Relationship of epidermal growth factor receptor activating mutations with histologic subtyping according to International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society 2011 adenocarcinoma classification and their impact on overall survival

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ABSTRACT

Background: There is limited Indian data on epidermal growth factor receptor (EGFR) gene activating mutations (AMs) prevalence and their clinicopathologic associations. The current study aimed to assess the relationship between EGFR AM and histologic subtypes and their impact on overall survival (OS) in a North Indian cohort. **Patients and Methods:** Retrospective analysis of nonsmall cell lung cancer patients who underwent EGFR mutation testing ($n = 186$) over 3 years period (2012–2014). EGFR mutations were tested using polymerase chain reaction amplification and direct sequencing. Patients were classified as EGFR AM, EGFR wild type (WT) or EGFR unknown (UKN). Histologically adenocarcinomas (ADC) were further categorized as per the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society-2011 classification. **Results:** Overall EGFR AM prevalence was 16.6%. The ratio of exon 19 deletions to exon 21 L858R mutations was 3.17:1. Female sex ($P = 0.002$), never smoking status ($P = 0.002$), metastatic disease ($P = 0.032$), and nonsolid subtype of ADC ($P = 0.001$) were associated with EGFR AM on univariate logistic regression analysis (LRA). On multivariate LRA, solid ADC was negatively associated with EGFR AM. Median OS was higher in patients with EGFR AM (750 days) as compared to EGFR-WT (459 days) or EGFR-UKN (291 days) for the overall population and in patients with Stage IV disease (750 days vs. 278 days for EGFR-WT, $P = 0.024$). On univariate Cox proportional hazard (CPH) analysis, smoking, poor performance status (Eastern Cooperative Oncology Group ≥ 2), EGFR-UKN status, and solid ADC were associated with worse OS while female sex and lepidic ADC had better OS. On multivariate CPH analysis, lepidic ADC (hazard ratio [HR] =0.12) and EGFR-WT/EGFR-UKN (HR = 2.39 and HR = 3.30 respectively) were independently associated with OS in separate analyses. **Conclusions:** Histologic subtyping of ADC performed on small biopsies is independently associated with EGFR AM and with better OS. EGFR AM presence is a positive prognostic factor for OS.

KEY WORDS: Adenocarcinoma, epidermal growth factor receptor, histology, India, mutations, overall survival

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Access this article online

Quick Response Code:

Website:
www.lungindia.com

DOI:
10.4103/0970-2113.180801

INTRODUCTION

Lung cancer has remained the most common cancer worldwide for several decades and represents 12.9% of all new cancers.[1] It is the most common type of cancer in men and remains the most common cause of cancer-related mortality in both sexes with a very high case fatality rate (mortality/incidence ratio of 0.81).[1] Although the lung cancer incidence rates in India are lower than in the developed world, most patients present with advanced disease and hence the relative mortality rates are higher, and this disparity results in a significant contribution to the world cancer deaths.[2-4]

The discovery of oncogenic driver mutations in the epidermal growth factor receptor (EGFR) gene (exons 18–21), and approval of agents targeted against these molecular drivers has revolutionized the management of nonsmall cell lung cancer (NSCLC).[5] Small molecule tyrosine kinase inhibitors (TKIs) namely gefitinib, erlotinib, and afatinib targeted against the EGFR significantly improve the response rates and progression-free survival when used in patients with activating mutations (AMs) of the EGFR gene.[6]

The prevalence of AMs in the EGFR gene (most common of which are exon 19 deletions and the exon 21 L858R point mutation) varies considerably based on the ethnicity of the population being evaluated.[7] The reported prevalence of EGFR AM is the highest among East Asians (30–60%)[8] and significantly lower in Caucasians (5–15%).[9] Previous studies from India have reported the frequency of EGFR AM to be between 22% and 40%, which is lesser than that reported from the East Asian populations.[8,10-12] Most of the previous Indian studies have involved patients from South and Central India. There is a paucity of data regarding the prevalence of EGFR mutations from North India.

Since the proposal of new pathologic classification of adenocarcinomas (ADC) by International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society (IASLC/ATS/ERS) in 2011,[13] several studies have shown that the histologic (histopathological examination [HPE]) subtyping predicts both the mutation status, as well as overall survival (OS).[14-18] However, most of these studies have been conducted on surgically resected specimens and have included patients with predominantly early stage disease (Stages I-IIIA). In developing countries like India, a majority of patients present at advanced stages (Stages IIIB-IV) because of lack of uniform access to healthcare facilities and lack of routine lung cancer screening programs. As a result, most patients are managed nonsurgically. Whether the results of studies done on surgical cohorts can be extrapolated to patients managed nonsurgically is open to speculation. Till date, there is no study from the Indian subcontinent on the prognostic and predictive value of the new histologic subclassification.

