Original Article

Feasibility of molecular testing in a multicenter study with geographical variation in India: Epidermal growth factor receptor mutation as a model molecular test

ABSTRACT

Context: Trends in epidermal growth factor receptor (EGFR) mutation based on ethnicity assist the initial selection of targeted therapy regimen. Reported incidence of EGFR mutation in Indian NSCLC patients is variable, ranging from 22% to 51.8%.

Aim and Settings and Design: This multicenter, noninterventional study evaluated the prevalence of EGFR mutation in Indian NSCLC patients, its association with patients' demographics, and for the first time used a central laboratory for molecular testing.

Subjects and Methods: Tissue samples from 252 NSCLC patients were tested at a Central Laboratory at Tata Memorial Hospital.

Statistical Analysis Used: Patient demographics, baseline characteristics including smoking status from routine examination were recorded in a single visit. Chi-square or Fisher's exact test was used for association of EGFR mutation status with gender, age, smoking status, and histological subtypes.

Results: The prevalence of EGFR mutation in Indian NSCLC patients was 23.4%. Among these, 55.9% patients had mutations in exon 19, 39% in exon 21, and 1.7% in exon 18. The incidence of EGFR mutation was higher in females than males (32.5% vs. 18.9%, respectively), and in 30.6% patients that had never smoked, 26.3% smokers, and 5.8% former smokers. The mean duration of transportation of tissue samples to the central laboratory was 48 h with an average turnaround time of 5 days for molecular testing.

Conclusions: Molecular testing at a central laboratory is a feasible option in India. Prevalence of EGFR mutation in Indian NSCLC patients was similar across western and southern centers in India. A statistically significant association between EGFR mutation and gender as well as the smoking status of the patients was observed. Majority of the patients had in-frame deletions in exon 19.

Keywords: Epidermal growth factor receptor mutation, nonsmall cell lung cancer, tyrosine kinase inhibitor, India

INTRODUCTION

Over two decades ago, molecular profiling of lung cancer led to the identification of driver mutations as oncogenic triggers in NSCLC. The incidence of epidermal growth

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Access this article online

Website:
www.asjo.in

DOI:
10.4103/ASJO.ASJO_104_16

Quick Response Code

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How to cite this article: Prabhash K, Rauthan A, Rajappa S, Desai C, Mistry R, Dutt A, *et al.* Feasibility of molecular testing in a multicenter study with geographical variation in India: Epidermal growth factor receptor mutation as a model molecular test. Asian J Oncol 2017;3:39-44.

factor receptor (EGFR) mutations has been reported to differ across ethnic groups.^[1-6] Mutations in exons 19–21 affect kinase activity which is fundamental to the activation of the receptor by autophosphorylation. Subsequently, the receptor gets blocked into a state of constitutive activation thereby signaling the cell to proliferate and resist apoptosis. The substitution at codon 858 (L858R) in exon 21 and in-frame deletions at exon 19 (del E746-A750 being the most common) are the most prevalent accounting for approximately 85%–90% of all EGFR mutations.^[7,8] Insertions in exon 20 and substitution at codon 719 (G719S) in exon 18 are rare, accounting for about 5% of the mutations.^[9]

These deeper understandings of the molecular pathways affected by these genetic mutations have heralded an era of targeted therapy. These drugs specifically target the pathways of oncogenesis mediated by EGFR hyperactivation. EGFR tyrosine kinase inhibitors (TKIs) gefitinib, erlotinib, and afatinib specifically targeting EGFR oncogenes have demonstrated an improved progression-free survival, overall survival, response rate (RR), and quality of life as well as a better tolerability when compared with first-line chemotherapy in several phase III randomized trials.^[10-16]

EGFR mutation rate is higher in Asians, women, never smokers, and patients with adenocarcinoma subtype.^[14,17] The incidence rate of EGFR mutation in Indian NSCLC patients reported in literature has been varied, ranging from 22% to 51.8%.^[18-21] Limitations of sample size and clinical selection of the participants have been attributed to the overestimation of incidence rate in some of these studies.^[22] In addition, most of these studies were single-center studies. The variability of samples accorded by a multicentric trial remains undisputed and most imperative from an epidemiological aspect. To determine the prevalence of EGFR mutation across the diverse population of India, we therefore undertook the multicentric study to answer this question.

