ORIGINAL ARTICLE

Profile of Exon 20 T790M Mutation Incidence Rate with Plasma ctDNA in Lung Adenocarcinoma Patients Receiving EGFR-TKI Treatment

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ARTICLE INFO

Article history:
Received 16 July 2022
Received in revised form
9 December 2022
Accepted 23 December 2022
Available online 30 January 2023

Keywords:
Cancer,
EGFR,
Exon 20 T790M,
Resistance.

Cite this as:
Praditya MH, Soeroso NN, Tarigan SP, *et al*. Profile of Exon 20 T790M Mutation Incidence Rate with Plasma ctDNA in Lung Adenocarcinoma Patients Receiving EGFR-TKI Treatment. *J Respi* 2023; 9: 12–17.

ABSTRACT

Introduction: Patients with lung adenocarcinoma following epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) treatment will develop acquired resistance after 7 to 16 months of starting the medication, indicated by the emergence of exon 20 T790M mutations. This study aimed to identify the clinical and demographical profile of acquired resistance in patients with lung adenocarcinoma receiving EGFR-TKI therapy through plasma circulating tumor deoxyribonucleic acid (ctDNA) examination.

Methods: This was a descriptive study with a retrospective cross-sectional design involving 108 lung adenocarcinoma patients who received EGFR-TKI for more than six months. Exon 20 T790M EGFR mutations were identified as a sign of acquired resistance using the digital droplet polymerase chain reaction (ddPCR) approach to examine plasma ctDNA Utilizing the Statistical Package for the Social Sciences, statistical tests were used to examine the data (SPSS). The data were analyzed by statistical tests using the Statistical Package for the Social Sciences (SPSS).

Results: A total of 31 patients were recruited as study participants. The majority of the research subjects were female (64.5%), aged 20-69 years old (58%), and non-smokers (67.7%). Exon 19 deletions were the most prevalent EGFR mutation (58.1%). The incidence of acquired resistance was found in 10 subjects (32.3%). Patients with acquired resistance were predominately female (70%), non-smokers (80%), and with gefitinib therapy (90%). The average time for EGFR-TKI treatment until acquired resistance occurred was 12.6 months.

Conclusion: The incidence of acquired resistance was mainly found in women without a smoking history after 12.6 months of treatment with EGFR-TKI.

INTRODUCTION

Lung cancer is the first cause of death in men (21.8%) and the second leading cause in women (9.1%) after breast cancer (21.4%). Non-small cell lung cancer (NSCLC) accounts for between 75-80% of lung cancer cases. Small cell lung cancer (SCLC) accounts for around 20% of all lung cancer occurrences or 30,000 new cases yearly. Adenocarcinoma, squamous carcinoma, and large-cell carcinoma are the three different kinds of NSCLC. The most prevalent type of lung cancer is adenocarcinoma, which is more prevalent

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Jurnal Respirasi (Journal of Respirology), p-ISSN: 2407-0831; e-ISSN: 2621-8372.
Accredited No. 200/M/KPT/2020; Available at https://e-journal.unair.ac.id/JR. DOI: 10.20473/jr.v9-I.1.2023.12-17

in women.[1]

Important therapeutic targets in lung cancer include epidermal growth factor receptor (EGFR), echinoderm microtubule-associated protein-like 4, vascular endothelial growth factor (VEGF) receptors, and anaplastic lymphoma kinase (EML4-ALK), among others. In contrast to chemotherapy, targeted therapy works by selectively inhibiting cell activity at the level of receptors and intracellular signaling cascades. Some drugs used as targeted therapy include erlotinib, gefitinib, bevacizumab, osimertinib, necitumumab, crizotinib, and dabrafenib. Erlotinib and gefitinib act on the EGFR receptor by inhibiting tyrosine kinase activity. This medication is advised as first-line therapy when treating lung adenocarcinoma patients with exon 21 and 19 EGFR mutations. Another mutation is T790M. Osimertinib is a brand-new EGFR inhibitor that can affect cells with the T790M mutation.[2]

