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EGFR mutations and *ROS1* and *ALK* rearrangements in a large series of non-small cell lung cancer in South India

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[Corrections added on 26 November 2020, after first online publication: The word "lung" has been added to the article title and the word "cell" included in the Aim statement within the abstract.]

Abstract

Background: Driver mutations are seen in 80% of lung adenocarcinomas, and they influence prognosis and choice of therapy.

Aim: Aim of this study was to analyse the frequency of epidermal growth factor receptor *(EGFR)* mutations, *ALK* and *ROS1* rearrangements and their association with age and gender in non-small cell lung cancer reported from a tertiary care center in South India.

Methods: Tumors from patients with non-small cell carcinoma of lung were evaluated for *EGFR* mutations, *ALK* and *ROS1* rearrangements and their association with age and gender were studied.

Results: Two thirds of non-small cell carcinomas had driver mutations or rearrangements. *EGFR* mutation was common and seen in 34.1%, whereas *ALK* rearrangement was seen in 11.1% and *ROS1* rearrangement in 2% patients. Among *EGFR* mutations, most common were Exon 19 deletion and L858R seen in 21.3% and 11% of patients, respectively. Adenocarcinoma was the histologic diagnosis in 81% to 85% of patients with exon 19 deletion and L858R mutation, respectively. *EGFR* mutation frequency in patients less than 36 years was 13.6%, whereas in older patients, it varied from 34% to 36%. Exon 19 deletion was seen in 29.8% females and 17.2% of males.

Conclusion: *EGFR* mutations are more common than *ALK* and *ROS1* rearrangements. They are more common in females. Patients less than 36 years have reduced frequency of *EGFR* mutations. Exon 19 deletion and L858R are most common and are more prevalent in lung adenocarcinomas. Rare *EGFR* mutations are seen in patients aged more than 50 years.

KEYWORDS

ALK, *EGFR*, non-small cell lung carcinoma, *ROS1*

1 | INTRODUCTION

Epidermal growth factor receptor (*EGFR*) mutations have been reported in pulmonary adenocarcinomas and can be targeted by tyrosine kinase inhibitors.[1,2] The most common *EGFR* mutations are exon 19 deletions and single-point substitution L858R in exon 21. They are seen in 41% to 44% of non-small cell lung cancer.[3] Less common mutations which are less responsive to tyrosine kinase inhibitors are G719X in exon 18, deletions in exon 20 (4% of cases) and L861Q in exon 21 (2% of cases).[4-6] Acquired resistance to tyrosine kinase inhibitors is known to occur during progression of which the commonest reported is the T790M mutation. This mutation has been observed in 60% of patients.[7,8]

There is variation in prevalence of *EGFR* mutations across countries and regions (20%-76% in Asia Pacific region, 6%-41% in Europe, 3%-42% in North America, 22%-27% in Indian subcontinent, and 9%-67% in South America).[9] In Middle East and Africa, the reported prevalence is around 21.2%.[10] In central Europe, the reported prevalence is 4.9%, and adenocarcinoma is the commonest histologic pattern.[11] Worldwide prevalence of activating mutations as well as resistance mutations in *EGFR* is higher in Asia than other regions.[12] In India, reported incidence of *EGFR* mutations is 31% to 51.8%.[13,14] *ALK* rearrangements have been reported in nearly 3% to 8% with prevalence in younger patients.[15-17] The reported prevalence in India is 2.7% to 3%.[18,19] *ROS1* rearrangements have been reported in 1% to 2% of non-small cell lung cancer.[20,21] In India, the reported incidence of *ROS1* rearrangement is around 2.8%.[22]

Currently, guidelines for non-small cell carcinoma recommend testing for *EGFR*, *ROS1*, *ALK*, *BRAF*, and *PD-L1*.[23-25] However, new emerging biomarkers, namely, *HER2*, *MET*, *RET*, *NTRK*, and *TMB*, necessitate comprehensive molecular studies including next generation sequencing.[26] Recent meta-analysis has investigated the role of other biomarkers such as Golgi phosphoprotein 3 and ERCC1 protein in non-small cell cancer of lung.[27,28]

The aim of this study was to analyse the frequency of EGFR mutations, *ALK* and *ROS1* rearrangements and their association with age and gender in non-small cell lung cancer.

2 | MATERIALS AND METHODS

Patients with histological diagnosis of non-small cell carcinoma lung were included in the study. Samples included biopsies from the primary site as well as metastatic sites.

Slides from paraffin-embedded tissue blocks were screened for presence of tumor. Once presence of tumor was confirmed, formalin-fixed paraffin-embedded (FFPE) sections were collected on glass slides. This was followed by proteinase K digestion. DNA was extracted from freshly cut FFPE tissue using QIAamp FFPE tissue DNA extraction kit (Qiagen) following the manufacturer's instruction and eluted in ATE buffer. *EGFR* mutational analysis was performed using Therascreen *EGFR* RGQ PCR Kit (Qiagen). The Therascreen *EGFR* RGQ PCR kit allows the detection of 29 somatic mutations in the *EGFR* oncogene by combining Scorpions and Amplification Refractory Mutation System dual primer probes. Samples were processed according to the manufacturer's protocol, using the Rotor-Gene Q real-time PCR cycler (Qiagen). The cycling times were 95°C for 15 minutes for 1 cycle, 95°C for 30 seconds and then 60°C for 60 seconds for 40 cycles. The obtained data were analysed with the Rotor-Gene Q Series Software (Qiagen).