SUBJECTS AND METHODS

Study design

The present study was designed as a noninterventional, multicenter, epidemiological study. It was conducted at seven centers across India with the objective to evaluate the prevalence of EGFR mutation in NSCLC patients in India (CTRI No.: CTRI/2013/10/004077). Treatment-naïve or treatment-experienced male or female patients diagnosed with NSCLC and with available tumor specimens from biopsy samples for genetic analysis were enrolled in this study. The samples were sent for EGFR testing to a Central Laboratory at Tata Memorial Hospital, Mumbai; a Tertiary Care Center in India. Patients, who in the opinion of the investigator were

unable to comply with study requirements, were excluded from the study. Written informed consent to perform the EGFR mutation analysis, using their available tumor samples, was obtained from all patients prior to their inclusion in the study. The Independent Ethics Committee or Institutional Review Board of all the participating centers approved the study protocol. The study was carried out in accordance with the Declaration of Helsinki and Good Clinical and Epidemiological Practices, guidelines, and relevant applicable regulatory requirements.

This study involved a single visit to capture information of patient demographics, baseline characteristics including smoking status and histopathology from routine examination performed in accordance with current guidelines and local standard of care. This was documented in the electronic CRF. The primary outcome measure was the incidence of EGFR mutation and the secondary outcome measures included types of EGFR mutation and association of mutation status with patients' demographics and histological subtype of NSCLC.

Sample size

There was no formal sample size calculation for this study. This was an epidemiological study; hence, a sample size of 250 patients would give meaningful insight into the scientific outcomes of this study as well as evaluate the feasibility of molecular testing at a central laboratory for samples collected from multiple centers across the country.

Collection of tissue samples

The patient samples were collected in the form of formalin-fixed paraffin-embedded (FFPE) tissue blocks from all the study centers and transported in ambient temperature through a courier service to the Central Laboratory at Tata Memorial Hospital.

DNA extraction and epidermal growth factor receptor mutation analysis

DNA was extracted from the FFPE blocks of the patients for EGFR mutation analysis. Hematoxylin and eosin stained tissue blocks were initially verified to constitute 75% of the tissue mass. Six sections of this tissue, sectioned using a microtome (Leica), were deparaffinized with xylene and the DNA extraction, and EGFR mutation analysis was conducted as described by Noronha *et al.*^[21] The mutations studied were in-frame deletions in exon 19, an L858R point mutation in exon 21, and the G719C point mutation in exon 18.

Statistical analysis

Statistical analysis was done using SAS version 9.1.3, SAS Institute Inc., Cary, NC, USA. EGFR mutation was categorized

as positive or negative qualitatively. Descriptive statistics were used to summarize the variables. Chi-square or Fisher's exact test was used, as appropriate, to find the association of EGFR mutation status and mutations in exon 18–21 across gender, age, smoking status, and histological subtypes as categories. The 95% confidence interval was constructed for primary and secondary endpoints, and a value of $P < 0.05$ was considered as the level of significance.

RESULTS

A total of 301 patients were screened from September 2013 to November 2014, of which, 252 patients were enrolled in the study and 49 patients were screen failures due to inadequate tissue sample. The patient and disposition chart are provided in Figure 1. Among the 252 patients, 169 (67.1%) were male and 83 (32.9%) were female, median age was 57 years with 186 (73.8%) patients in the age group of 18–64 years. Data on their smoking status, stage of disease, and NSCLC histology are presented in Table 1.

The mean duration of transportation of tissue samples to and from the central laboratory was 48 h, and the average turnaround time for molecular testing was 5 days.

Of the 252 patient samples analyzed, 59 (23.4%) were EGFR mutation positive and 193 (76.6%) were negative for EGFR mutation. EGFR mutations were identified in exon 19 in 33 (55.9%) patients, in exon 21 in 23 (39%) patients, and in exon 18 in 1 (1.7%) patient [Table 2].

In terms of geographic regions, of the 198 patients from the study centers in the western part of India, 48 (24.2%) were EGFR mutation positive and out of the fifty patients from the study centers in the southern part of India, 11 (22%) were

EGFR mutation positive. Four patients were enrolled from the northern part of India and none of whom were mutation positive [Table 2]. There were no centers in eastern part of India.

Patients in the age category of 40–49 years had a higher incidence of EGFR mutation (31%) as compared with other age categories. However, there was no statistically significant association between age and EGFR mutation ($P = 0.6106$). There was a statistically significant association between EGFR mutation status and gender ($P = 0.0166$) and smoking status [$P = 0.0003$, Table 3].

