Of Epidermal Growth Factor Receptor Mutation In The Present Study With Similar Studies

Study	Frequency in lung adenocarcinomas (%)	Age range	Median age of patients (yrs)	Males (%)	Females (%)
Present study (n=86)	17.44	30-77	50	46.6	53.3
Arafat Tfayli et al (n=205)	15.6	NA	63.4	8.8	29
Hee-Young Yoon et al (n=1020)	38.0	NA	66	39.7	60.3
J.Matthew et al (n=121)	19	NA	NA	NA	NA

Among the different Non small cell lung carcinomas (NSCLC), adenocarcinoma is most commonly tested for EGFR mutation status [9]. Bronchioalveolar carcinoma (BAC), which is a subtype of adenocarcinoma is frequently associated with EGFR mutation than other types of adenocarcinoma [9].

In the present study the well differentiated adenocarcinoma (6%) showed the least frequency of expression of EGFR. While the moderately differentiated adenocarcinoma showed the highest EGFR expression (53.3%). Poorly differentiated adenocarcinoma showed 40% expression.

Of the 86 samples tested for EGFR mutation, 15 cases had mutation (17.44%). Amongst them, the predominant histological pattern in our study is acinar (53.3%), followed by solid (26.6%), lepidic (13.3%) and papillary (6.6%). This is in concordance with the study by Gupta P et al [1]. (Figure 1-4)
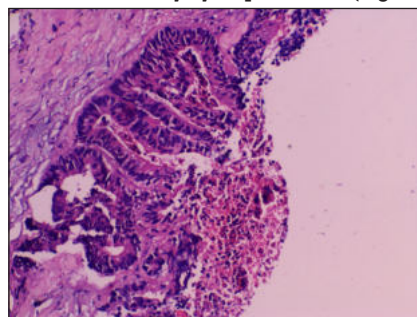

Figure 1: Glandular pattern ; H & E - 100X
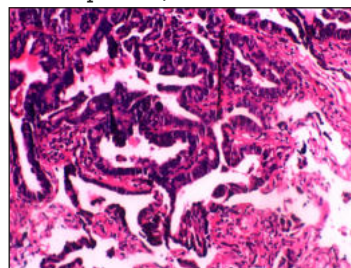

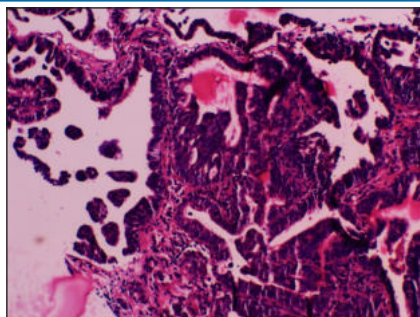**Figure 2:** Papillary pattern ; H & E- 200X

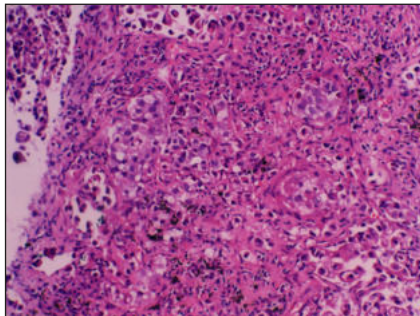**Figure 3:** Micropapillary pattern; H&E- 100X

Figure 4: Solid pattern; H&E- 400X

The major mutations analysed were deletions in exon 18,19,20,21. The commonest being Exon 19 deletions for most mutant positive patients (66.6%) followed by exon 21 mutation (20%) and 2 cases by exon 20. This was similar to other studies by Tfayli A et al [10], Ho HL et al [12] and Reinersman JM et al. [6]

The EGFR mutant lung adenocarcinomas have a better survival than the wild type, when treated with Tyrosine kinase inhibitors [4,12]. While the same study also demonstrated good prognosis in patients with E19 deletion and adverse outcomes in patients with E21 mutation.

Anaplastic Lymphoma Kinase
The frequency of ALK expression is 7.5% in our study. Song Z et al [7] in his study found the incidence of ALK to be 12.2%. (Table 3-4).

ROS1
The frequency of ROS1 is 5% in our study which is higher than the study by Bergethon K et al [13] in young never smokers .(Table 3-4). The crizotinib chemotherapy which is targeted against ALK and ROS1 mutation is beneficial for the young patients.

Table 3. Frequency Of ALK And ROS1 Mutation In Lung Adenocarcinoma.

IHC	SAMPLES TESTED	SAMPLES POSITIVE	Prevalence in present study
ALK IHC D5F3 CLONE	40	3	7.5%
ROS1 IHC D4D6 CLONE	40	2	5%

Table 4. Comparison Of Frequency Of ALK And ROS 1 Mutations.

Test	Present study (n=40)	Arafat Tfayli et al (n=205)	Z song et al (n=732)
ALK IHC D5F3 CLONE	7.5%	1.9%	12.2%
ROS1 IHC D4D6 CLONE	5%	Not studied	4.4%

Other Findings
Among our patients of lung adenocarcinoma, 24 had metatstatic disease, either distant or nodal metastases. Distant metatstatic sites were to the bone, brain etc.

Out of total of 24 cases which had metastases, 4 tested positive for EGFR mutation (16.66%) and one positive for ALK mutation which again emphasizes the importance of targeted chemotherapy.

CONCLUSION
The study conducted in lung adenocarcinoma, in our cancer research institute represents an overall picture of EGFR, ALK, ROS mutation testing, in a tertiary centre in Kerala.

Choosing to test for mutation will increase the proportion of the patients who get benefitted from specific therapy. In conclusion, the discovery of many theranostic molecular biomarkers in NSCLC has greatly changed the classification of lung cancer, and thus its management.

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