ALK gene rearrangement was detected by fluorescence in situ hybridisation (FISH) technique using Vysis *ALK* Break Apart FISH Probe Kit (CE-IVD marked, Abbot Molecular). Five micrometers thin paraffin-embedded tissue sections were mounted on positively charged slides, dehydrated in xylene and alcohol, hybridized with the probe and incubated overnight. They were then counterstained with DAPI (4,6 diamidino-2-phenylidole) and visualized under fluorescence (Cytovision system capture station software 7.4 v Leica fluorescent microscope) microscope. Signals from 50 cells were counted, and rearrangement was considered to be present if more than 25 cells recorded positive. If 5 to 25 cells were positive, then it was considered equivocal (Figure 3). *ROS1* rearrangements were detected by FISH using 6q22 *ROS1* Break Apart FISH Probe RUO Kit. Deparaffinized tumor sections were dehydrated and hybridized with the probe, counterstained and visualized under fluorescence. Fifty cells were scored, and rearrangement was considered to be present if

TABLE 1 Distribution of various *EGFR* mutations by gender in the study group

EGFR mutation (n = 748)	Number (%)	Males n = 510 (%)	Females n = 238 (%)	Median age in years	*P* value
No mutation	488 (65.5)	359 (70.3%)	129 (54.2)	62	<.001
Exon 19 deletion	159 (21.3)	88 (17.2)	71 (29.8)	60.5	<.001
L858R	82 (11)	51 (10)	31 (13)	63	.2
G719X	12 (1.6)	6	6	64	—
Exon 20 mutation	3 (0.4)	2	1	67	—
L861Q	3 (0.4)	3	0	63	—
S768I	1 (0.1)	1	0	—	—

Note: Among 510 males, 151 (29.6%) had *EGFR* mutation of which commonest was exon 19 mutation seen in 17.2% males, followed by L858R mutation seen in 10.0%. The remaining four mutations (G719X, Exon 20 mutation, L861Q, and S768I) together were seen in 2.3% of males. Among 238 females, 109 (45.7%) had *EGFR* mutation of which commonest was exon 19 mutation seen in 29.8% females, followed by L858R mutation seen in 13.0%. The remaining four mutations (G719X, Exon 20 mutation, L861Q, and S768I) together were seen in 2.9% of females. Females significantly outnumber males in exon 19 deletion ($P < .001$).

TABLE 2 Histological diagnosis of tumors with *EGFR* mutations

Mutation/deletion	Adenocarcinoma, n = 602	Adenosquamous, n = 8	Squamous, n = 3	Metastases, n = 64	Carcinoma/poorly differentiated/ non-small cell, n = 71
No mutation (n = 488)	386 (79.0%)	5 (1.02%)	2 (0.40%)	46 (9.4%)	49 (10.0%)
Exon 19 (n = 159)	134 (84.2%)	1 (0.6%)	0	7 (4.4%)	17 (10.6%)
L858R (n = 82)	70 (85.3%)	1 (0.12%)	0	8 (9.7%)	3 (3.6%)
G719X (n = 12)	8 (66.6%)	1 (8.3%)	0	2 (16.6%)	1 (8.3%)
L861Q (n = 3)	2 (66.6%)			1	
S768I (n = 1)	1 (100%)				
Exon 20 mutation (n = 3)	1 (33.3%)		1 (33.3%)		1 (33.3%)

Note: 66.6% to 85.3% of patients with exon 19 deletion/L858R mutation/G719X mutation were diagnosed to have adenocarcinoma histologically. 9 to 22.5% of patients diagnosed with adenosquamous carcinoma/metastases/poorly differentiated carcinoma had either exon 19 deletion or L858R mutation or G719X mutation. Percentages in each cell have been calculated for the overall number of mutations in each row.

25 or more cells showed positivity. If less than five cells showed positive pattern, then the tumor is negative for *ROS1* rearrangement (Figure 4).

2.1 | Statistical analysis

The data analysis was generated using the Real Statistics Resource Pack software (Release 6.8). Copyright (2013-2020). Data were segregated into categorical and continuous variables. Categorical variables were expressed as percentages. Continuous variables were expressed as mean when normally distributed and as median when the distribution was not normal. Categorical variables were compared using Chi-square test, whereas continuous variables were compared using Kruskal-Wallis test (for data which were not normally distributed). NCSS2020 software was used for cluster analysis.

