Original Research Article

https://doi.org/10.53339/aimdr.2026.12.1.8
E-ISSN: 2395-2822 | P-ISSN: 2395-2814

Molecular study of non-small cell carcinoma of lung in a tertiary-level laboratory in Bangladesh

Md. Iqbal Karim, Shamoli Yasmin

Department of Pathology, Armed Forces Institute of Pathology, Dhaka, Bangladesh

Address for correspondence: Dr. Md. Iqbal Karim, Department of Pathology, Armed Forces Institute of Pathology, Dhaka, Bangladesh. E-mail: iqbalhistopath@gmail.com

Abstract

Introduction: Lung cancer is the most common cause of cancer related deaths in the world as well as in Bangladesh. In both male and female Non Small Cell Lung Carcinoma (NSCLC) are the major type of Lung cancer. Molecular and genetic profiling has been made in the recent times, which has led to a significant improvement in treatment outcomes, survival, and quality of life, including epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (ROS1) rearrangements, and programmed death ligand 1 (PD-L1) expression, which were evaluated by reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC).

Methods: It is a retrospective study that was done at the Armed Forces Institute of Pathology, Dhaka, between January 2022 and December 2023, with 220 patients diagnosed with NSCLC. RT-PCR was done to analyze EGFR mutation, and IHC was done to analyze ALK, ROS1, and PD-L1 by Ventana systems. The Statistical Package for the Social Sciences version 20 was used to conduct statistical analysis, and patient outcomes after targeted therapy with or without chemotherapy were analyzed.

Results: The mean age was 63.5 (range of 41–80) years with a male-to-female ratio of 2.6:1. The best-described subtype was adenocarcinoma (81.8%). The prevalence of EGFR mutation was observed in 20% of cases, and then, ALK (2.7%), ROS1 (1.8%), and PD-L1 positivity followed (3.6%). The most common EGFR mutation was exon 19 deletion (50%). Patients of Stage II and III had a better therapeutic response as compared to those of Stage IV. Targeted therapy showed better results in contrast to standard chemotherapy.

Conclusion: Although the economic constraints limit the use of molecular testing as a universal method in Bangladesh, patients who underwent molecular testing responded positively to either targeted or immunotherapies, especially patients with EGFR exon 18 and 19 mutations and PD-L1 positive cases.

Keywords: Chemotherapy, epidermal growth factor receptor, mutation, non-small cell cancer

Introduction

Lung carcinoma is one of the leading causes of death worldwide. The world data indicate 2.5 million newly diagnosed cases, 1.8 million deaths of patients.[1] Lung cancer occurs in non-small cell lung carcinoma (NSCLC) and small cell lung cancer. NSCLC and small cell carcinoma account for approximately 80–85% and 5–10% lung cancers, respectively. Other categories of cancers, such as small cell carcinoma, large cell carcinoma, etc.[2] The way the treatment of the patient will be different is also not the same, and that depends on prognosis and the final outcome of the life of the patient; hence, it is important to detect the molecular study of lung cancer to assist the physician in treating the patient.[3] The World Health Organization 22 blue book shows that non-

small cell cancers are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, which constitute approximately 40% of lung cancer.

Each tumor contains some type of driver mutations, without which the existence of the type of tumor would have been impossible. The mutations are complex in their molecular pathogenesis of cancer that is essential in tumor progression. The major alterations are epidermal growth factor receptor (EGFR), Kirsten rat sarcoma virus, anaplastic lymphoma kinase (ALK), and programmed death-ligand 1 (PD-L1). Targeted treatment against some mutation is decisive in assisting the patient with the lung tumor to survive. In case we consider the lung cancer mutation and tumor biology, a targeted therapeutic implementation is reliant on targeting specific gene mutation, deletion, insertion, or ALK translocation and ROS1 mutation. Disturbances in EGFR signaling pathways are the main alterations that become dysregulated in the NSCLC that facilitate cell survivability, its growth, and predisposition to metastasis. It is now known that the treatment of NSCLC is dependent on its modifications. The first case of a tyrosine kinase inhibitor (TKI) was in 2004 in lung cancer. Hence, lung carcinoma with NSCLC and well-sensitized TKI drugs. It has been found, based on the reaction of TKI, that mutation in the *EGFR* gene falls under the category of exon18 (G719X), exon19 deletions, exon20 (T790X, S768I, exon20insGGT/CAC, exon20ins9), and exon21 (L858R and L861Q) mutations.