In this study, we aimed to (a) determine the prevalence of EGFR AM in a cohort of patients from North India; (b) determine the associations, between EGFR AM and the new HPE subtyping of ADC; and, (c) determine the impact of EGFR AM and the HPE subtypes on OS.

PATIENTS AND METHODS

This was a retrospective analysis of patients with cytologically or histopathologically proven lung cancer diagnosed at our institute who underwent testing for EGFR gene mutations over a 3 years period (2012–2014). All patients were of North Indian origin. The case records were retrieved, and the clinical details including age, sex, smoking status, EGFR and anaplastic lymphoma kinase (ALK) gene rearrangement status, tumor stage, histopathologic type, treatment received, objective radiological responses, and OS were entered in a standard data extraction sheet. The detailed methodology has been described by us previously.[3,4,19] Patients with adenocarcinoma on histology were tested for the presence of EGFR gene mutations irrespective of the age, sex, and smoking status, whereas patients with squamous cell lung cancer were tested only if the patient was a never smoker or on patients request. Patients were considered as never-smokers if they had not smoked any cigarette/bidi (the hand rolled form of tobacco wrapped in the dried tendu leaf) in his/her lifetime. Those who had left smoking ≥12 months prior to diagnosis of lung cancer were considered as reformed smokers and those who were continuing to smoke or had left <12 months prior were considered as current smokers. Ever-smokers (reformed and current smokers) were further classified based on their smoking index (SI). SI was defined as the product of the number of cigarettes/bidis smoked per day and the number of years smoked.[4] Patients with an SI of <300 were considered as light smokers and those with an SI of ≥300 were considered as heavy smokers. The tumor was staged and stage grouped according to the seventh edition of the tumor, node, metastasis [TNM] staging of malignant tumors. Chemotherapy regimens and management protocols used at our center have been described in detail previously.[20-24] OS was calculated as the time (in days) from initiation of treatment to date of death/last follow-up. Written informed consent was taken from patients at the time of starting treatment, and the study was approved by the Institutional Ethics Committee.

Histopathologic subtyping

Hematoxylin and eosin stained sections of lung adenocarcinoma diagnosed on endobronchial biopsy, transbronchial lung biopsy, pleural biopsies, and as a metastatic tumor in the lymph nodes were reviewed. Based on 2011 IASLC/ATS/ERS classification, we attempted to classify the cases on these small biopsies into the following histological patterns: Acinar, solid, papillary, micropapillary, and lepidic. The lepidic pattern was classified in transbronchial lung biopsy specimens only. The cases were classified based on the consensus opinion of two pathologists (AB and AD).

Epidermal growth factor receptor mutation analysis

Genomic DNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue using three 10 um sections by Qiagen kit (QIAamp DNA FFPE Tissue Kit, Cat No. 56404) after ensuring adequacy of tumor cells in the sections. The quantity and quality of genomic DNA was checked at 0.8% Agarose gel or by NanoDrop. DNA was amplified for exon 18, 19, 20, and 21 using 100 ng genomic DNA. The primers used were as follows:

EGFR EXON 18: 5'-AGGGCTGAGGTGACCCTTGT-3' (forward primer),
　　　　　　　5'-TCCCCACCAGACCATGAGAG-3' (reverse primer)
EGFR EXON 19: 5'-ACCATCTCACAATTGCCAGTTAAC-3' (forward primer),
　　　　　　　5'-GAGGTTCAGAGCCATGGACC-3' (reverse primer)
EGFR EXON 20: 5'-GAAGCCACACTGACGTGCCT-3' (forward primer),
　　　　　　　5'-CCCTTCCCTGATTACCTTTGCGA-3' (reverse primer)
EGFR EXON 21: 5'-TCACAGCAGGGTCTTCTCTGTTT-3' (forward primer)
　　　　　　　5'-ATGCTGGCTGACCTAAAGCC-3' (reverse primer).

The polymerase chain reaction (PCR) product was purified using PCR purification kit (QIA quick PCR Purification Kit, Cat No. 28104). Both forward and reverse sequencing was done using 2.5 ng of PCR product and 1.0 pmol of forward or reverse primer in the Applied Biosystems Inc. genetic analyzer. The sequence was compared with the wild type (WT) sequence available in the National Center for Biotechnology Information (NCBI) database using NCBI BLAST.

