Original Article

The Association of Acquired Resistance EGFR Exon 20 T790M Mutation and Treatment Response in Lung Adenocarcinoma Patients Receiving EGFR-TKI

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Abstract

Background: Lung adenocarcinoma patients receiving EGFR-TKI may develop acquired resistance within 7-16 months of treatment initiation, which is characterized by the presence of exon 20 T790M mutations in treatment response patients and can be assessed objectively by CECT and then evaluated by RECIST 1.1. The purpose of this study is to look into the association between acquired resistance EGFR Exon 20 T790M mutation and treatment response in lung adenocarcinoma patients receiving EGFR-TKI.

Method: This research is an analytic study with a retrospective cohort design carried out at the Oncology Polyclinic at Haji Adam Malik Hospital from October 2020 to January 2021 in all patients with adenocarcinoma lung cancer who were treated with EGFR-TKI for more than 6 months. After that, an evaluation was carried out based on RECIST 1.1 and then examined for EGFR mutations from liquid biopsy specimens in the form of circulating tumor plasma DNA (ct-DNA) with the droplet digital Polymerase Chain Reaction (ddPCR) method to detect EGFR exon 20 T790M mutations as a marker of acquired resistance.

Results: It was found that the majority of subjects were female (64.5%), aged 20-69 years (58%), and non-smokers (67.7%). The most common EGFR mutation was exon 19 deletion (58.1%). The incidence of acquired resistance was found in 10 subjects (32.3%). The distribution of RECIST 1.1 results on positive acquired resistance includes progressive diseases of 35.2%; stable disease of 11.1%; partial response of 33.4%; and 100% complete response. Negative acquired resistance includes 64.8% progressive disease, 88.9% stable disease, 66.6% partial response, and 0% complete response (P=0.93).

Conclusion: There is no significant association between the incidence of acquired resistance mutations EGFR exon 20 T790M and treatment response in patients with lung adenocarcinoma who received EGFR-TKI therapy.

Keywords: Exon 20 T790M mutation, Acquired resistance, EGFR, Tyrosine Kinase Inhibitor, RECIST 1.1

INTRODUCTION

Lung cancer is the second most common cancer and the leading cause of cancer death in the United States. Around 247,270 new cases of lung cancer are estimated to occur in 2020, with 130,340 male cases and 116,930 female cases.[1] Indonesia ranks third after China and India, with the incidence of lung cancer, reaching approximately 25,322 cases with a mortality rate of 22,522 cases.[2]

Histopathologically, lung tumors are divided into two major parts, that is Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC) which will later be divided into 2 subtypes, i.e. adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and unclassified lung carcinoma,[3] with the highest prevalence is the type of adenocarcinoma.[4–7]

Some people with lung cancer have mutations. Several studies have found a close relationship between mutations in the Epidermal Growth Factor Receptor (EGFR) gene, which is found in 15-20% of lung adenocarcinoma cases. In East Asia, the presentation of EGFR gene mutations in lung adenocarcinoma cases was higher, i.e. 35%, especially in stage III and IV. In India, EGFR gene mutations are expressed in 89% of lung adenocarcinoma cases.[8] Meanwhile, in Indonesia, research on adenocarcinoma lung cancer shows 44.4% EGFR mutations, namely common EGFR mutations (ins/dels exon 19, L858R), and uncommon EGFR mutations (G179X, T790M, L861Q) of around 57.1% and 29%.[9] In H. Adam Malik General Hospital, research on EGFR profile and RECIST 1.1 shows that at 3 months of Gefitinib treatment found Partial Response 38.5%, Stable Disease 46.2% and Progressive disease 15.4% while Erlotinib Partial Response 100%, Stable Disease 0%, Progressive disease 0%.[10]

EGFR mutations are usually found in the first four exons of the tyrosine kinase domain of the EGFR gene. The frequent mutations are as follows: Substitution for G719 in the nucleotide-binding loop of exon 18, in-frame deletion in exon 19, in-frame duplication and/or insertion in exon 20, and substitution for L858 or L861 in the activation loop of exon 21.[11] More than 80% of EGFR domain-mutated kinases have in-frame deletions at exon 19 or L858R exon 21.[12]

METHOD

This research is an analytic study with a retrospective cohort design that was held at the Oncology Polyclinic at Haji Adam Malik Hospital for 4 months from October 2020 to January 2021 in 31 patients with adenocarcinoma lung cancer and receiving Gefitinib, Erlotinib, or Afatinib who met the inclusion and exclusion criteria.