Approximately 3–5% of NSCLC possess *ALK* gene reciprocal translocation, and 1–25 have ROS Proto-oncogene encoded membrane protein molecules. Any mutation in the *ROS1* gene also plays a role in tumor formation and development. Research established that 1–2% of young female patients carry the ROS1 reengagement gene. The survival rate has been improved by immunotherapy against PDLI-positive patients, who are the recent ones. Hence, EGFR, ALK, ROS1, and PD-L1 research has been conducted in one center.

To personalize the therapeutic implementation of lung cancer biology, it is required to comprehend the foundation of the molecular nature of the tumor, targeted on gene mutations, deletion or insertions and their carcinogenesis mechanisms.[4] The sensitivity to EGFR TKIs was initially discovered in 2004 to treat lung cancer, and the pathway involves activation of the EGFR and its further regulation that results in apoptosis, proliferation, and angiogenesis.[5] EGFR activation and regulation cause a subsequent increase in cell survival, proliferation, angiogenesis, and tend to metastasize.[6,7]

The EGFR mutation in lung cancer patients is sensitive to TKI drugs.[8] It may be categorized into four sensitive types (G719X, L858R, L861, and exon19 deletion) and two resistant types (insertion in exon 20 and T790M) based on the treatment effects. The EGFR mutation, *ALK* gene rearrangement, as well as the ROS proto-oncogene encode a membrane protein that has tyrosine kinase activity.[9] A mutation in the *ROS1* gene rearrangement also plays a role in the development and further progression of tumors.[10] Approximately 12% of young, female, and never-smoked NSCLC patient has ROS1 rearrangement.[11,12] In recent years, with the approval of immunotherapy against PD-L1, the overall survival rate has increased.[13] As such, this study has included EGFR, ALK1, ROS1, and PD-L1 studies.

Methods

In the current study, all patients diagnosed with non-small cell carcinoma between January 2022 and December 2023 were subjects of this study. The enlisted clinical data and diagnosis of pulmonary non-small cell carcinoma patients were statistically analyzed with the use of the Statistical Package for the Social Sciences (SPSS) version 20.0. All cases diagnosed with non-small cell carcinoma were included as per the inclusion criteria of the study. The case sheets provided all the clinical data, such as gender, age at diagnosis, and smoking history. The cases that lacked clinical data and those diagnosed with diseases other than NSCLC were excluded from the study. Two out of the three well-experienced pathologists did all the

histopathological confirmation of diagnosis, and terminology was done in reference to the 2021 World Health Organization classification of tumors of the lung.

Immunohistochemistry (IHC) was used to classify the tumor as NSCLC and small cell cancer by thyroid transcription factor 1 (TTF-1)/Napsin A, p63, p40, CD56, and synaptophysin. Based on TTF-1/Napsin patient diagnosed with adenocarcinoma, p63 and p40 positivity patient diagnosed with squamous cell carcinoma, CD56 and synaptophysin positivity patients diagnosed with small cell carcinoma of the lung.

The molecular and genetic laboratory mutation analysis was performed in DNA extraction, then, the EGFR mutation analysis kit is planned to identify EGFR exon 18,19,20, and 21 somatic mutations, which was done by real-time polymerase chain reaction technique. IHC was done on ALK-1, ROS, and PDLI. The quality control analysis was performed with the help of the internal control of certain mutations. The statistical analysis was done using SPSS version 20.0. The Chi-square test was used to find out the frequency of EGFR mutation status.

Results

This table summarizes the clinicodemographic characteristics, treatment modalities, comorbidity burden, and survival outcomes of patients with NSCLC according to EGFR mutation status. Of the 220 patients, 44 (20%) were EGFR-positive, and 176 (80%) were EGFR-negative. Significant differences were observed between the two groups in age, sex distribution, smoking status, disease stage, and treatment patterns (all $P < 0.05$). EGFR positivity was more frequent among females and never smokers and was associated with lower disease stage at presentation. The Charlson Comorbidity Index was slightly higher in the EGFR-positive group ($P = 0.042$). Median overall survival was significantly longer in EGFR-positive patients compared with EGFR-negative patients (36.4 vs. 16.8 months, $P < 0.001$) [Table 1].

