

Rate of EGFR mutation in patients with pulmonary adenocarcinoma

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Abstract

Purpose: Contemporary literature on lung adenocarcinoma has demonstrated a genetic difference of the epidermal growth factor receptor (EGFR) pathway conferring to ethnicity, such as higher frequency of activated EGFR mutations in East Asian population. This information is missing in some developing countries, and we intend to address this gap in the literature. **Methods:** We examined the rate of EGFR mutations among Pakistani patients with adenocarcinoma of the lung. Fine-needle aspiration samples were gathered from 73 patients. Polymerase chain reaction was performed on extracted DNA for mutational analysis of EGFR exons 19 and 21. **Results:** EGFR mutations were discovered in 18 of 73 (24.6%) patients. We did not find any significant difference in EGFR mutation rate with regard to patient's age, sex, smoking history, clinical stage of lung cancer, subtypes of adenocarcinoma, and tumor differentiation. **Conclusion:** Our investigation shows that the EGFR mutation rate in our patient population with adenocarcinoma of the lung was higher than in African-American, Arabian, and white Caucasian patients, and was lower than the East Asian population.

Keywords: Adenocarcinoma; EGFR; Mutation; Lung Cancer

Introduction

The rate of epidermal growth factor receptor (EGFR) mutation in non-small cell lung cancer is known to vary throughout ethnic groups, with nearly 10% of patients with non-small cell lung cancer (NSCLC) in the US and 35% in East Asia have tumor associated EGFR mutations.¹⁻⁴ These mutations occur within EGFR exons 18-21, which encodes a percentage of the EGFR kinase domain. About 90% of the mutations are exon 19 deletions or exon 21 L858R point mutations.⁵ The influence of ethnicity on the rate of the mutation is poorly understood.

Contemporary literature suggests that EGFR mutations offer survival benefit independent of therapy.^{6,7} Literature concentrating on East Asian population indicates that the incidence of classical EGFR mutations is predictive of survival benefit following EGFR tyrosine kinase inhibitor (TKI) therapy.⁸

After a meticulous review of the literature, we did not find any reports of genetic backgrounds of lung cancer in Pakistan. Therefore, we studied the rate of major types of activating mutations in exons 19 and 21 of EGFR in Pakistani patients with adenocarcinoma of the lung.

Methods and Materials

Seventy-three patients underwent computed tomography (CT)-guided fine-needle aspiration (FNA) biopsy at the Karachi Institute of Radiotherapy and Nuclear Medicine (KIRAN), and Atomic Energy Medical Centre (AEMC) at Jinnah Post Graduate Medical Centre (JPMC) Karachi from 2011 to 2013. The study complies with the institutional review board committee policies. Informed consents were obtained from all participants.

None of the study participants were exposed to chemotherapy prior to FNA. All pathological diagnoses were conducted by pathologist's blind to the clinical information, and verified by one pathologist in accordance with the WHO classification system.

FNA and DNA samples

Tumor specimen was obtained using the approved protocols of the IRB through CT-guided FNA biopsies using 23-gauge needles in all participants. Appropriateness for DNA extraction was documented after pathologists confirmed more than 70% tumor cells in each sample.

FNA samples were instantly stored in tubes containing 99.5% ethanol at -80°C. Genomic DNA was isolated from

tissue by removal of ethanol after high-speed centrifugation. A modified procedure using the commercial DNAzol reagent was successfully applied to extract genomic DNA (DNAzol® BD, Molecular Research Center, Cincinnati, USA).

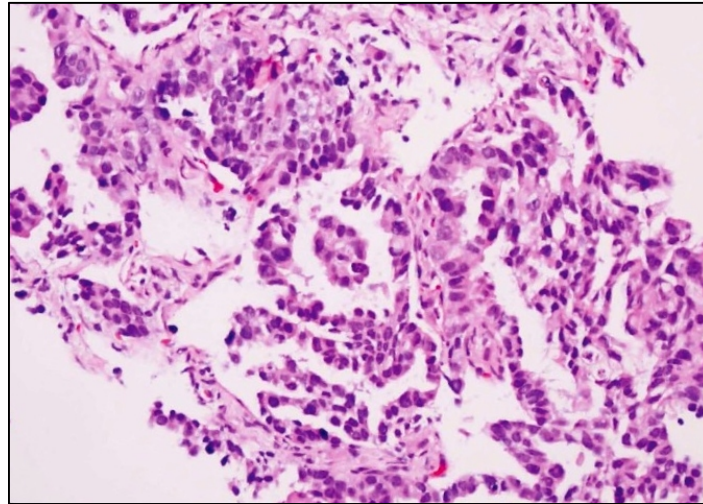
Polymerase chain reaction single-strand conformation polymorphism was utilized to identify mutations in exons 19 and 21 of the EGFR gene. PCR was done by the AmpliTaq Gold PCR Master Mix (Life Technologies, USA). **Table 1** illustrates the primers and PCR conditions used. After denaturing PCR products, electrophoresis was done with the GenePhor System and GeneGel Excel 12.5/24 (GE Healthcare, Sweden) at 18°C, 650 V for 80 minutes. The gels were stained using the DNA Silver Staining Kit (Promega, Madison, WI, USA).