Statistical analysis

Data were analyzed using SPSS statistical software (version 22.0, IBM Corp., USA). Descriptive data is presented as mean (standard deviation [SD]), median (interquartile range), or as percentages. Comparison between the groups was done using the Chi-square/Fishers exact test (for categorical variables), unpaired Student's t-test (for continuous variables with a Gaussian distribution) or the Mann–Whitney U-test (for continuous variables with a nonGaussian distribution). Factors associated with EGFR AM were assessed using the univariate and multivariate logistic regression analysis (LRA) and results expressed as odds ratio (OR) with 95% confidence interval (CI). Crude ORs were derived from the univariate analysis and if found significant ($P < 0.10$), these variables were then entered into a multivariate model to derive adjusted ORs and 95% CIs. Survival probability and median OS were calculated by Kaplan–Meier method and group differences analyzed using the log-rank test. Factors affecting OS were assessed using the univariate and multivariate Cox proportional hazards regression analysis and calculation of hazard ratio (HR) with 95% CI. For all analyses, a $P < 0.05$

was taking as a significant except for Cox univariate and multivariate regression analyses where a $P < 0.1$ was taken as being significant.

RESULTS

A total of 186 patients were tested for EGFR AM during the study period. Of these, 135 underwent testing on small biopsy specimens (endobronchial biopsy, transbronchial lung biopsy, thoracoscopic pleural biopsy, or computed tomography [CT]-guided lung biopsy) and the rest underwent mutation testing on cell blocks made from cytology specimens (pleural fluid, CT-guided aspiration, or transbronchial needle aspiration specimens). The clinical and demographic parameters of the study population

Table 1: Clinical and demographic characteristics of the study population (n=186)

Characteristic	Number (percentage)
Age (years)	58.26±12.10
Sex	
Male	121 (65.1)
Female	65 (34.9)
Smoking status (n=158)	
Never smoker	76 (48.1)
Ever smoker	82 (51.9)
Reformed smoker	30 (19)
Current smoker	52 (32.9)
Light smoker (SI ≤300)	26 (16.5)
Heavy smoker (SI >300)	56 (35.4)
SI (ever smokers)	582.4±592.3
Number of patients tested for EGFR	186 (100)
EGFR mutation uninterpretable	29 (15.6)
EGFR mutation interpretable	157 (84.4)
EGFR mutation positive*	26 (16.6)
Exon 18 mutation	0
Exon 19 deletion	19 (12.1)
Exon 20 mutation	1 (0.6)
Exon 21 mutation	6 (3.8)
EGFR mutation negative*	131 (83.4)
Number of patients tested for ALK rearrangement	88
ALK rearrangement positive**	2 (2.3)
ALK rearrangement negative**	86 (97.7)
Histopathology	
ADC	174 (93.5)
SCC	8 (4.3)
Adenosquamous	1 (0.5)
NSCLC-NOS	3 (1.6)
TNM stage (n=160)	
Stages I-IIIA	25 (15.6)
Stage IIIB	21 (13.1)
Stage IV	114 (71.3)
ECOG score (n=157)	
ECOG <2	79 (50.3)
ECOG ≥2	78 (49.7)

*Numbers expressed as a percentage of those with interpretable EGFR status, **Numbers expressed as a percentage of those tested for ALK rearrangement. Values expressed as n (%) or mean±SD. ADC: Adenocarcinoma, ALK: Anaplastic lymphoma kinase, ECOG: Eastern Cooperative Oncology Group, EGFR: Epidermal growth factor receptor, NSCLC-NOS: Nonsmall cell lung cancer not otherwise specified, SCC: Squamous cell lung cancer, TNM: Tumor node metastasis, SD: Standard deviation, SI: Smoking index

are summarized in Table 1. The mean age of the study population was 58 years (SD: 12.1 years). A majority of the patients were men (*n* = 121, 65.1%), had adenocarcinoma on histology (*n* = 174, 93.5%) and metastatic disease at presentation (*n* = 114, 71.3%). Of the 135 patients who were tested on biopsy specimens, four had squamous cell carcinoma and the remaining 131 patients with adenocarcinoma were subclassified as per the new IASLC/ATS/ERS criteria [Supplementary Figure 1]. The predominant histologic subtype was acinar (*n* = 64, 48.9%) followed by solid (*n* = 53, 40.5%), lepidic (*n* = 13, 9.9%), and papillary (*n* = 1, 0.8%). None of the cases showed a micropapillary pattern.

