International Journal of Medical Science and Clinical Invention 8(11): 5795-5801, 2021
DOI:10.18535/ijmsci/v8i11.07
https://valleyinternational.net/index.php/ijmsci

Research Article,

A Hospital-Based Study of EGFR and ALK Mutations in Patients with Lung Adenocarcinoma in Odisha

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Abstract:

Background: Patients with advanced non-small cell lung cancer (NSCLC) have considerably benefited from the molecular identification of epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) mutations and subsequent targeted therapy against these biomarkers. Few studies have been undertaken in the Indian population on the analysis of both EGFR and ALK mutations in lung adenocarcinoma cases.

Aim and Objectives: The aim of this study was to determine the prevalence of EGFR and ALK mutations in lung adenocarcinoma patients, as well as to link mutational status with age, sex, and smoking history.

Materials and Methods: This single hospital-based retrospective study was conducted at the Department of Medical Oncology, Acharya Harihar Post Graduate Institute of Cancer, Cuttack, on histologically proven lung adenocarcinoma cases over a duration of two years from 01.08.2019 to 31.07.2021.

Results: Out of a total of 164 cases, males comprised 89 (54.26%) of the 164 lung adenocarcinoma cases, while females comprised 75 (45.73%). EGFR mutations were found in 42 (26.75%) of the patients. In 9 cases, the ALK gene rearrangement was also determined to be positive (5.66%). In terms of EGFR and ALK mutations, there was no statistically significant relationship between patient age and gender. (P-value < 0.05). In our research, we found a link between nonsmokers and EGFR and ALK mutations. (P-value <0.05). The deletion of exon 19 (76.19%) was the most prevalent mutation, followed by the exon 21 L858R mutation (14.28%).

Conclusion: This study was found to have a significantly higher rate of EGFR and ALK mutation in the Indian population with adenocarcinoma of lung compared to Western populations. To get the maximum benefit from targeted therapies, all patients of adenocarcinoma of the lung should have mutational testing for EGFR and ALK as part of a broad molecular pannel.

Keywords: Anaplastic lymphoma kinase, epidermal growth factor receptor, lung adenocarcinoma

Introduction:

Lung cancer (LC), also known as bronchogenic carcinoma, is a kind of cancer that begins in the parenchyma or bronchi of the lungs. It is one of the most commonly diagnosed malignancies and the leading cause of cancer-related deaths worldwide, with an estimated 2 million new diagnoses and 176 million deaths per year. [1] In India, lung cancer accounts for 5.9% of all cancers and 8.1% of all cancer-related deaths.[2] The pathophysiology of LC is exceedingly complex and poorly understood. Lung epithelial dysplasia is caused by repeated exposure to chemicals, mainly cigarette smoke. If the exposure continues, it leads to genetic mutations and affects protein synthesis. The cell cycle is interrupted as a result, which promotes carcinogenesis. [3] Small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) are the two types of lung cancer, with NSCLC accounting for approximately 75–80 percent of all lung carcinomas.[4]

Since the early 2000s, better knowledge of the molecular biology of NSCLC has resulted in significant improvements in the treatment of LC.

New molecular targets and driver mutations are being discovered.

The EGFR tyrosine kinase domain undergoes active mutation, making it a therapeutic target. EGFR exons 18–21 of the EGFR gene's tyrosine kinase domain are associated with a high likelihood of responsiveness to EGFR tyrosine kinase inhibitors. [5]

A decade ago, a fraction of non-small cell lung cancer (NSCLC) patients had genetic rearrangements in the anaplastic lymphoma kinase (ALK) receptor tyrosine kinase. [6]

These mutations are mutually exclusive. After the introduction of molecularly targeted medicines, the median survival duration increased from 12–14 months to 24–36 months.