Evaluation of EGFR mutations is still required in standard clinical practice. This is because the EGFR mutational status is relevant to the selection of the most appropriate therapy. A therapeutic intervention may be possible by identifying the molecular changes causing both primary and acquired resistance to EGFR-TKIs. Utilizing surrogate deoxyribonucleic acid (DNA) sources, such as blood, serum, and plasma samples—which typically contain DNA from circulating free tumors (CFT) or circulating tumor cells (CTCs)—is a novel strategy for genotyping cancers. Numerous studies have examined the analysis of isolated circulating tumor cells, however circulating tumor DNA (ctDNA) is more readily available and simpler to use. In 30 patients with metastatic breast cancer, Murtaza, *et al*. (2013) studied ctDNA, CTC, and CA15-3. They discovered that while ctDNA was detected 97% of the time, CTC and CA15-3 were only detected 78% and 87% of the time, respectively. According to Bettegowda, *et al*. (2014), 206 patients with metastatic cancer had a ctDNA detection sensitivity of 87.2%.[3–5]

This study aimed to identify the incidence of exon 20 T790M mutations that have received EGFR-TKI in adenocarcinoma lung cancer patients at Haji Adam Malik General Hospital, Medan.

METHODS

This was a retrospective descriptive study examining the frequency of exon 20 T790M mutations in lung adenocarcinoma patients receiving EGFR-TKI treatment at Haji Adam Malik Hospital, Medan. This study was conducted at the Oncology Polyclinic at Haji Adam Malik Hospital, Medan. This study was conducted for four months.

The sample was taken using the total sampling technique. All patients with adenocarcinoma-type lung cancer from 2019-2020 who underwent treatment with EGFR-TKIs were included in this study. The inclusion criteria were patients aged >18 years old who had EGFR mutations at baseline diagnosis before treatment (with compound uncommon and common mutations), received EGFR-TKI therapy as first-line treatment for lung cancer, had taken EGFR-TKI medications for at least three months, and had undergone at least one response evaluation criteria in solid tumors (RECIST) evaluation. The exclusion criteria were adenocarcinoma patients who received EGFR-TKI treatment but died before the first RECIST evaluation. These adenocarcinoma patients received EGFR-TKI treatment and progressed RECIST results but were unwilling to undergo rebiopsy or liquid biopsy examinations. These patients took EGFR-TKI but underwent treatment changes due to severe side effects.

This study received ethical clearance from the Health Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara, Medan (No. 756/KEP/USU/2020). All patients with adenocarcinoma lung cancer who met the inclusion and exclusion criteria were included as research subjects. The EGFR gene mutation test findings were examined along with patient medical records. Patients with EGFR no mutation or EGFR mutation exon 20 T790M examination results at the beginning of the diagnosis were excluded from the study. The patient's medical record data was followed to see the progress of the patient's disease during treatment.

Patients who experienced the results of RECIST progressive disease evaluation then underwent a rebiopsy or liquid biopsy to re-examine the EGFR mutation. The data was obtained from examining EGFR exon 20 T790M mutations after treatment with EGFR-TKI. The incidence of exon 20 T790M mutations with liquid biopsy was found in lung adenocarcinoma patients receiving EGFR-TKI treatment at Haji Adam Malik Hospital, Medan. The data obtained were assessed for validity based on the inclusion criteria of the study. The data were then categorized according to the predetermined categories. After the data were collected, the next step was data processing.

Cancer prognosis and diganosis through liquid biopsy were assessed using ctDNA as a novel

biomarker. While protein markers in plasma can have a half-life of several weeks, ctDNA has a half-life of less than two hours. ctDNA from the same patient at various phases of the disease can be used to dynamically monitor genetic alterations as cancer develops. ctDNA has a 45.0% sensitivity and a 92.5% specificity for detecting EGFR mutations, respectively.

EGFR ctDNA mutation is a test to detect the presence of EGFR gene mutations in lung adenocarcinoma patients using plasma ctDNA to detect deletions in exon 19, point mutations (L858R) in exon 21, and T790M mutations in exon 20 in genes EGFR using digital droplet polymerase chain reaction (ddPCR) method. The sample type is EDTA Plasma or PAXGene Plasma.

EDTA plasma is stable for one month at <(-20)°C. EDTA blood specimens are stable for a maximum of 2 hours at room temperature (15-25°C) and 8 hours at 2-8°C before the plasma is separated. PAXgene blood specimens were stable for 72 hours at room temperature (15-25°C) before being separated from the plasma.

RESULTS

A total of 34 patients of Haji Adam Malik General Hospital, Medan were examined for T790M at Prodia Medical Laboratory using the ddPCR method. A total of three patients were excluded because the results of the examination exceeded the time limit, two patients due to lysis material, and one patient whose medical record data were incomplete. The number of research samples was 31 people.