EGFR mutation status was uninterpretable (EGFR unknown [UKN], EGFR-UKN) in 29 patients (15.6%). Of the patients with interpretable mutation status, EGFR mutations were detected in 26 patients (16.6%). The most common EGFR mutation was exon 19 deletion (*n* = 19, 12.1%) followed by exon 21 L858R point mutation (*n* = 6, 3.8%). Exon 20 mutation was seen in only one patient, and none had mutations in exon 18. Among the 88 patients who simultaneously underwent testing for ALK gene rearrangements using either Vysis™ Break Apart FISH (*n* = 47) or Ventana™ anti-ALK antibody (D5F3) by immunohistochemistry (*n* = 41), ALK gene rearrangements were detected in two patients (2.3%).

Treatment details of patients with and without EGFR AM are shown in Supplementary Tables 1 and 2, respectively. Patients with EGFR AM were treated with EGFR-TKIs and those without EGFR AM and with EGFR-UKN status were treated with platinum-based doublet chemotherapy.

Factors predicting presence of epidermal growth factor receptor activating mutations

A comparison of the clinicopathologic characteristics of the study population stratified according to the EGFR mutation status is shown in Table 2. On univariate LRA [Table 3], the factors associated with EGFR mutations were sex (*P* = 0.002), smoking status (*P* = 0.002), disease stage (*P* = 0.032), and histologic subtype of adenocarcinoma (*P* = 0.027), and these are briefly summarized below. EGFR mutations were significantly higher in females as compared to males (29.3% vs. 9.1%), and in never-smokers compared to ever-smokers (31.3% vs. 8.8%). The EGFR mutation frequency was however similar across the various subgroups of ever smokers (light smokers [13.0%] vs. heavy smokers [7.1%], *P* = 0.399; reformed smokers [4.3%] vs. current smokers [11.1%], *P* = 0.656). EGFR mutations were also significantly higher in patients with metastatic disease at presentation as compared to those without (17.2% vs. 7.5%). Among the various subgroups of adenocarcinoma, EGFR mutations were least common in solid predominant adenocarcinoma (6.3%) and most frequent in the lepidic predominant adenocarcinoma (36.4%).

Other factors not significantly correlating with EGFR status on univariate LRA were age (*P* = 0.523), primary tumor stage (*P* = 0.255), lymph nodal status (*P* = 0.236), presence of malignant pleural effusion (*P* = 0.301), and Eastern Cooperative Oncology Group (ECOG) performance status (PS) (*P* = 0.662). On multivariate LRA, histopathologic subtyping was the only factor predictive of EGFR AM with the incidence of mutations being significantly low in patients with a solid subtype of adenocarcinoma (OR: 0.23, 95% CI: 0.06–0.90) [Table 3].

Factors predicting overall survival

Median OS was highest in patients with EGFR AM (750 days [431–1069]), intermediate in patients

Table 2: Clinical and demographic characteristics of the study population stratified according to EGFR mutation status

Characteristic	*n*	EGFR mutation positive	EGFR mutation negative	*P*
Age (years)	157	59.81±13.46	58.14±11.94	0.468
Sex				
Male	99	9 (9.09)	90 (90.9)	0.001
Female	58	17 (29.31)	41 (70.69)	
Smoking status				
Never smoker	64	20 (31.25)	44 (68.75)	0.001
Ever smoker	68	6 (8.82)	62 (91.18)	
SI (ever smokers)	132	343.3±160.8	602.1±623.3	0.156
Histopathology				
Nonsquamous NSCLC	149	26 (17.45)	123 (82.55)	0.354
Squamous NSCLC	8	0	8 (100)	
Histopathology subtype				
Acinar/papillary	62	14 (21.31)	48 (78.69)	0.013
Solid	48	3 (6.25)	45 (93.75)	
Lepidic	11	4 (36.36)	7 (63.64)	
Metastatic disease				
Yes	94	23 (17.16)	71 (52.98)	0.030
No	40	3 (7.5)	37 (92.5)	
ECOG group				
ECOG <2	66	12 (18.18)	54 (81.82)	0.662
ECOG ≥2	66	14 (21.21)	52 (78.79)	

Values expressed as *n* (%) or mean±SD. ECOG: Eastern Cooperative Oncology group, EGFR: Epidermal growth factor receptor, NSCLC: Nonsmall cell lung cancer, SD: Standard deviation, SI: Smoking index

Table 3: Logistic regression analysis for factors associated with presence of EGFR activating mutations