The prevalence of EGFR mutations varies among different populations, and these mutations are present at higher frequencies in women, in light or never-smokers, and in East Asian patients. [7]. the frequency of ALK rearrangements is approximately 5% in lung adenocarcinomas and is higher in light or never-smokers and in younger individuals. [6]

Identification of mutations in the epidermal growth factor receptor (EGFR) and (ALK) fusion genes has been recommended by the National Comprehensive Cancer Network (NCCN) in all lung adenocarcinoma patients as these markers are guiding the use of recently developed specific targeted therapies. [8] [9] Varied populations have different rates of EGFR and ALK mutations. Few studies have been undertaken in the Indian population on the analysis of both EGFR and ALK mutations in lung cancer cases. [4] The objective of this study was to determine the prevalence of EGFR and ALK mutations in lung cancer patients, as well as to link mutational status with age, sex, and smoking history.

Materials and methods:

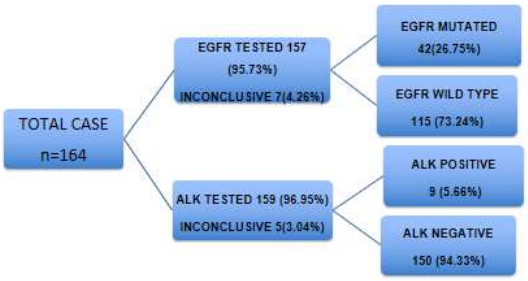
This single hospital-based retrospective study was conducted at the Department of Medical Oncology, Acharya Harihar Post Graduate Institute of Cancer, Cuttack for a duration of two years from 01.08.2019 to 31.07.2021.

This study included all lung cancer patients diagnosed with adenocarcinoma in histology during the time period and who were then tested for both EGFR and ALK mutations.Inadequate materials and unsatisfactory samples were excluded from this study.

The clinical data for the cases that were included was obtained from hospital records. Diagnostic procedures were performed according to the proposed 2011 adenocarcinoma classification by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society. [10] Tissue biopsies from both the primary and metastatic locations were included in the study. PCR and gene sequencing were used to check for EGFR mutations. Microdissected cells from Formalin-Fixed Paraffin-Embedded (FFPE) tissue blocks were used for testing. The test was carried out on a tumor-rich area of FFPE tissue with > 40–50% cancer cells. Mutations in exons 18–21 of the EGFR gene were investigated. Fluorescence in Situ Hybridization (FISH) was used to detect the ALK gene rearrangement. The EGFR and ALK mutational analysis was outsourced and completed according to the previous protocol. [11]

Statistics:

All statistical calculations were done using the SPSS 17.0 statistical software. The chi-square test was used to compare the frequency of two groups. In all analyses, p-values were two-sided; values of less than 0.05 were considered statistically significant.



Graph 1: Distribution of molecular testing of lung adenocarcinoma cases in study population

Table 1: Frequency of type of EGFR mutations in lung adenocarcinoma

Type of exon	Mutation	Frequency (%)
Exon 18	G719 A,C	2 (4.76%)
Exon 19	delE746-A750	32 (76.19%)
Exon 20	S7681	2 (4.76%)
Exon 21	L858R	6 (14.28%)

Results:

This study included 164 lung cancer patients who had their genes tested for EGFR mutations and ALK gene rearrangements.

Males made up 89 (54.26%) of the population, while females made up 75 (45.73%). At the time of diagnosis, the patients' ages ranged from 36 to 86. The percentage of smokers and former smokers in the male population was higher than in the female population.There were 70 (42.68%) nonsmokers, out of which 63 were (84%) females and seven were (7.86%) males.The majority of our patients (9155.48%) had a lesion on the right side of their lungs.

Forty-two (26.75%) cases were positive for EGFR mutations out of 157 blocks analyzed. However, in 7 cases (4.26%), the result was inconclusive due to scanty or improperly processed tissue **[Grapg 1]**. The frequency of EGFR mutational subtypes is shown in **[Table 1]**.

ALK gene rearrangement was also found to be positive in 9 of the 159 cases. The positivity rate for ALK gene rearrangement was 5.66% after removing five (3.04%) cases that had unsatisfactory results on molecular analysis. **Table 2** shows the EGFR mutation and ALK gene rearrangement status of lung cancer patients with clinical correlation.