Table 2 shows that histopathological findings of adenocarcinoma were predominant, 81.81% ($n = 180$), followed by squamous cell carcinoma 14.54% ($n = 32$), adenosquamous cell carcinoma 2.27% ($n = 5$), and large cell carcinoma 1.36% ($n = 3$) [Table 2].

This table shows the distribution of molecular alterations among the study population. EGFR mutations were the most common, identified in 44 patients (20%), followed by PD-L1 expression in 8 patients (3.6%). ALK rearrangements and ROS1 mutations were less frequent, observed in 6 (2.7%) and 4 (1.8%) patients, respectively [Table 3].

Figure 1 shows that, regarding EGFR mutation ($n = 44$), the most common mutation was exon 19 deletion, 50% ($n = 22/44$), 18.18% ($n = 08/44$) in exon 18, 27.28% ($n = 12/44$) in exon 20, among them 18.2% and 9.17% in T790M and S7681 mutation, respectively. The L858R mutation was 4.55% ($n = 2/44$) in exon 21. Most of the mutations found in adenocarcinoma of the lung (98%).

Discussion

The molecular mutation analysis of EGFR is obligatory in the present-day treatment scheme of lung cancer, prior to targeted therapy specifically using TKI-based therapy. Recent research indicates that there is a high variation in EGFR molecular tests across geographical locations across the world.[13] However, the evidence of EGFR mutation analysis in South East Asia is insufficient, especially in Bangladesh.

This paper demonstrates that 20% of lung cancer cases had EGFR mutations. The prevalence of EGFR mutation is different among various demographic distributions. One study has indicated that 60% of the patients with lung cancer have demonstrated positive EGFR mutation, and only 10% of patients with lung cancer had EGFR mutation in non-smokers.[14,15] In an Indian study, the frequency of EGFR mutation is 22–52%. A notable relationship between EGFR mutations and smoking status was also identified in our study. It has been demonstrated that 68% of non-

Table 1: Demographic distribution of EGFR-positive and EGFR-negative non-small cell lung carcinoma

Variables	EGFR positive	EGFR negative	Total	*P*-value
Numbers (*n*)%	44 (20)	176 (80)	220	
Age				
Male	63.5±8.5	55.6±10.2	59.5±9.3	<0.01
Female	61.2±7.8	58.2±8.8	59.7±12.7	<0.01
Sex (%)				
Male	28 (17.5)	132 (82.5)	160 (72.72)	<0.01
Female	16 (26.6)	44 (73.4)	60 (27.27)	<0.01
Smoking status (%)				
Smoker	14 (6.3)	78 (35.45)	92 (41.81)	<0.001
Never smoker	30 (13.63)	98 (44.54)	128 (58.18)	
Stage (%)				
II	20 (9.1)	90 (40.9)	110 (50)	<0.001
III	15 (6.8)	42 (19.1)	57 (25.9)	<0.001
IV	09 (4.1)	44 (20)	53 (24.1)	<0.001
Treatment (%)				
Chemotherapy	25 (11.36)	195 (88.63)	220 (100)	<0.01
Tyrosine kinase inhibitor	36 (16.36)	184 (83.64)	220 (100)	<0.001
Immunotherapy	08 (3.6)	212 (96.4)	220 (100)	<0.001
Radiation therapy	20 (9.1)	200 (90.0)	220 (100)	
Charlson comorbidity index	10.4±2.6	9.6±3.4	10±3	0.042
Median overall survival, month (95% confidence interval)	36.4 (32.2–38.6)	16.8 (12.6–18.2)	-	<0.001

EGFR: Epidermal growth factor receptor

Table 2: Distribution of non-small cell carcinoma (*n*=220)

Type of cancer	Number (*n*=220)	Percentage
Adenocarcinoma	180	81.81
Squamous cell carcinoma	32	14.54
Adenosquamous cell carcinoma	05	2.27
Large cell carcinoma	03	1.36

Table 3: Distribution of lung molecular panel (*n*=62)