Variable	Univariate regression			Multivariate regression		
	OR	95% CI	*P*	OR	95% CI	*P*
Sex						
Male	1			1		
Female	4.15	1.71-10.08	0.002	1.457	0.35-5.44	0.575
Smoking status						
Ever smoker	1			1		
Never smoker	4.70	1.74-12.65	0.002	2.073	0.52-8.23	0.300
SI (ever smokers)						
Heavy smoker	1					
Light smoker	2.10	0.39-11.34	0.389			
Metastatic disease						
No	1			1		
Yes	3.99	1.13-14.18	0.032	2.359	0.59-9.37	0.223
Histopathology subtype						
Acinar/papillary	1			1		
Solid	0.23	0.06-0.85	0.027	0.230	0.06-0.90	0.034
Lepidic	1.96	0.50-7.67	0.334	1.370	0.31-6.14	0.681

OR: Odds ratio, CI: Confidence interval, EGFR: Epidermal growth factor receptor, SI: Smoking index
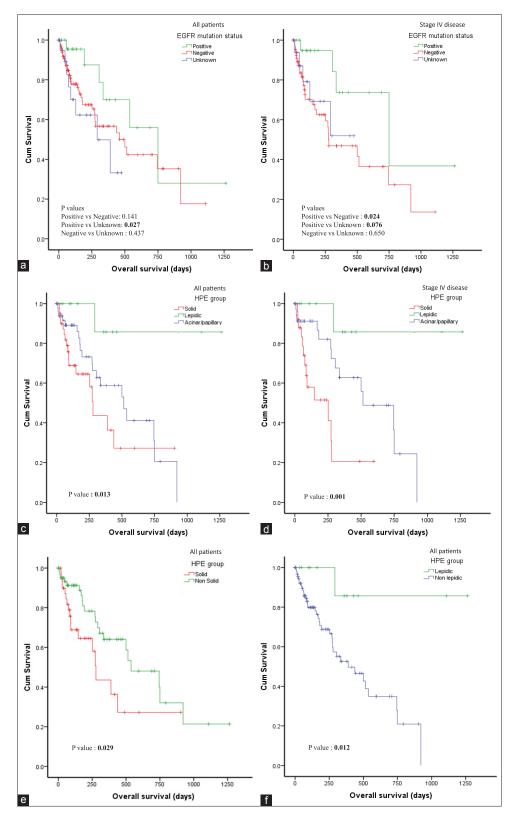
Figure 1: Kaplan–Meier survival curves for overall survival in patients stratified according to (a) epidermal growth factor receptor mutation status (all patients included); (b) epidermal growth factor receptor mutation status (Stage IV disease alone); (c) predominant histologic subgroup (all patients included); (d) predominant histologic subgroup (Stage IV disease alone); (e) presence or absence of solid predominant adenocarcinoma (all patients included); and, (f) presence or absence of lepidic predominant adenocarcinoma (all patients included)

Table 4: Cox's proportional hazard analyses for factors affecting overall survival

Univariate analysis			Multivariate analysis (model 1)			Multivariate analysis (model 2)		
Variable	HR (95% CI)	*P*	Variable	HR (95% CI)	*P*	Variable	HR (95% CI)	*P*
EGFR status			EGFR status			EGFR status		
Yes	1		Yes	1		Yes	1	
No	1.91 (0.80-4.51)	0.144	No	1.15 (0.45-2.95)	0.771	No	2.39 (0.97-5.88)	0.057
Unknown	2.70 (0.93-7.90)	0.069	Unknown	1.12 (0.26-4.83)	0.884	Unknown	3.30 (1.10-9.88)	0.033
Age	1.02 (0.99-1.04)	0.191				Age	1.02 (1.00-1.05)	0.086
Sex		0.001	Sex					
Male	1		Male	1				
Female	0.30 (0.15-0.62)		Female	0.63 (0.24-1.67)	0.354			
Smoking		0.005	Smoking			TNM stage		
Never smoker	1		Never smoker	1		Stage I-IIIA	1	
Ever smoker	2.34 (1.30-4.22)		Ever smoker	1.44 (0.64-3.26)	0.380	Stage IIIB	0.98 (0.32-2.98)	0.975
Smoking groups						Stage IV	1.27 (0.56-2.91)	0.570
Never smoker	1							
Light smoker	1.00 (0.37-2.71)	0.995						
Heavy smoker	3.21 (1.74-5.92)	<0.001						
ECOG PS		0.047	ECOG PS			ECOG PS		
ECOG <2	1		ECOG <2	1		ECOG <2	1	
ECOG ≥2	1.77 (1.01-3.10)		ECOG ≥2	1.54 (0.77-3.08)	0.221	ECOG ≥2	1.74 (0.98-3.10)	0.061
Metastatic disease		0.528						
No	1							
Yes	1.23 (0.65-2.31)							
HPE subtype			HPE subtype					
Solid ADC	1	0.032	Solid ADC	1				
Nonsolid ADC	0.48 (0.25-0.94)		Lepidic ADC	0.12 (0.01-0.99)	0.049			
Lepidic ADC	1	0.034	Acinar ADC	0.77 (0.37-1.59)	0.481			
Nonlepidic ADC	8.83 (1.18-66.3)							