3 | RESULTS

There were 748 patients with histologically diagnosed lung cancer. 510 (68.1%) were males and 238 (31.8%), females. Age varied from 20 to 90 years with a median of 62 years. Primary tumor sites were tested in 684 (91.4%) and metastatic sites in 64 (8.5%) patients. Histologic diagnosis was adenocarcinoma in 602 (80.4%) patients, poorly differentiated/non-small cell carcinoma in 71 (9.4%), adenosquamous in 8 (1.0%) and squamous cell carcinoma in 3 patients (0.4%).

EGFR mutation (Table 1): 260 (34.1%) were positive for *EGFR* mutation. There were 151 (58.0%) males and 109 (41.9%) females with age varying from 29 to 90 years. 29.6% of males in the study (151/510) and 45.7% (109/238) females in the study had *EGFR* mutations. Among 260 patients, adenocarcinoma was the commonest histology in 213 patients (81.9%). Exon 19 deletion was detected in 159 patients (61.1% of patients with *EGFR* mutations), L858R in 82 (31.5% of *EGFR* imutations), G719X in 12 (4.6% of *EGFR* mutations), exon 20 insertion and L861Q in three patients each (1.1% of *EGFR* mutations) and S768I in one patient (0.3% of *EGFR* mutations) (Table 1). Among 159 patients with exon 19 deletion, there were 88 males and 71 females with age varying from 29 to 90 years. In patients with exon 19 deletion, 84.2% were diagnosed to have adenocarcinomas, 10.6% were diagnosed as poorly differentiated carcinomas, 4.4% as metastases, and 0.6% as adenosquamous carcinoma (Table 2). Among patients with exon

TABLE 3 Distribution of *ALK* and *ROS1* rearrangements in the study

Mutation/*EGFR*/ tumor type and demographic characteristics	*ALK* positive (number tested)	*ROS1* positive (number tested)
No mutation/deletion, n = 488	34 (198)	4 (127)
Exon 19 deletion, n = 159	0 (71)	0 (49)
L858R, n = 82	0 (26)	0 (16)
G719X, n = 12	0 (5)	0 (1)
L861Q, n = 3	0 (2)	0 (2)
Exon 20 mutation, n = 3	0 (2)	—
Age range in years	27-78	40-63
Male: Female ratio	1.8:1	4 (2%)*
Adenocarcinoma, n = 21 (%)	19 (55.8%)	2 (50%)
Metastases, n = 12	12 (35.2%)	0
Poorly differentiated carcinoma, n = 5	3 (8.8%)	2 (50%)

Note: ALK rearrangement by FISH was seen in 34/304 patients (11.1%). *ROS1* rearrangement by FISH was seen in 4/195 patients (2.0%). Among 34 patients positive for *ALK* rearrangement, 55.8% were histologically diagnosed to have adenocarcinoma, whereas, 44.1% were diagnosed to have metastases or poorly differentiated carcinoma. In four patients with *ROS1* rearrangement, two were diagnosed to have adenocarcinomas, and the remaining two were diagnosed to have poorly differentiated carcinoma. Among 64 patients with metastases, 30 (46.8%) had one of the driver mutations (28.1% with *EGFR* and 18.7% with *ALK* rearrangement). 38.0% of poorly differentiated carcinomas had one of the three driver mutations (30.9% with *EGFR*, 4.2% with *ALK* rearrangement and 2.8% with *ROS1* rearrangement).
Abbreviation: FISH, fluorescence in situ hybridization.
*In this group, all four were males and no male female ratio could be calculated.

19 deletion, four (2.5%) patients had T790M mutation. Among the 82 patients with L858R mutation, there were 51 males and 31 females varying in age from 37 to 90 years. The histological diagnosis in these patients was adenocarcinoma in 85.3%, metastases in 9.7%, poorly differentiated carcinoma in 3.6% and adenosquamous carcinoma in 1.2%. Three patients (3.6%) had T790M mutation in addition to L858R mutation.

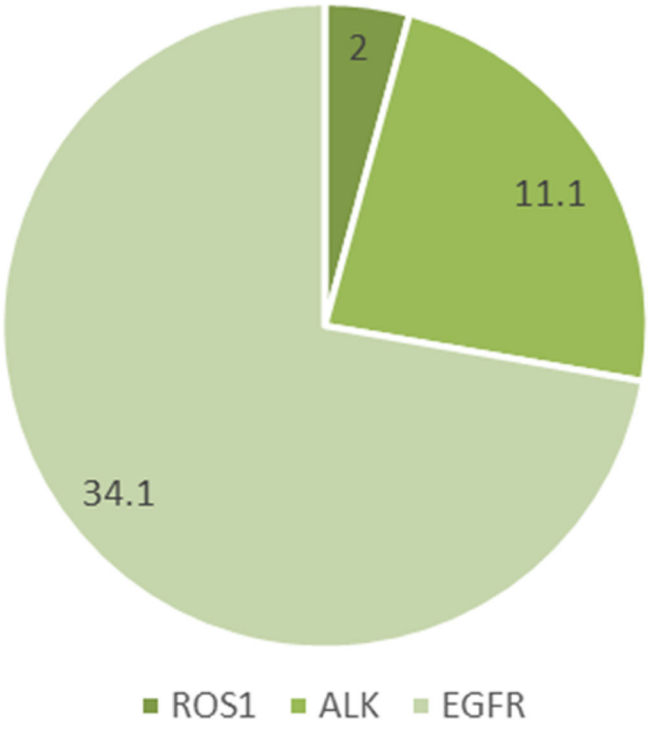

FIGURE 1 Frequency of the three driver mutations in non-small cell lung cancer. *EGFR* is most common (34.1%), followed by *ALK* (11.1%) and *ROS1* (2%)