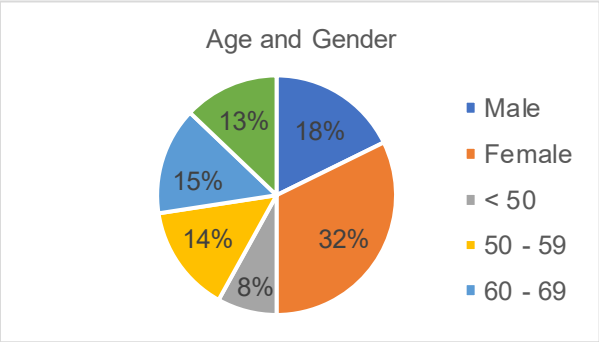

Figure 1. Age and gender frequency distribution

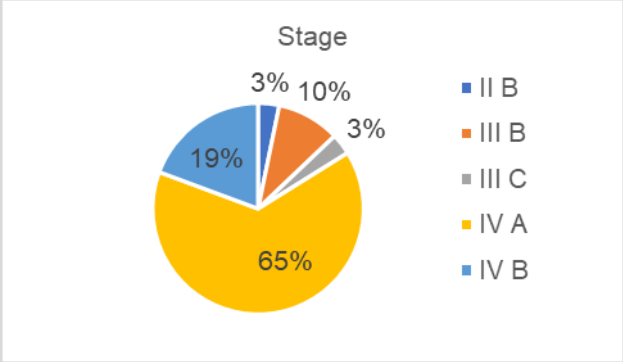

Figure 2. Frequency distribution of lung cancer stages

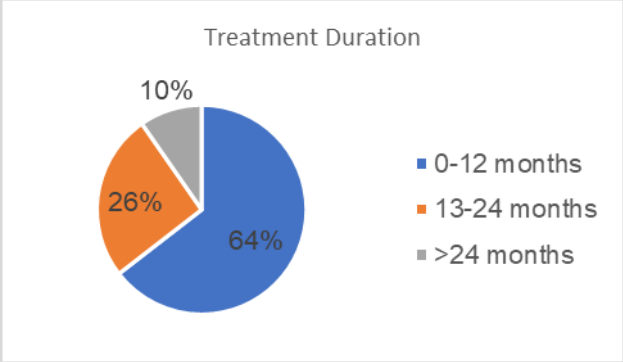

Figure 3. Frequency distribution of EFGR-TKIs treatment duration

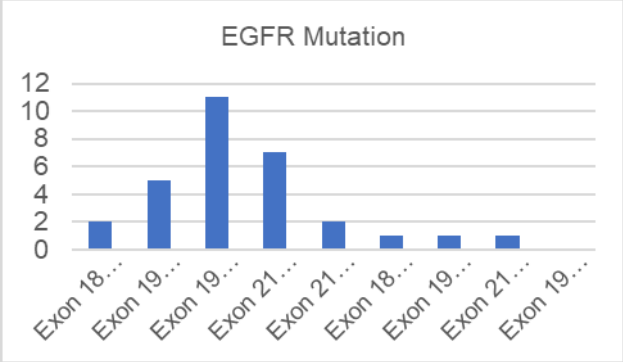

Figure 4. Frequency distribution of EFGR mutation

Table 1. Characteristics of patients with acquired resistance

Characteristics		n	%
History of EFGR mutations	Exon 18	2	20
	Exon 19	4	40
	Exon 21	4	40
Type of EGFR TKI	Afatinib	0	0
	Erlotinib	1	10
	Gefitinib	9	90
	Osimertinib	0	0
Gender	Male	3	30
	Female	7	70
Brinkman Index	Severe	1	10
	Moderate	1	10
	Mild	0	0
	Do not smoke	8	80
Stage	II B	0	0
	III B	1	10
	III C	1	10
	IV A	6	60
	IV B	2	20
Age	Mean	62 years old	
	Min-Max	52–81 years old	
TOTAL		31	100.0
Time of acquired resistance	<6 months	0	0
	6-9 months	5	50
	10-12 months	1	10
	>12 months	4	40
	Mean	12.6 months	
	Min-Max	7–33 months	

DISCUSSION

The basic characteristics of young-age adenocarcinoma patients who were found in this study were women and non-smokers. The proportion of women at young age with adenocarcinoma was 58.3% and men 41.7%.[6] The largest epidemiological study conducted by Subramanian, *et al*. (2010) on young people in the United States also found that the proportion of women was higher in younger patients than in elderly patients.[7]