Type of mutation	Number	Percentage
EGFR	44	20
ALK	06	2.7
ROS-1	04	1.8
PD-L1	08	3.6

EGFR: Epidermal growth factor receptor, ALK: Anaplastic lymphoma kinase, ROS-1 (ROS Proto-Oncogene 1), PD-L1: Programmed death-ligand 1

smokers and 32% of smokers had EGFR mutations. In contrast, 47% in Africa and 35% in Europe have EGFR mutation in non-smokers. In Africa and Europe, 47% and 35%, respectively, possess EGFR mutation among non-smokers.[15]

The EGFR mutation frequency difference in our study versus the other one can be due to the difference in the genetic and environmental factors that are related to cancer. The various frequencies of EGFR mutations in the various geographical locations indicate the necessity of treatment for the patients.

The most common deletion mutations in this research included deletion in the exon 19 (50%) and deletion in exon 18 and exon 20 (T790M)

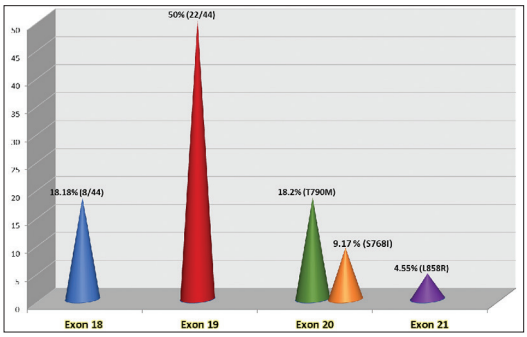

Figure 1: Epidermal growth factor receptor mutation distribution chart ($n = 44$)

mutations (18.2% and 18.2%, respectively). The mutations at L858R and S768I were 4.5% and 9.17%, respectively. Very recent research discusses NSCLC *ALK* gene rearrangements, which is also significant to comprehend the tumor's molecular biology and the development of targeted inhibitors. In this case, ALK-rearranged NSCLC represents 2–5% of total cases of NSCLC.[16] 2.7% of cases ($n = 6$) in this study were ALK positive. signet-ring cell types of adenocarcinomas, the signs of the ALK-rearranged gene are present. The first approved targeted therapy against ALK-positive NSCLC is ALK inhibitor crizotinib, which, in one study, was demonstrated to be superior to traditional chemotherapy, and in second-line therapy, pemetrexed has shown an advantage over docetaxel.[17,18]

The ROS1 rearranged oncogene was initially reported in glioblastoma multiforme patients, then subsequently in lung adenocarcinomas. ROS1 rearrangements may be determined in both IHC and fluorescence *in situ* hybridization. Rimkunas *et al*. documented both EGFR mutations observed in two ROS1-rearranged tumors in lung adenocarcinoma.[19] In our study, all the ROS1 translocations were detected independent oncogenic alterations, but both ALK1 and ROS1 mutations were observed in two cases. We have also had a few cases of double mutation in addition to the common mutations. In our study, five cases were found to possess double mutation, of which two cases express a combination of resistant and sensitive mutations, in exon 19 deletion and T790M mutation, respectively.

Recently, the presence of antibodies against immune checkpoint inhibitors, such as the PD-1 and PD-L1, has demonstrated to enhance the overall survival of NSCLC patients.[20] NSCLC patients expressing positive PD-L1 were found to predominate among the EGFR, ALK, and ROS1 negative patients, and probably in the advanced stages. Therefore, prognosis and survival rate are poorer in positive PD-L1 patients but higher than in all molecular negative cases. According to this study, 3.8% of tumors express PD-L1. Research established that the median survival rate is augmented using immune checkpoint therapy.

Conclusion

Lung cancer is a heterogeneous disease involving genetic and environmental factors, and immunological complexities that have many molecular biomarkers associated with variations in treatment protocol, particularly targeted therapy. An EGFR mutation, ALK, ROS1, and PD-L1 study is necessary to detect appropriate molecular biomarkers in lung cancer. Targeted therapy is associated with optimal combination methods that improve the efficacy and therapeutic effects of anticancer therapy. In this study, we have analyzed the molecular markers of non-small cell carcinoma patients and evaluated their therapeutic response to targeted therapy. Targeted therapy, particularly by TKIs, has improved the patient's life significantly.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
2. Tokgun O, Karakas DE, Tan S, Karagür ER, İnal B, Akca H, *et al*. Novel ruthenium and palladium complexes as potential anticancer molecules on SCLC and NSCLC cell lines. Chem Pap 2020;74:2883-92.
3. Turner RM, Chen YW, Fernandes AW. Validation of a case-finding algorithm for identifying patients with non-

small cell lung cancer (NSCLC) in administrative claims databases. Front Pharmacol 2017;8:883.