There were six males and six females with G719X mutation with age varying from 41 to 82 years. Among these 12 patients with G719X mutation, 66.6% had histologically diagnosed adenocarcinomas, 16.6% had metastases, 8.3% had poorly differentiated carcinoma, and 8.3% had adenosquamous carcinoma. L861Q mutation was seen in three male patients in the seventh decade. Among these patients, adenocarcinoma was the histological diagnosis in 66.6% and metastases in 33.3%. S768I mutation was seen in one male patient aged 63 years, and the histological diagnosis was adenocarcinoma. Exon 20 mutation was seen in three males in the seventh decade, and the histological diagnosis was adenocarcinoma in 33.3%, squamous cell carcinoma in 33.3% and poorly differentiated carcinoma in 33.3%.

EGFR mutation was detected in 260 (34.1%) patients. *ALK* was positive in 34 cases (11.1%), and *ROS1* was positive in four cases (2.0%) (Table 3 and Figure 1). In 98 patients (76 male, 22 female with median age of 65 years), *EGFR* mutation and *ROS1* and *ALK* rearrangement were absent. By hierarchical clustering, the study group formed nine clusters (Figure 2) with respect to tumor type (adenocarcinoma, poorly differentiated carcinoma, metastases, adenosquamous, and squamous cell carcinoma). However, none of the clusters were specific for any mutation or rearrangement. Demographic characteristics, histological diagnosis and FISH images of cases with *ALK* and *ROS1* positivity are summarized in Table 3 and Figures 3 and 4.

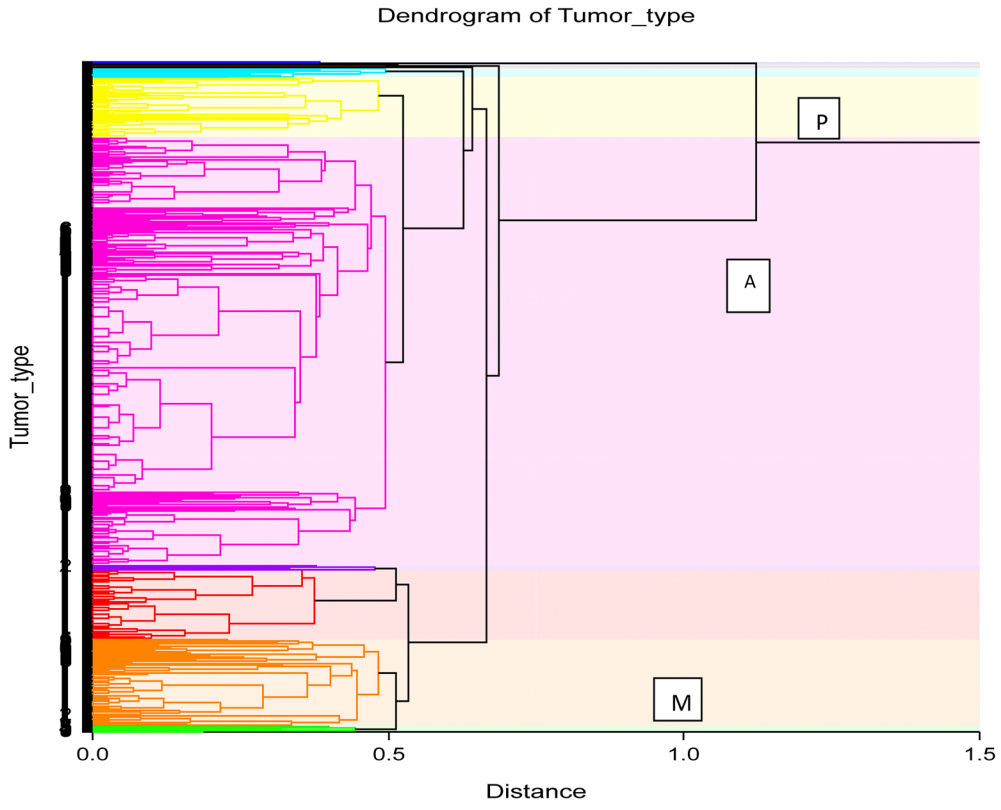

FIGURE 2 Dendrogram displaying clusters by tumor types (adenocarcinoma, poorly differentiated carcinoma, squamous cell carcinoma, and adenosquamous carcinoma). At distance of 0.5, we get nine clusters (cophenetic correlation = 0.67, agglomerative hierarchical clustering with Euclidean distance). The largest clusters are labeled as: A, adenocarcinoma; M, metastases; P, poorly differentiated carcinoma. Smaller clusters are formed by adenosquamous and squamous cell carcinoma