Table 2: EGFR mutation and ALK gene reaarrangement status of the lung adenocarcinoma patients with clinical correlation

Charecteristics	Total Cases N(%)	EGFR mutation status		P value	ALK gene rearrangement (n=159)		P value
		Mutated N(%)	Wild type N(%)		Positive N(%)	Negative N(%)	
Median age (range) in years	58.3 (36-87)	60 (36-82)	59.87 (36-87)		51.43 (36-71)	56 (36-79)	
Sex							
Male	89 (54.26)	27(64.28)	58(50.43)	.181	6(66.66)	81(54)	.458
Female	75 (45.73)	16(38)	56(48.69)		3(33.33)	69(46)	
Age							
<50	53 (32.31)	17(40.47)	32(27.82)	.129	5(55.55)	44(29.33)	.097
>50	111(67.68)	25(59.52)	83(72.17)		4(44.44)	106(70.66)	
Smoking status							
Smoker/ Former smoker	94(57.31)	20(47.61)	77(66.95)	.027*	2(22.22)	87(58)	.035*
Non smoker	70(42.68)	22(52.38)	38(33.43)		7(77.77)	63(42)	
Total	164	42	115		9	150	

* Result is showing significant at p value <0 .05.

Table 3: The comparision between present study with previous study report about the frequency of EGFR mutation and ALK gene rearrangement of lung adenocarcinoma cases

Charecteristics	Rana et al.[11]	Dattatreya et al.[5]	Noronha et al.[21]	Kalal Iravarthy et al.[28]	Dutta et al.[20]	Chougle et al.[12]	Present study
Total cases	152	446		267	3351	907	164
Male	60.5%	68%				70.78%	54.26%
Female	39.5%	32%				29.21%	45.73%
Median age(year)	57.5	60					58.3
Range	25-86%						36-87
EGFR mutated	35.5%	24%		20.59%	28.19%	23%	26.75%
Exon 18(G719A,C)	4.2%		2.5%			7%	4.76%
Exon 19(%)	70.8%	73%	74%	56%	72.99%	50%	76.19%
Exon 20(%)	4.2%						4.76%
Exon 21(%)	20.8%	21.6%	23%			42%	14.28%
ALK positive(%)	7.6%	2.1%		4.11%	2.53%		5.66 %.

Discussion:

Males outnumbered females in this study. Other research has shown similar results. [4,11,12] Unlike other research, the cause of lung cancer in non-smokers has remained unknown.

According to the study reports, secondhand smoke, environmental exposures such as asbestos, arsenic, and radon, viruses such as the human papillomavirus, lung illnesses such as idiopathic pulmonary fibrosis, and indoor air pollutants such as fumes and smoke released from a coal burner are all attributable risk factors in nonsmokers.[3] In our study, the right lung was found to be more commonly involved. This finding was consistent with previous studies done by Mohan et al. (52.3%). [13] EGFR mutations occur at a rate of 10–15% in North Americans and Europeans, 19% in African-Americans, and 26–30% in various East Asian countries. [14-17] Limited literature is available regarding the Indian population. The study involving the largest numbers of Indians has reported the incidence of EGFR mutations to be 26% in cases of lung adenocarcinoma, which is comparable to the rates in East Asian countries. A few studies from India reported a frequency of EGFR mutation ranging from 29% to 51.8% along with evidence of female dominance.[18-19] In our analysis, the prevalence of EGFR mutations was found to be 26.75%, which is similar to the prevalence reported in other studies. [5,12,20] However, some researchers have reported that the incidence is 35.5%. [11,21] In contrast to the prior investigation, we found no statistically significant link between patient age and sex and the EGFR mutation. [11] Other research has demonstrated the female gender's dominance in cases of EGFR mutation. [12,19] According to various research, EGFR mutations are more common in nonsmokers than in smokers. In our study, we discovered a significant association between nonsmokers and EGFR mutations, despite the fact that no such link was detected in another. [11]

Anti-EGFR therapy response has been linked to the existence of activating or driver mutations in exons 18–21 of the EGFR gene. In the literature, 45–54% of EGFR mutations are in-frame deletions in exon 19, 40 % are missense mutations in L858R in exon 21, and 4–9% are EGFR mutations in exon 20. [22] The prevalence of our EGFR mutation and ALK gene rearrangement in lung cancer cases is compared to previous research in **[Table 3]**.