ADC: Adenocarcinoma, ECOG: Eastern Cooperative Oncology Group, HPE: Histopathology examination, PS: Performance status, CI: Confidence interval, HR: Hazard ratio, TNM: Tumor node metastasis

with WT EGFR (459 days [227–692], *P* = 0.141 vs. EGFR positive group), and least in patients with EGFR-UKN status (291 days [29–553], *P* = 0.027 vs. EGFR positive group). As there were significantly higher number of patients with metastatic disease in patients with EGFR AMs, OS was compared for patients with Stage IV disease alone. When patients with Stage IV disease were compared, the median survival of the group with EGFR AM was significantly higher as compared to the EGFR negative group (750 days [155–1345] vs. 278 days [30–526], *P* = 0.024). The difference in OS between those with WT EGFR and EGFR-UKN status was not significantly different [Figure 1a and b].

Median OS was also significantly different across the different histologic subgroups of adenocarcinoma (*P* = 0.013). Median OS was highest in patients with lepidic predominant ADC (median-not achieved), intermediate in those with acinar/papillary ADC (514 days [284–744]), and least in patients with a solid ADC (278 days [237–319]) [Figure 1c,e and f]. The difference was significant (*P* = 0.001) even when patients with Stage IV disease alone were compared [Figure 1d].

Factors associated with a worse survival on Cox univariate regression analysis were smoking, poor ECOG PS (≥2), EGFR mutation status, and a solid ADC, and those associated with a better survival were female sex and lepidic ADC. Two different models were used for multivariate analysis [Table 4]. In Model 1, factors which were significant on univariate analysis (*P* < 0.1) were

included. In this model, the only factor independently associated with a better OS was a lepidic subtype of adenocarcinoma (HR = 0.12, 95% CI = 0.01–0.99). In Model 2, we excluded the factors which were associated with EGFR mutation status (sex, smoking status, and histologic subgrouping) and included other variables which are usually associated with survival (age, TNM stage grouping, and PS). In this model, the factors independently associated with a worse OS were EGFR mutation negativity (HR = 2.39, 95% CI = 0.97–5.88), increasing age (HR = 1.02, 95% CI = 1.00–1.05), and poor PS (HR = 1.74, 95% CI = 0.98–3.10).

Similar survival analyses were performed including only patients with Stage IV disease [Supplementary Tables 3 and 4]. The factors associated with OS were similar both for univariate as well multivariate analyses.

DISCUSSION

The prevalence of EGFR AMs and ALK gene rearrangements was 16.6% and 2.3%, respectively, in our study cohort. The only factor independently associated with both the presence of EGFR AM, as well as OS was the new histopathologic subtyping of adenocarcinoma as per the IASLC/ATS/ERS 2011 classification.

The prevalence of EGFR AM (16.6%) in our North Indian cohort is less than that reported earlier from India (22–40%) and more similar to that seen in the Caucasians.[11,12,25-27] As the percentage of females (30–40%) and ADC (>90–100%)

reported in these earlier studies was similar to that seen in our study cohort, the possible reasons for the apparently lower prevalence of EGFR AM could be related to differences in (a) ethnicity, (b) percentage of never smokers, (c) method used, and (d) sample type tested.