The proportion of non-smokers in this study can be considered as very high, reaching 67% of the subjects. It is not much different from the findings of Elhidsi, *et al*. (2016) at Persahabatan National Respiratory Referral Hospital who found a quite high incidence of lung adenocarcinoma at a young age, namely 66.7% non-smokers and 33.3% smokers. Brenner, *et al*. (2010) also found that the proportion of lung cancer in young non-smokers was high, reaching 75%.[8]

According to the PIONEER study, 22-64% of lung adenocarcinomas in seven Asian nations tested positive for the EGFR mutation. The young age group (70.8%) had a larger percentage of positive EGFR mutations than the elderly (51.6%). This confirms the association of EGFR mutations as oncogene drivers in young lung adenocarcinoma. The proportion of wild type (29.2%) at a young age also needs further analysis because it does not mean that there are no gene mutations.[9]

For staging and performance status in this study, it is in line with a study by Mohan (2020). The NSCLC staging in North India from January 2008 to March 2018 was indicated by Stage IV based on TNM version 7 (before 1 January 2017) at 65.9% and followed by Stage III at 29.1%. Meanwhile, the TNM system version 8 (after 1 January 2017) showed Stage IV at 66.3% and Stage III at 30.2%. The performance status of patients based on the Eastern Cooperative Oncology Group (ECOG) in India was dominated by a score of 0 to 1, which was 50.8%.[10]

Adenocarcinoma patients with EGFR mutations initially respond strongly to EGFR-TKI therapy but develop secondary resistance or resistance to the drug for a median period of 9-14 months.[11,12] In the AURA3 research trial, which used DNA testing without cells, found that about 51.2% of the patients had T790M mutation. This brought on EGFR T790M mutation at exon 20, which has been connected to molecular changes and manifests in 60% of patients treated with EGFR-TKI.[13] Meanwhile in Indonesia, research on adenocarcinoma lung cancer showed 44.4% EGFR mutations, namely common EGFR mutations (ins/dels exon 19, L858R) and uncommon EGFR mutations (G179X, T790M, L861Q), around 57.1% and 29%, respectively.[14]

This study found that the incidence of acquired resistance in lung adenocarcinoma patients who received TKI therapy was 32.3%. This figure is slightly lower compared to other studies. Merinda and Wulandari (2019) found that the incidence of acquired resistance mutations in exon 20 T790M in patients at Dr. Soetomo General Academic Hospital, Surabaya, was 46.2%. Previous studies generally show that the incidence of acquired resistance can reach 50-60% of the total lung adenocarcinoma patients.[13]

Prior to 1st generation of EGFR-TKI therapy, it is thought that a small percentage of cancer cells may already have a secondary T790M mutation in addition to an active EGFR mutation. These cancer cells may then gradually establish dominance (e.g. erlotinib and gefitinib). According to recent studies, T790M-positive cells can potentially develop from a single T790M-negative cell at first through genetic evolution.[15]

One of the many mechanisms of resistance to EGFR-TKI includes the activation of alternative pathways (c-Met, HGF, AXL), abnormalities at the end of the pathway (K-RAS mutation, loss of PTEN), disruption of the EGFR-TKI-mediated apoptotic pathway (polymorphisms in the form of deletion of the 11/BIM gene such as BCL2), histological changes, ATP-binding fusion transporter site (ABC), and others.[13,16]

The etiology of EGR-TKI resistance can be broken down into many categories depending on the cell signal transduction pathway.[17,18] NSCLC survivors who initially react to EGFR-TKI therapy may later acquire resistance to EGFR inhibitors, which can result in treatment failure. Most studies have found that the EGFR mutation occurs at exon 20, resulting in a substitution of methionine for threonine in codon 790 (T790M).[19] This mutation causes a tridimensional change in the tyrosine kinase domain structure and prevents the binding of erlotinib and gefitinib to the EGFR.[20]

CONCLUSION

The incidence of acquired resistance in lung adenocarcinoma patients who received TKI therapy was 32.3%. The profile of lung adenocarcinoma patients who have acquired resistance was dominated by women without a smoking history. The average length of EGFR-TKI treatment until resistance occurred was 12.6 months with a range of 7–33 months.

Acknowledgments
The authors express their deepest gratitude to Prodia Medical Laboratory, which has provided scientific equipment and tools.

Conflict of Interests
The authors declared there is no conflict of interest.

Funding
The authors would like to thank AstraZeneca Indonesia for supporting the funding of this study.

Authors' Contributions
Conceiving and designing the study, conducting research, providing research materials, collecting and organizing data: MHP. Analyzing and interpreting data: MHP, NNS, and SPT. Writing initial and final draft of article and providing logistic support: MHP, TA, and DWL. All authors critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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