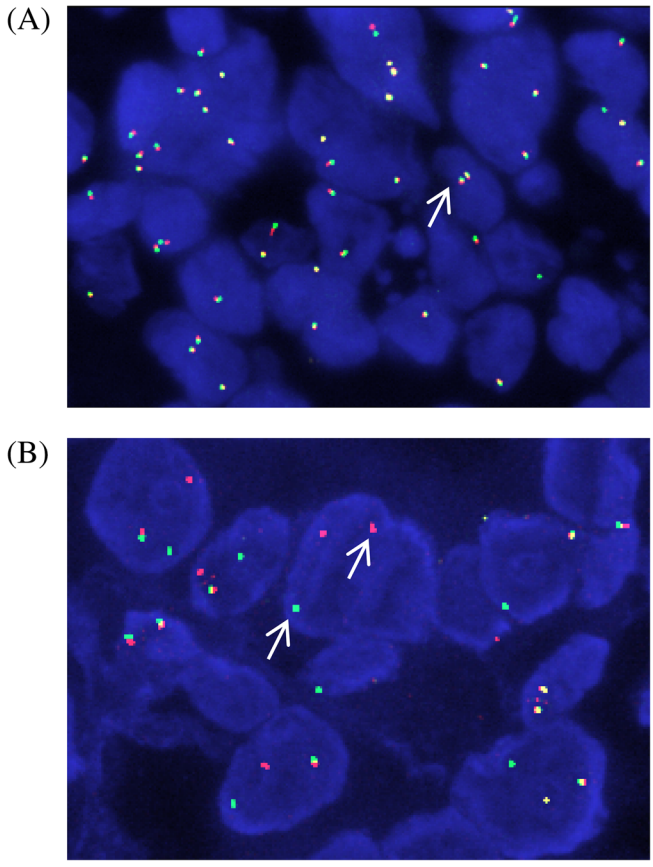

FIGURE 3 FISH analysis using *ALK* dual-color break apart FISH probes to detect *ALK* fusion as split orange and green signals. A, Sections were considered negative for rearrangement when orange and green signals appeared adjacent (indicated by arrow) to each other or yellow (fused) signals were seen. B, Rearrangement was considered to be present when the green and orange signals are two to three signals apart (indicated by arrows) or one orange signal without the corresponding green signal along with another fused signal is seen (magnification ×630). FISH, fluorescence in situ hybridization

Among the different age groups, *EGFR* mutations were equally distributed among the various age group except in the 20- to 35-year age group (13.6%). Exon 19 deletion was most common in the 20-to 35-year age group (100%) which reduced with increasing age (Table 4 and Figure 2). L858R mutation was not seen in the 20-to 35-year age group and gradually increased with age. Other mutations G719X, L861Q, S768I, exon 20 insertion and T790M were not seen in the 20-to 35-year age group. *ALK* and *ROS1* rearrangements were most common in 36- to 50-year age group (23% and 7.4%, respectively). Exon 19 deletion frequency in males (17.2%) was less than females (29.8%), chi square = 15.3, $P < .001$, whereas, absence of mutations in *EGFR* was significantly more common in males (70.3%) than females (54.2%), chi square = 18.7, $P < .001$. L858R mutation was equally seen in males (10%) and females (13%), chi square = 1.5, $P = .2$. Age distribution among the different groups (excluding exon 20 mutation, L861 Q and S768I as