TABLE 1: Association of EGFR gene mutations to clinicopathological features.

Variable	Total patients (n=73)	EGFR gene mutation status		
		Mutated (n=18)	Wild-type (n=55)	P-value
Age, median (range)	58.9(18-84)	61.6 (43-79)	56.2 (18-84)	
<60 years of age	47	8 (17%)	39	0.12
≥60 years of age	26	10 (38.4%)	16	
Gender				0.39
Male	53	14 (26.4%)	39	
Female	20	4 (20%)	16	
Smoking status				0.95
Never-smoker	27	7 (26%)	20	
Former-smoker	12	4 (33.3%)	8	
Current-smoker	34	7 (20.5%)	27	
Clinical stage				0.46
Stage I	10	4 (40%)	6	
Stage II	20	6 (30%)	14	
Stage III	18	3 (16.6%)	15	
Stage IV	25	5 (20%)	20	
Subtypes of adenocarcinoma				0.63
Bronchio-alveolar	2	0	2	
Papillary	4	0	4	
Solid	2	0	2	
Mixed	65	18	47	
Acinar component				0.54
Present	41	8 (19.5%)	33	
Absent	32	7 (21.8%)	25	
Bronchio-alveolar component				0.87
Present	14	2 (14.2%)	12	
Absent	59	13 (22%)	46	
Papillary component				0.05
Present	37	6 (16.2%)	31	
Absent	36	12 (33.3%)	24	
Solid component				0.23
Present	16	3 (18.7%)	13	
Absent	57	15 (26.3%)	42	
Tumor differentiation				0.23
Well	9	2 (22.2%)	7	
Moderate	24	5 (20.8%)	19	
Poor	40	14 (35%)	26	

TABLE 2: PCR primers and parameters.

EGFR gene	Forward (5'–3')	Reverse (5'–3')	Product size (bp)	Number of cycle	Annealing condition
Exon19	CGTCTTCCTTCTCTCTCTGT	CCACACAGCAAAA-GCAGAAAC	148	35	55 °C, 15 s
Exon21	AGGGCATGAAC-TACTTG	CCTCCTTACTTT-GCCTCCTTC	167	35	55 °C, 15 s

**FIG. 1:** Adenocarcinoma of the lung.

Statistical analysis

The incidence of EGFR mutation status along with the corresponding important predictors of incidence of EGFR mutations were additionally identified by logistic regression with a forward-model selection procedure. The factors included in the model selection were age, sex, histology, and stage of the tumor. Continuous measurements between two groups were examined by the t test or Fisher's exact test, whereas, the Mann-Whitney U test determined the differences between the continuous variables. All tests were two-sided. A p-value < 0.05 was considered statistically significant.

Results

Of the 73 tumor patients, mutations in exons 19 and 21 of the EGFR gene were detected in 11 (15%) and 7 (9.5%) tumors. Estimated mutation rate was 24.6% (18 of 73). Mutation male to female rate was 26.4 % (14 of 53) and 20% (4 of 20) **Table 1. Table 2** reviews the association of EGFR gene mutations to clinicopathological features. A higher incidence of EGFR mutation was appreciated in adenocarcinomas with papillary component (0.05). We did not find any other significant relationship between EGFR mutation rate and patient characteristics.

Discussion

According to our literature search, our study is the only report available comprising information on EGFR mutations in

a Pakistani patient population. Considering that our recruiting site is one of the principal cancer centers in the country, it is likely that our findings closely reflect the overall occurrence of EGFR mutations in the population of Pakistan.

Our study shows that the EGFR mutation rate in Pakistani patients with adenocarcinoma of the lung was higher than the African-American⁹, Arabian¹⁰, and white Caucasian patients^{1, 2}, and was lower than in patients in East Asia and other countries of South Asia.¹¹⁻¹³ This is probably the result of the fundamental differences between cohorts.

It is worth mentioning that a latent tumor stage at diagnosis may have affected the reported ethnic disparity in EGRF mutation rate, since Pakistan has no mandatory national health insurance policy yet, and therefore, Pakistani patients often present at advanced stages of the lung cancer [**Figure 1**].

While our sample size is not sufficient to draw any conclusion, our results however may predict a higher likelihood of important responses to EGFR-TKIs in Pakistani patients with adenocarcinoma of the lung than in African-American, Arabian, and white Caucasian patients. This hypothesis is particularly of interest for patients in Pakistan, where lung cancer cases are diagnosed at advanced stages and surgery is no longer a therapeutic option. Additionally, the conclusions of our study may benefit future prospective clinical trials in-

crease the number of patients to fully investigate the relationship between the presence of activating EGFR mutation and response to TKIs.

Conclusion

Epidermal growth factor receptor mutations were found in 18 of 73 (24.6%) Pakistani patients. Our investigation shows that the EGFR mutation rate in our patient population with adenocarcinoma of the lung was higher than in African-American, Arabian, and white Caucasian patients, and was lower than the East Asian population.

Conflict of interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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