This study included patients of the North Indian ethnicity while earlier studies which have reported the prevalence of EGFR AM in Indians has mainly included patients from South/Central India. Most Indian groups descend from a mixture of two genetically divergent populations: Ancestral North Indians related to Caucasians and Europeans; and ancestral South Indians not closely related to groups outside the subcontinent.[28] This differing ancestral origin might be one reason for the low prevalence of EGFR AM seen in our cohort. Similar differences in the EGFR AM prevalence between North and South Indians (68% vs. 41%) has been shown in a smaller study which included 55 patients with adenocarcinoma who were never/ex-smokers.[29] This geographical diversity among Indians also exists in the predominant histologic type of lung cancer detected (squamous vs. ADC) and the percentage of smokers and male sex in newly diagnosed lung cancers (higher in North Indians).[30]

Second, the percentage of never smokers in our study (49%) is less than that in the earlier studies (55–80%).[25-27] As EGFR AM are more commonly seen in never smokers, the lower percentage of never smokers in our cohort could be another reason for the lesser prevalence of EGFR AM. The other two factors responsible for the lower prevalence of EGFR AM in our study are possibly related to differences in the method (Scorpion ARMS vs. direct sequencing) and the sample (fresh specimens vs. FFPE tissues) used for EGFR mutation analysis. Earlier studies have shown that targeted methods like the Scorpion ARMS are more sensitive than direct sequencing in detecting EGFR AM.[31-33] Similarly, testing on FFPE blocks has been shown to have a greater rate of uninterpretable results and a lesser sensitivity as compared to fresh specimens. Studies from Indian patients using scorpion ARMS

method for detection of EGFR mutations have shown the prevalence of EGFR mutations to be higher (40–50%),[10,11] as compared to those studies in which DNA sequencing has been used (25–35%).[12,27]

This ratio of exon 19 deletions to exon 21 mutations is highly variable across populations and earlier studies from our subcontinent have shown this ratio to vary from 1.3:1 to 4.6:1.[12,25] The ratio in our cohort of 3.2:1 falls within this range.

Since the discovery of EGFR AM, they have been shown to be associated with specific clinicopathological characteristics namely female sex, never smokers, and adenocarcinoma histology.[34-36] Similar to these earlier reports, females and never smokers in our cohort had a higher prevalence of EGFR AM. In addition to these factors, studies have also described associations with age, tumor stage, smoking intensity, and duration since stopping smoking.[37] Two large epidemiologic studies, one each from Asians[8] and the Caucasians[37] have shown the frequency of EGFR AM to be higher in patients with Stage IV and Stages IIIB/IV, respectively. The frequency of EGFR AM in this study was observed to be higher in patients with Stage IV disease. The association between increasing age and EGFR AM is more controversial with studies showing conflicting reports. Some report no association between age and EGFR status,[8,37,38] whereas others report increasing[39,40] or decreasing[34,35,41] prevalence of EGFR AM with increasing age. In our study, we found no association between age at diagnosis and the frequency of EGFR AM. Whether the frequency of EGFR AM differs in current versus reformed smokers is also not clear. Similar to the study by Kim *et al.*,[41] we found no difference in the EGFR AM when people were stratified as current and reformed smokers. However in the study by Girard *et al.*, time since quitting smoking was shown to be an independent predictor of EGFR AM on multivariate analysis.[37]

In this study, we attempted to histologically subtype ADC on small biopsies. In clinical practice, treatment decision making depends on the histological type and subtype

Table 5: Studies reporting an association of IASLC/ATS/ERS 2011 adenocarcinoma histologic subtyping (when performed on small biopsy specimens) with EGFR mutations and survival

Author, year	Country	Number of patients with HPE subtyping	Predominant TNM stage	Sample used for HPE subtyping	Association of HPE subtype with EGFR status	Association of HPE subtyping with survival
Girard *et al.*, 2012[37]	USA	2392	Stages I-IV	Resected specimens and small biopsies	Papillary (46.4%) and Lepidic (32.2%) positively associated with EGFR AM on multivariate analysis	Not assessed
Kim *et al.*, 2014[41]	Korea	135	Stages I-IV	Small biopsies	Papillary (77.8%) and lepidic (61.1%) have higher prevalence as compared to Solid (16.7%)	Not assessed
Campos-Parra *et al.*, 2014[50]	Mexico	257	Stages IIIB-IV	Small biopsies	No significant association seen	OS, PFS and ORR better in high-grade ADC (solid/MP) as compared to intermediate grade ADC (acinar/lepidic)
Current study	India	131	Stages IIIB-IV	Small biopsies	Solid ADC (6.3%) negatively associated with EGFR AM on multivariate analysis	Lepidic ADC associated with better OS and solid ADC with worse OS

ADC: Adenocarcinoma, AM: Activating mutations, EGFR: Epidermal growth factor receptor, HPE: Histopathology examination, MP: Micropapillary, ORR: Objective response rate, OS: Overall survival, PFS: Progression-free survival, IASLC: International Association for the Study of Lung Cancer, ATS: American Thoracic Society, TNM: Tumor node metastasis

reported in small biopsy specimens which are assumed to represent the whole tumor. However in view of the heterogeneity observed in cases of lung adenocarcinoma, the relevance of applying the new IASLC/ATS/ERS classification to small specimens can be debated. Trejo Bittar *et al.* showed discrepancies between patterns in intraoperative frozen and permanent sections and attributed it to inadequate sampling and poor quality of frozen sections.[42] Moreover, there are no publications which have correlated biopsy patterns with patterns in the resected specimens.