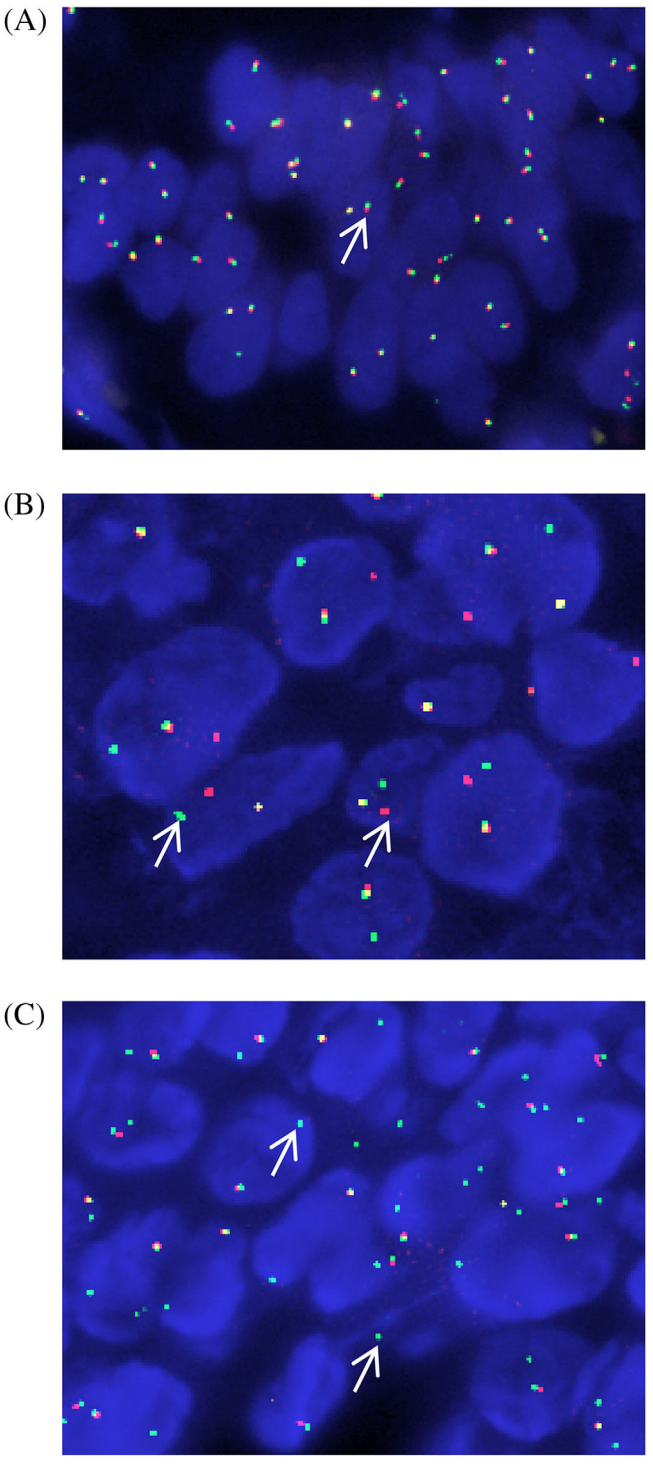

FIGURE 4 FISH analysis using *ROS1* dual-color break apart FISH probes to detect *ROS1* fusion. A, Negative patterns were identified as two fused signals per nuclei (marked by arrow). Positive patterns include one fused signal along with one separate orange and green signal (arrows mark the separated spectrum orange and spectrum green signal) or when one isolated green signal (indicated by arrow) and one fused signal is seen. B,C, Spectrum orange binds telomeric to *ROS1* gene, and spectrum green binds centromeric to it (magnification ×630). FISH, fluorescence in situ hybridization

TABLE 4 Distribution of the three driver mutations across various age groups

Age group in years	Number of patients, n = 748	*EGFR* mutation, n = 748	*ALK*, n = 304	*ROS1*, n = 195
20-35	22 (2.9%)	3 (13.6%)	7 (5.3%)	0
36-50	101 (13.5%)	35 (34.6%)	9 (23.0%)	2 (7.4%)
51-65	254 (33.9%)	127 (36.3%)	11 (8.3%)	2 (2.4%)
66-80	349 (46.6%)	88 (34.6%)	7 (6.3%)	0 (0)
81-95	22 (2.9%)	8 (36.3%)	0 (0)	0 (0)

Note: *EGFR* frequency is almost similar across all age groups (34.6%-36.3%) except for lower frequency (13.6%) in patients aged 35 years or less. *ALK* rearrangement peaks at 36 to 50 years, whereas, *ROS1* rearrangement is infrequent.

the number of patients in these groups were very less) was not significantly different by Kruskal-Wallis test, $H = 4.48$,[3] $P = .21$.

4 | DISCUSSION

As early as 2005, higher frequency of *EGFR* activating mutations had been reported in Asian females with no smoking history.[29] In a large study including many Asian countries, EGFR mutation frequency was nearly 60%.[30] Lower incidence of *EGFR* mutations were reported in non-Asians when compared to Asian patients (25% vs 39%).[31] Exon 18 mutations of *EGFR* are seen more frequently in Asia. Exon 19 deletions and L858R mutations have been commonly reported in southern Asia, and L861Q mutations have been reported from northern Asia.[12] In a European study on 552 patients, *EGFR* mutations were seen in 4.9%.[11] In this study, exon 19 deletions were seen in 56%, exon 21 mutations in 30% and exon 18 mutations in 11%.[11] Women had higher frequency of *EGFR* mutations than men (8.5% vs 2.8%). Among non-small cell lung cancers, the commonest histological pattern with *EGFR* mutations was adenocarcinoma (8.5%). A small proportion of squamous cell carcinomas (1.1%) showed *EGFR* mutations.[11] No significant age difference was observed in patients with *EGFR* mutation (mean 70.3 years) and patients with wild-type EGFR (mean 66.7 years). In a large Spanish study on 2105 patients with non-small cell cancer, *EGFR* mutations were seen in 16.6%. *EGFR* mutations were more common in women (69.7%), and the predominant histological type was adenocarcinoma (80.9%). Among patients with mutations, 27.1% were less than 57 years, 30.1% were between 56.7 and 69.1 years and 42.8% were more than 69 years of age.[32] In an Indian study by Sahoo and others, *EGFR* mutations were seen in 51.8% of non-small cell carcinoma. Commonest mutations were exon 19 deletion (52%) and L858R deletion (26%).[14] In another Indian study, using immunohistochemistry, exon 19 and L858R mutations were seen in 26.6% patients.[33] Among 907 Indian patients, *EGFR* mutations were seen in 23% with a female preponderance (29.8% vs 20.4% in males), and the predominant histological pattern was adenocarcinoma (25.9%).[34] In the same study, *EGFR* mutations were seen in 3.8% squamous cell carcinoma. Data from Japan and East Asia indicate a prevalence of 27% to 30% *EGFR* mutation positivity in non-small cell lung cancers.[35]