Table 1: Patient demographic and clinical characteristics

Variables	Number of patients (n=252)
Median age (years)	57 (27-86)
Age categories (years), n (%)	
18-64	186 (73.8)
65-84	64 (25.4)
85+	2 (0.8)
Gender, n (%)	
Male	169 (67.1)
Female	83 (32.9)
Smoking status, n (%)	
Smoker	19 (8.1)
Former smoker	69 (29.4)
Never smoked	147 (62.6)
Disease stage, n (%)	
Stage I	7 (2.8)
Stage II	9 (3.6)
Stage IIIA	4 (1.6)
Stage IIIB	17 (6.7)
Stage IV	215 (85.3)
Histology of NSCLC, n (%)	
Adenocarcinoma	213 (84.5)
Squamous cell carcinoma	29 (11.5)
Large cell carcinoma	2 (0.8)
Other	8 (3.2)

NSCLC - Non-small-cell lung cancer

Figure 1: Patient disposition flow chart. Of the 252 patients enrolled, 59 were epidermal growth factor receptor mutation positive

Table 2: Epidermal growth factor receptor mutation status and type

Variable	Number of patients (n=59)
EGFR mutation status, n (%)	
Positive	59 (23.4)
Negative	193 (76.6)
EGFR mutation type, n (%)	
Exon 18	1 (1.7)
Exon 19	33 (55.9)
Exon 21	23 (39.0)
Geographical region of study center, n (%)	
West (n=198)	48 (24.2)
South (n=50)	11 (22)

Western India includes two study centers in Mumbai and one in Ahmedabad, southern India includes one study center in Hyderabad, one in Bengaluru, one in Vishakhapatnam, and one study center in northern India (New Delhi) enrolled four patients but all were EGFR negative. EGFR - Epidermal growth factor receptor

Table 3: Association of epidermal growth factor receptor mutation status with age, gender, and smoking status

Variables	Category	Total number of patients (n=252)	EGFR mutation positive (n=59) (%)	EGFR mutation negative (n=193) (%)	P
Age	20-29	2	0	2 (100.0)	0.6106 [^]
	30-39	14	1 (7.1)	13 (92.9)	
	40-49	42	13 (31.0)	29 (69.0)	
	50-59	89	22 (24.7)	67 (75.3)	
	60-69	74	15 (20.3)	59 (79.7)	
	70-79	24	6 (25.0)	18 (75.0)	
	80-89	7	2 (28.6)	5 (71.4)	
Gender	Male	169	32 (18.9)	137 (81.1)	0.0166*
	Female	83	27 (32.5)	56 (67.5)	
	Total (n)	252	59 (23.4)	193 (76.6)	
Smoking status	Smoker	19	5 (26.3)	14 (73.7)	0.0003*
	Former smoker	69	4 (5.8)	65 (94.2)	
	Never smoked	147	45 (30.6)	102 (69.4)	
	Total (n)	235	54 (23.0)	181 (77.0)	
	Data not available	17	5 (29.4)	12 (70.6)	
Pathological stage	Stage I	7	2 (28.6)	5 (71.4)	0.4765*
	Stage II	9	4 (44.4)	5 (55.6)	
	Stage IIIA	4	1 (25)	3 (75)	
	Stage IIIB	17	1 (5.9)	16 (94.1)	
	Stage IV	215	51 (23.7)	164 (76.3)	
	Total (n)	252	59 (23.4)	193 (76.6)	
Histology of NSCLC	Squamous cell carcinoma	29	5 (17.2)	24 (82.8)	0.7940 [^]
	Adenocarcinoma	213	53 (24.9)	160 (75.1)	
	Large cell carcinoma	2	0	2 (100)	
	Other	8	1 (12.5)	7 (87.5)	

*Chi-square/Fisher's exact test was used appropriately to find the effect of EGFR mutation. Denominator for calculation of percentage was the total number of patients in respective category. EGFR - Epidermal growth factor receptor; NSCLC - Nonsmall-cell lung cancer; [^] - Not statically significant

Among the Stage IV disease patients, 23.7% patients were EGFR mutation positive. Due to the low number of patients in other stages, the comparative analysis of mutation status across stages of the disease was inconclusive. Adenocarcinoma patients had a higher incidence of EGFR mutation (24.9%) as compared to squamous cell carcinoma patients (17.2%). However, the difference was not statistically significant [Table 3].

DISCUSSION

Worldwide a growing body of literature indicates a significant benefit from TKIs in this subset of the population. In India, NSCLC patients with activating mutation of EGFR demonstrated 74% RR to oral TKI compared to 5% in patients with wild-type EGFR.^[21] Identification of patterns in population genetics of NSCLC patients across different ethnicities will assist oncologists in prescribing the optimum treatment regimen.