According to the study, exon 21 mutations were discovered to be more common in never-smoker females, while exon 19 mutations were found to be more common in non-smoker males, according to the study. [12,23]

Within India, the EGFR mutation frequency is distributed evenly across the country. Additionally, although exon 19 mutations are more common in nonsmokers, exon 21 mutations are more common among EGFR mutation-positive male smokers of Indian heritage. [12]

According to research, exon 18 EGFR mutations are found more commonly in younger people. Exon 18 mutations in lung cancer should not be disregarded in clinical practice. Although currently available in vitro diagnostic tools cannot detect all exon 18 mutations, these instances are best treated with afatinib or neratinib. [24] According to a study, mutations in exon 21 were more common in females than in males, and one patient had a double mutation involving exons 20 and 21. In exon 20, one of the patients had an insertion-type deactivating mutation. [11] There have been reports of uncommon EGFR mutations, and they are a heterogeneous group. As a result, it's crucial to learn more about each subgroup in order to figure out the best therapy alternatives. According to a study, when Indian patients with EGFR activating mutations were treated with EGFR-targeted therapies, their response rate, progression-free survival, and overall survival all increased significantly. [21] The total incidence of ALK gene rearrangement in NSCLC is estimated to be between 2 and 7%. [25] According to certain research, the East Asian population has an incidence ranging from 5–13 percent. [26] There are only a few published studies on ALK gene rearrangement in India, with an incidence rate of around 3%. [27] Previously, ALK gene rearrangement was found in 2.1%, 2.53%, and 4.11% of people, respectively.[5,20,28] While conducting our research, we discovered a significant rate of ALK gene rearrangement, which was consistent with a recent publication. [11] The high prevalence of ALK gene rearrangement in our patients highlights the need for molecular research to ensure that patients receive the most benefit from targeted medication.

According to study data, the ALK gene rearrangement was shown to be positive in younger male patients, with a statistically significant link. [11,25,26,29] However, no substantial difference was found in our research.

Prior investigations were conducted into all of the cases that were evaluated for both EGFR and ALK mutations, and they were determined to be mutually

exclusive. (5, 11) This was also noticed in our research. According to the literature study, there have been 6–7 people worldwide who have had both mutations. Zhang et al. [30] described it in a paper published in 2010. They discovered a patient who had both EGFR and ALK mutations. This case was a Chinese woman who had histologically confirmed adenocarcinoma. Tanaka et al. [31] described a case of a 39-year-old man with acinar adenocarcinoma who had an EGFR mutation and an ALK fusion gene in 2012. In other research, two patients were discovered to have both mutations. The patients were 60 and 62-year-old men, respectively. Both displayed an acinar pattern, with histological grade I in one and histological grade II in the other. [4] When considering the use of targeted therapies such as EGFR inhibitors and/or ALK inhibitors, it's critical to know the mutation status of both tumor-driving receptor genes in a single tumor. We also discovered equivocal results due to insufficient tissue or faulty processing, unlike earlier reports. [11] We also discovered equivocal results due to insufficient tissue or faulty processing, unlike earlier reports. [11] It is also recommended to test ALK and EGFR mutations simultaneously in light of the approval of targeted crizotinib therapy for ALK gene rearrangement and to maximise the use of limited tumor samples.

Conclusion:

This study found to have a significantly higher rate of EGFR and ALK mutation in Indian population with adenocarcinoma of lung compared to Western populations.To get the maximum benefit from targeted therapies, all patients of adenocarcinoma of lung should have mutational testing for EGFR and ALK as part of a broad molecular pannel.

Conflict of interest: Nil
Funding source: Nil

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