In our study, *EGFR* imutations were present in 34.1% which is nearly similar to the data from Japan and east Asia[35] and Indian population.[33] However, it is slightly higher than that reported in the study by Chougule A (23%) and is less than that reported by Sahoo et al.[14] The prevalence of *EGFR* mutations is more than the Spanish population (16.6%) and European population (4.9%).[11,32] However, this variation in prevalence and higher incidence in Asia have been previously reported in literature.[9] In the present study, females had a higher prevalence of *EGFR* mutations (45.7%) compared to males (29.6%). This is similar to data from Europe, Spain and other Indian studies.[11,32,34] Like other studies,[11,32,34] adenocarcinoma was the commonest histological pattern in our study (81.9%). In the present study, the commonest mutations were exon 19 deletion and L858R mutation seen in 21.3% and 11% patients, respectively, which is similar to previous studies.[11,14,33]

A few observers reported increasing incidence of *EGFR* mutations with age.[36] Others reported a reduced frequency of *EGFR* mutations in patients less than equal to 50 years ($P = .04$) and a higher frequency of uncommon mutations ($P = .03$).[37] However in our study, we found uncommon mutations of *EGFR* (G719X, L861Q, S768I, and exon 20 insertion) in patients aged above 50 years. This difference may be due to the fact that 83.5% of our patients were aged above 50 years.

Globally, *ALK* rearrangements have been reported in 2.7% to 8% of non-small cell lung cancers.[15-19] In our study, we found *ALK* rearrangements in 11.1%. The slightly higher prevalence may be explained by the fact that we did not test the whole study group and we used FISH for detection of *ALK* rearrangements. *ALK* rearrangements have been seen more commonly in younger age group with median age of 51 years.[17] Similarly, in our study, the median age group of patients was 48 years with a range from 27 to 78 years. In the study by Kwak et al, adenocarcinoma was the most prevalent histology in 96% of *ALK* positive lung cancers.[17] In our study, adenocarcinomas were seen in 55.8% (Tables 2,3). We did not classify metastatic tumors histologically, which could be the reason for the lower incidence of adenocarcinoma as compared to previous study.[17] Our prevalence of *ROS1* rearrangements (2%) is similar to that reported elsewhere in literature.[20,21]

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Anil Tarigopula: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; supervision; validation; visualization; writing-original draft; writing-review and editing. **Gayathri Ramsubban:** Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing-original draft; writing-review and editing. **Vani Chandrashekar:** Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing-original draft; writing-review and editing. **Perumal Govindasami:** Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing-original draft; writing-review and editing. **Chitra Chandran:** Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing-original draft; writing-review and editing.