Despite these limitations, the strongest and the only independent predictor of EGFR AM in our study was the histologic subtype of adenocarcinoma. In the multivariate analysis, when stratified by histologic subtyping, all other factors including sex and smoking status were no longer significant. Association between histologic subtypes of adenocarcinoma and EGFR mutation status has been described earlier in literature [Supplementary Table 5].[14,37,41,43-50] All the studies uniformly show that solid ADC is negatively associated with EGFR AM. Lepidic, papillary, micropapillary, and acinar subtypes are all positively associated with EGFR AM with different studies showing different subtypes to be associated with EGFR AM. Most of these studies have been from East Asian countries with limited reports from the Western world. Ours is the first study describing the association of EGFR mutations with HPE subtyping in Indian patients. Moreover, unlike the earlier studies in which HPE subtyping was done on resected specimens and included patients with Stages I-IIIA NSCLC, we included mainly patients with advanced stage NSCLC (Stages IIIB-IV) and classified the histology on small biopsy specimens. There are only a few studies which have assessed the predictive value of HPE subtyping on small biopsy specimens of which only one by Campos-Parra *et al.* had exclusively included advanced NSCLC patients (Stages IIIB-IV) patients [Table 5].[37,41,50]

Several earlier studies have also shown the new IASLC/ATS/ERS classification to be an independent predictor of survival and disease recurrence.[14-18,51-53] The results of these studies uniformly suggest that lepidic ADC has the best survival, whereas the solid/micropapillary ADCs have the least survival and a higher chance of recurrence. Most of these studies have been on surgically resected specimens and included patients predominantly in Stages I-IIIA. To the best of our knowledge, ours is the first study from Asia in which HPE subtyping has been independently associated with OS in a cohort of patients with predominantly advanced NSCLC (85% having Stage IIIB/IV disease) and managed nonsurgically. The Campos-Parra study, the only other study assessing the association of HPE subtyping on small biopsy specimens with OS-had shown high-grade ADC (solid and micropapillary ADC) to be having better progression-free survival and OS as compared with intermediate grade ADC (lepidic and

acinar) something which is in contradiction to earlier published reports, as well as with our results.[50] It is unlikely that differences in ethnicity of patient populations or in chemotherapy protocols used between ours and the Campos-Parra study (pemetrexed-based [current study] vs. nonpemetrexed-based regimen) could account for the contradictory results observed. The results of our study also highlight the fact that accurate subclassification of ADC subtype as per the IASLC/ATS/ERS classification, even when done on small biopsy specimens, has both prognostic value, as well as a predictive value for EGFR mutations.

There are a few limitations of this study. First, the frequency of noninterpretable EGFR reports (15%) is higher than that reported from the Western world. However, earlier studies from the Indian subcontinent have shown almost similar rates (11%) of uninterpretable samples.[8] This could be because of poor processing, especially of the cytology samples and use of a less sensitive method to detect EGFR mutations (DNA sequencing versus ARMS, and FFPE specimens vs. fresh biopsy samples). Second, the current cohort of patients is not a consecutive patient analysis. Hence, it can be argued that the estimated prevalence of EGFR AM might not represent the true population prevalence. Unfortunately, the earlier studies from India describing the prevalence of EGFR AM were also not on consecutive patients. However, as the percentage of females (34.9%) and never smokers (48%) in this study is similar to that reported by us in a recent epidemiologic study involving consecutive lung cancer patients (31% females and 42% nonsmokers among adenocarcinoma),[4] the patient cohort in this study is likely a true representation of the entire lung cancer population at our center.

CONCLUSION

The results of this study suggest that the prevalence of EGFR AM in North India is similar to that reported among Caucasians, and that presence of EGFR AM is a positive prognostic marker for OS. The IASLC/ATS/ERS 2011 histologic subtyping of adenocarcinoma, even when performed on small biopsy specimens, is an independent predictor of the presence of EGFR AM and also of better OS in patients with advanced NSCLC.

Conflicts of interest

There are no conflicts of interest.

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