The two unique characteristics of our study were the multicenter design and the use of a central laboratory. The centers were selected from various parts of India for this

study to ensure representation from different regions of the country. A pan-India survey conducted to deduce the pattern of EGFR mutation testing across different tertiary care centers reported significant differences between centers having in-house testing facility compared to centers that outsourced the testing.^[23] One of the potential shortcomings of a multicenter design are the minor differences in execution of laboratory methodologies while following the same protocol. Therefore, to overcome this shortcoming, the samples obtained from various centers across the country were analyzed at a Central Laboratory, a Tertiary Care Center in India. To the best of our knowledge, this is the first time a central laboratory was used in an epidemiological study for EGFR mutation status in India. This study demonstrated the successful execution of molecular testing at a central laboratory for EGFR mutation status. The FFPE blocks were transported from the center to the central laboratory within 48 h and the average turn-around time for molecular testing was 5 days. This was significantly lower than the average 15–17 days reported in majority of the patients in the Indian Tertiary Care Centers and the PIONEER study.^[24,25] This turnaround time for molecular testing at a central laboratory can assure

timely initiation of optimum treatment for patients as well as extend the benefits of targeted therapy to patients in tier two cities in India which may not have such testing facilities.

An important observation was the inadequacy of tissue samples in 49 out of 301 patients screened, which prevented molecular testing. This underlines an inherent problem of tissue biopsy in procurement of tissue samples and the limitations of tumor analyses. Liquid biopsy, a noninvasive method which assesses the genetic makeup of a tumor through a biofluid sample, has the potential to help clinicians screen for disease as well as monitor treatment response and resistance mechanisms in the tumor.^[26]

The results of our study are in line with the global trend with the incidence of EGFR mutation higher in females (32.5%), nonsmokers (30.6%), adenocarcinoma (24.9%), and Stage IV disease (23.7%). Chougule *et al.* reported the prevalence (23.2%) of EGFR mutation in a sample size of 907 patients.^[22] The frequency of EGFR mutation in exon 19 (55.9%) in our study was also similar to 50% reported by Chougule *et al.*^[22] but lower than 61.3% reported by Prabhakar *et al.*^[27] In this study, 29 patients had squamous cell carcinoma of which 17.2% were EGFR mutation positive. In reported literature, EGFR mutation positivity rate in the Asian population ranges from 0% to 20.5%.^[28-30] It will be important to investigate this finding further.

Similar to the overall incidence rate observed in India, the regional incidence rate of EGFR mutation was comparable between the western centers (24.2%) and the southern centers (22%). This was in contrast to the higher incidence of EGFR mutation (39.6% and 68%) reported in southern India.^[19,31]

In-frame deletion in exon 19 was predominant (55.9%) in our study similar to observed in other Indian studies which reported an incidence of 50%–74%. Similarly, incidence of missense mutation in exon 21 was 39% in our study as compared to 25%–42% reported in previous studies in India.^[20,22] Tumors expressing an exon 19 mutant EGFR isoform have been reported to exhibit better TKI response (86% vs. 67%; $P = 0.171$), and improved survival has been compared with patients whose tumors expressed an exon 21 mutation.

One of the limitations of this study was the retrospective design for data collection. The data on the treatment outcomes were either not available or inadequately captured during the retrospective data collection. A prospective study will allow one to follow the patient's response to treatment

and thereby evaluate its relationship with their mutation status. Another shortcoming was the lack of a study center in the eastern part of India and limited enrollment in the northern India study center.

Molecular testing at a central laboratory, with an average turn-around time of 5 days, is a feasible option in India. The overall prevalence of EGFR mutation in Indian NSCLC patients was 23.4% with a similar incidence across western and southern centers in India. EGFR mutation was predominant in females, never-smokers, adenocarcinoma, and disease Stage IV patients. A statistically significant association between EGFR mutation and gender as well as the smoking status of the patients was observed. Majority of the patients had in-frame deletions in exon 19.

CONCLUSIONS

Molecular testing at a central laboratory is a feasible option in India. Prevalence of EGFR mutation in Indian NSCLC patients was similar across western and southern centers in India. A statistically significant association between EGFR mutation and gender as well as the smoking status of the patients was observed. Majority of the patients had in-frame deletions in exon 19.

Acknowledgment

The authors would like to acknowledge the contribution of the following from Roche Products (India) Pvt.: Dr. Balaji Ugile for his valuable inputs on the draft; Anant Dethe for data acquisition and Priyanka Bhattacharya for medical writing support.

Financial support and sponsorship

This study was funded by Roche Products (India) Pvt. Ltd.

Conflicts of interest

The authors would like to disclose that Dr. Amit Rauthan, Dr. Rajesh Mistry, and Dr. P.K. Das are Advisory Board Members for Roche Products (India) Pvt. Ltd. The remaining authors have no conflict of interest.

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