ETHICS STATEMENT

Data were obtained from hospital information system and anonymized. Samples were collected after obtaining informed consent from patients. Approval for the study and publication was obtained retrospectively from the Institution Ethics Committee (approval number: AMH-C-S-013/07-20).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350(21):2129-2139.
2. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304(5676):1497-1500.
3. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene*. 2009;28:S24-S31.
4. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol*. 2012;13:e23-e31.
5. Arrieta O, Cardona AF, Corrales L, et al. CLICaP the impact of common and rare EGFR mutations in response to EGFR tyrosine kinase inhibitors and platinum-based chemotherapy in patients with non-small cell lung cancer. *Lung Cancer*. 2015;87:169-175.
6. Baek JH, Sun JM, Min YJ, et al. Efficacy of EGFR tyrosine kinase inhibitors in patients with EGFR-mutated non-small cell lung cancer except both exon 19 deletion and exon 21 L858R: a retrospective analysis in Korea. *Lung Cancer*. 2015;87:148-154.
7. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res*. 2013;19:2240-2247.
8. Riely GJ, Yu HA. EGFR: the paradigm of an oncogenedriven lung cancer. *Clin Cancer Res*. 2015;21:2221-2226.
9. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). *Am J Cancer Res*. 2015;5(9):2892-2911.
10. Benbrahim Z, Antonia T, Mellas N. EGFR mutation frequency in Middle East and African non-small cell lung cancer patients: a systematic review and meta-analysis. *BMC Cancer*. 2018;18(1):891.
11. Boch C, Kollmeier J, Roth A, et al. The frequency of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC): routine screening data for Central Europe from a cohort study. *BMJ Open*. 2013;3(4):e002560.
12. Graham RP, Treece AL, Lindeman NI, Vasalos P, Shan, M, Jennings LJ, Rimm DL. Worldwide frequency of commonly detected egfr mutations. *Arch Pathol Lab Med*. 2018;142(2):163-167. http://dx.doi.org/10.5858/arpa.2016-0579-cp.
13. Noronha V, Prabhash K, Thavamani A, et al. EGFR mutations in Indian lung cancer patients: clinical correlation and outcome to EGFR targeted therapy. *PLoS One*. 2013;8:e61561.
14. Sahoo R, Harini VV, Babu VC, Patil Okaly GV, Rao S, et al. Screening for EGFR mutations in lung cancer, a report from India. *Lung Cancer*. 2011;73:316-319.
15. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-*ALK* fusion gene in non-small-cell lung cancer. *Nature*. 2007;448:561-566.
16. Johnson BE, Kris MG, Berry LD, et al. A multicenter effort to identify driver mutations and employ targeted therapy in patients with lung adenocarcinomas: the lung cancer mutation consortium (LCMC). *J Clin Oncol*. 2013;31:8019.
17. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer [published correction appears in N Engl J Med. 2011 Feb 10;364(6):588]. *N Engl J Med*. 2010;363(18):1693-1703.
18. Desai SS, Shah AS, Prabhash K, Jambhekar NA. A year of anaplastic large cell kinase testing for lung carcinoma: pathological and technical perspectives. *Indian J Cancer*. 2013;50:80-86.
19. Doval D, Prabhash K, Patil S, et al. Clinical and epidemiological study of EGFR mutations and EML4-*ALK* fusion genes among Indian patients with adenocarcinoma of the lung. *Onco Targets Ther*. 2015;8:117-123.
20. Lin JJ, Shaw AT. Recent advances in targeting *ROS1* in lung cancer. *J Thorac Oncol*. 2017;12:1611-1625.
21. Bergethon K, Shaw AT, Ou SH, et al. *ROS1* rearrangements define a unique molecular class of lung cancers. *J Clin Oncol*. 2012;30:863-870.
22. Suryavanshi M, Panigrahi MK, Kumar D, et al. *ROS1* rearrangement and response to crizotinib in stage IV non-small cell lung cancer. *Lung India*. 2017;34:411-414.
23. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2019;129:iv192-iv237.
24. Kalemkerian GP, Narula N, Kennedy EB, et al. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. *J Clin Oncol*. 2018;36:911-919.
25. NCCN. Non-small cell lung cancer. *NCCN Clinical Practice Guidelines in Oncology*. Cold Spring Harbor, New York: Harborside Press, LLC; 2018. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf.
26. Garrido P, Conde E, de Castro J, et al. Updated guidelines for predictive biomarker testing in advanced non-small-cell lung cancer: a

National Consensus of the Spanish Society of Pathology and the Spanish Society of Medical Oncology. *Clin Transl Oncol*. 2019;22:989-1003. https://doi.org/10.1007/s12094-019-02218-4.
27. Shi W, Feng W, Wang J, et al. Clinicopathologic features and prognostic implications of Golgi phosphoprotein 3 in non-small cell lung cancer: a meta-analysis. *J Cancer*. 2019;10(23):5754-5763.
28. Li G, Chen D. Meta-analysis of ERCC1 protein expression and platinum chemosensitivity in non-small-cell lung cancer. *Evid Based Compl Alt Med*. 2020:7376568.
29. Hsieh RK, Lim KH, Kuo HT, Tzen CY, Huang MJ. Female sex and bronchioloalveolar pathologic subtype predict EGFR mutations in non-small cell lung cancer. *Chest*. 2005;128(1):317-321.
30. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009;361(10):947-957.
31. Girard N, Sima CS, Jackman DM, et al. Nomogram to predict the presence of EGFR activating mutation in lung adenocarcinoma. *Eur Respir J*. 2012;39(2):366-372.
32. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med*. 2009;361(10):958-967.
33. Jain D, Iqbal S, Walia R, et al. Evaluation of epidermal growth factor receptor mutations based on mutation specific immunohistochemistry in non-small cell lung cancer: a preliminary study. *Indian J Med Res*. 2016;143(3):308-314.
34. Chougule A, Prabhash K, Noronha V, et al. Frequency of *EGFR* mutations in 907 lung adenocarcioma patients of Indian ethnicity. *PLoS One*. 2013;8:e76164.
35. Yang P-C, Yuankai S, Au J S-k, et al. Molecular epidemiological prospective study of EGFR mutations from Asian patients (pts) with advanced lung adenocarcinoma. *J Clin Oncol*. 2012;30:1534-1534.
36. Ueno T, Toyooka S, Suda K, et al. Impact of age on epidermal growth factor receptor mutation in lung cancer. *Lung Cancer*. 2012;78(3):207-211. https://doi.org/10.1016/j.lungcan.2012.09.006.
37. Wu SG, Chang YL, Yu CJ, Yang PC, Shih JY. Lung adenocarcinoma patients of young age have lower EGFR mutation rate and poorer efficacy of EGFR tyrosine kinase inhibitors. *ERJ Open Res*. 2017;3(3):00092-2016.

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