4. Luo SY, Lam DC. Oncogenic driver mutations in lung cancer. Transl Respir Med 2013;1:6.
5. Gazdar AF, Minna JD. Deregulated EGFR signaling during lung cancer progression: Mutations, amplicons, and autocrine loops. Cancer Prev Res (Phila) 2008;1:156-60.
6. Yuan M, Huang LL, Chen JH, Wu J, Xu Q. The emerging treatment landscape of targeted therapy in non-small-cell lung cancer. Signal Transduct Target Ther 2019;4:61.
7. Francis H, Solomon B. The current status of targeted therapy for non-small cell lung cancer. Intern Med J 2010;40:611-8.
8. Chmielecki J, Foo J, Oxnard GR, Hutchinson K, Ohashi K, Somwar R, *et al*. Optimization of dosing for EGFR-mutant non–small cell lung cancer with evolutionary cancer modeling. Sci Transl Med 2011;3:90ra59.
9. Da Cunha Santos G, Shepherd FA, Tsao MS. EGFR mutations and lung cancer. Annu Rev Pathol 2011;6:49-69.
10. Araujo JM, Gomez AC, Pinto JA, Rolfo C, Raez LE. Profile of entrectinib in the treatment of ROS1-positive non-small cell lung cancer: Evidence to date. Hematol Oncol Stem Cell Ther 2021;14:192-8.
11. Azelby CM, Sakamoto MR, Bowles DW. ROS1 targeted therapies: Current status. Curr Oncol Rep 2021;23:94.
12. Bubendorf L, Büttner R, Al-Dayel F, Dietel M, Elmberger G, Kerr K, *et al*. Testing for ROS1 in non-small cell lung cancer: A review with recommendations. Virchows Arch 2016;469:489-503.
13. Graham RP, Treece AL, Lindeman NI, Vasalos P, Shan M, Jennings LJ, *et al*. Worldwide frequency of commonly detected EGFR mutations. Arch Pathol Lab Med 2018;142:163-7.
14. 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;5:2892.
15. Moorjani P, Thangaraj K, Patterson N, Lipson M, Loh PR, Govindaraj P, *et al*. Genetic evidence for recent population mixture in India. Am J Hum Genet 2013;93:422-93.
16. Rodig SJ, Mino-Kenudson M, Dacic S, Yeap BY, Shaw A, Barletta JA, *et al*. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res 2009;15:5216-23.
17. Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, *et al*. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 2014;371:2167-77.
18. Lee JO, Kim TM, Lee SH, Kim DW, Kim S, Jeon YK, *et al*. Anaplastic lymphoma kinase translocation: A predictive biomarker of pemetrexed in patients with non-small cell lung cancer. J Thorac Oncol 2011;6:1474-80.
19. Rimkunas VM, Crosby KE, Li D, Hu Y, Kelly ME, Gu TL, *et al*. Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: Identification of a FIG-ROS1 fusion. Clin Cancer Res 2012;18:4449-57.
20. Okita R, Maeda A, Shimizu K, Nojima Y, Saisho S, Nakata M. PD-L1 overexpression is partially regulated by EGFR/HER2 signaling and associated with poor prognosis in patients with non-small-cell lung cancer. Cancer Immunol Immunother 2017;66:865-76.

How to cite this article: Karim MI, Yasmin S. Molecular study of non-small cell carcinoma of lung in a tertiary-level laboratory in Bangladesh. Ann. Int. Med. Den. Res. 2026;12(1):45-50.

Source of Support: Nil, **Conflict of Interest:** None declared

Received: 29-Dec-2025; **Revised:** 24-Jan-2026; **Acceptance:** 12-Feb-2026; **Published:** 10-Mar-2026