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Original Article

A study of therapy targeted EGFR/ALK mutations in Indian patients with lung adenocarcinoma: A clinical and epidemiological study

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ABSTRACT

Background: Established predictive biomarkers for Non-Small Cell Lung Carcinoma (NSCLC) include sensitizing Epidermal Growth Factor Receptor (EGFR) mutations and Anaplastic Lymphoma Kinase (ALK) fusion oncogene. The primary aim of the study is to ascertain the prevalence of EGFR mutation and ALK gene rearrangement in patients of lung adenocarcinoma in Indian population and the second objective is to impress upon the importance of adequate processing of limited tissue samples.

Methods: Histopathologically confirmed cases of lung adenocarcinoma, whose tumour had been tested for both EGFR and ALK gene mutations, were included in this study. The EGFR mutations were analyzed using PCR and Gene Sequencing. ALK fusion oncogene was found by Fluorescence In Situ Hybridization (FISH) technique using kit of Vysis LSI ALK Dual colour Break Apart Rearrangement probe.

Results: A total of 152 cases of lung adenocarcinoma were included. Out of which, 92 (60.5%) were male and 60 (39.5%) were female. After exclusion of 17 cases due to unsatisfactory result, EGFR mutations were found positive in 35.5% cases (48/135). ALK gene rearrangement was found in 7.6% (10/131) after excluding 21 cases with unsatisfactory result.

Conclusion: EGFR mutations and ALK gene rearrangement was found to be mutually exclusive. Incidence of EGFR mutations (35.5%) is much higher in Indian population than in Caucasians (13%) and is close to the incidence in East Asian countries. The 7.6% incidence of ALK fusion oncogene in Indian patients establishes the importance of molecular studies to give maximum benefit of targeted therapy to the patients.

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Introduction

Targeted therapies have given new lease of life to cancer patients. Latest to join this field of individualized therapy are patients with lung malignancy. Lung cancers are the leading cause of death all over the world. Depending on their biology, therapy and prognosis, lung cancers have been divided by the WHO in two categories, i.e. Small Cell Lung Carcinoma (SCLC) and Non-Small Cell Lung Carcinoma (NSCLC).[1] Several biomarkers have been recognized as prognostic and predictive markers for NSCLC. Established predictive biomarkers are sensitizing Epidermal Growth Factor Receptor (EGFR) mutations and Anaplastic Lymphoma Kinase (ALK) fusion oncogene.[2,3] As per the guidelines of the National Comprehensive Cancer Network (NCCN) version 5.2015, testing for EGFR mutations and EML4-ALK is recommended in the NSCLC treatment algorithm for all patients of lung adenocarcinoma.[4] The Asian population with NSCLC show much higher rate of EGFR mutations as compared to Caucasian.[5] There have not been many large studies conducted in Indian population for both EGFR and EML4-ALK mutations. Routine molecular testing of tumour samples is an important paradigm shift in NSCLC therapy and highlights the role of diagnostic faculty in obtaining adequate and representative tissue sample and its proper analysis. The primary objective of the study was to find the prevalence of EGFR mutation and EML4-ALK fusion in patients of lung adenocarcinoma in Indian population and the second objective was to impress upon the importance of proper and adequate processing of critical tissue sample for the total benefit to the patient.

Material and methods

All the lung carcinoma patients having Adenocarcinoma histology diagnosed during the period February 2013 to February 2015 and, whose tumour had been tested both for EGFR mutational status and ALK gene rearrangement, were included in this study conducted at our centre. Clinical details of the patients were taken from MDTC registry of our centre and from other treating oncophysician of Pune region. The molecular investigations were outsourced.

The EGFR mutations were tested using PCR and Gene Sequencing. Testing was done on micro-dissected cells from Formalin-Fixed, Paraffin-Embedded (FFPE) tissue blocks. The assay was done on tumour rich region of the FFPE tissue with >40–50% cancer cells. Exon 18–21 of EGFR gene was screened for mutations. Vysis LSI Dual colour Break Apart Rearrangement probe kit was used in Fluorescence In Situ Hybridization (FISH) for ALK fusion oncogene detection. It has a spectrum green (green) labelled 300 kb probe at the 5′ end and a spectrum orange (red) labelled 250 kb probe at the 3′ end of ALK. On evaluation of signals for each probe, two types of ALK rearrangement were identified (Fig. 1). One has split green and red signals with split distance being more than or equal to two signal diameters. The second pattern was loss of green signal because of deletion in the 5′ ALK region in association with 2p inversion. Minimum of 50 tumour cells were assessed and cases were termed positive for ALK rearrangement only when more than 15% of the cells revealed single red signals or split signals.[3] If no signals were detected even after repeat test, the report of ''test uninterpretable'' was issued. During the later period of study standardized immunohistochemistry (IHC) instead of FISH was used for screening of ALK gene rearrangement in few patients due to financial constrains. IHC was carried out on the Benchmark XT Autostainer, Roche Diagnostic's using Rabbit monoclonal antibody D5F3 clone, company Ventana. Optiview DAB IHC detection kit and Optiview amplification kit was used for high sensitivity. The positive and negative tissue controls were run simultaneously. Presence or absence of cytoplasmic granular staining in tumour cells was taken as positive or negative for ALK mutation.[6]

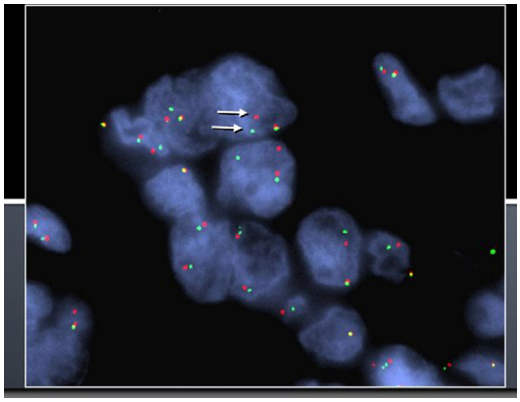

Fig. 1 – Fluorescence microscopic image (100×) showing presence of ALK rearrangement in form of break apart red and green signals.

Statistical analysis was done by using Pearson's Chi-squared or Fischer's exact test, whichever was appropriate for categorical variables. The power of study for EGFR mutation was found to be is 96.4% and for ALK mutation it was 100% at 5% level of significance using one sample formula for percentage. A two sided $P < 0.05$ was considered significant. Statistical analysis was done using IBM SPSS statistic V22.0 software.

Results

Total of 152 cases of lung adenocarcinoma were included in this study that had undergone molecular studies for EGFR mutations and ALK gene rearrangement status. Out of 152 cases, 92 (60.5%) were male and 60 (39.5%) were female with median age of 57.5 years (range 25–86 years). Baseline patients' characteristics are given in Table 1. Median age and age range in years along with distribution of genders are depicted.

The EGFR and ALK gene rearrangement status of cases are shown in Table 2. Forty-eight cases were positive for EGFR mutations out of 152 blocks analyzed. However, in 17 cases (11.2%) result could not be obtained due to scanty or improperly processed tissue. So out of 135 cases with available results the positivity rate of EGFR mutation is 35.5%. Eighty-seven patients had EGFR of wild type. EML4-ALK fusion gene found positive in 10 cases out of 152 however after taking out

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