Medical records were reviewed for patient data, including the results of the EGFR gene mutation examination. 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 with progressive disease from evaluation results or who have been on EGFR-TKI for at least 6 months were rebiopsied or liquid biopsied ct-DNA utilizing dd-PCR technique conducted at Prodia Laboratorium Medan to re-examine EGFR mutations. Data was obtained from the examination of EGFR exon 20 T790M mutations after treatment with EGFR-TKI (acquired resistance).

The univariate analysis was carried out to determine the profile and characteristics of lung adenocarcinoma patients with acquired resistance mutations EGFR exon 20 T790M and the description of the RECIST 1.1 results for the patients. The analysis was continued with a bivariate analysis that examined the relationship between the acquired resistance mutation EGFR exon 20 T790M and the patient's RECIST 1.1 results. Data analysis was done through the Chi-Square test or Fisher exact test.

In January 2009, a revised version of the RECIST 1.1 guide was introduced was introduced as a method of evaluating treatment response of oncology patients by assessing subjectively as a method of evaluating treatment response of oncology patients by assessing subjectively, semi-subjectively, and objectively using serial CECT to measure target lesions, non-target lesions, and new lesions.[13]

RESULT

There were 106 patients with a diagnosis of lung adenocarcinoma who had received targeted EGFR-TKI therapy. It was found that four patients who visited were still starting treatment in 2017. The four patients stated that their RECIST 1.1 results were for Progressive Disease.

A study was conducted to determine the relationship between the incidence of acquired resistance and RECIST 1.1 results in lung adenocarcinoma patients receiving tyrosine kinase inhibitor therapy. The demographic characteristics of the research subjects showed in Table 1.

Table 1. Demographic characteristics of research subjects

Characteristics	N	%
Gender		
Male	11	35.5
Woman	20	64.5
Age		
< 50 years	5	16.1
50 - 59 years	9	29.0
60 - 69 years	9	29.0
≥ 70 years	8	25.8
Ethnic group		
Batak	20	64.5
Jawa	8	25.8
Padang	3	9.7
Smoking Status		
Smoker	10	32.3
Never Smoker	21	67.7
Stage		
II B	1	3.2
III B	3	9.7
III C	1	3.2
IV A	20	64.5
IV B	6	19.4
Total	31	100.0

The molecular profiling and EGFR-TKI therapy response of the research subjects can be seen in Table 2.

All research subjects underwent gene analysis to detect mutations in EGFR by sequencing techniques. The results showed that of the 31 research subjects, the majority of subjects the majority of subjects (58.1%) had mutations in exon 19. Furthermore, mutations that were also

found in research subjects were mutations in exon 21, which were found in 10 subjects (32.3%). There were only 3 people (9.7%) of the subjects who had mutations in exon 18.

The majority of research subjects, namely 17 people (54.8%), got RECIST 1.1 progressive disease results. There were 9 patients whose RECIST 1.1 results were stable disease. There were 3 patients whose RECIST 1.1 results showed a partial response, and there were only 2 patients (6.5%) who managed to experience a complete response from the results of the RECIST 1.1 evaluation.

Table 2 demonstrates that, of the ten persons who had acquired resistance, six had progressing illness based on their RECIST 1.1 results. In other words, based on the results of the RECIST 1.1 review, the majority of patients who develop acquired resistance after receiving tyrosine kinase inhibitor treatment experience disease deterioration.

Table 2. Molecular profiling characteristics of research subjects

Characteristics	N	%
EGFR mutation		
Exon 18 G719X	2	6.5
Exon 19 Deletion	11	35.5
Exon 21 L858R	7	22.6
Exon 21 L861Q	2	6.5
Exon 18 G719D and Exon 21 L858R	1	3.2
Exon 19 Deletion and Exon 21 L861Q	1	3.2
Exon 21 L858R and Exon 21 L861Q	1	3.2
Exon 19 Ins/Del, Exon 21 L858R and Exon 21 L861Q	1	3.2
Types of EGFR-TKI		
Afatinib	2	6.5
Erlotinib	4	12.9
Gefitinib	24	77.4
Osimertinib	1	3.2
Length of Consumption TKI		
0-12 months	20	64.5
13-24 months	8	25.8
>24 months	3	9.7
RECIST 1.1 Results 1.1		
Progressive[8]	17	54.8
Stable	9	29.0
Partial	3	9.7
Complete	2	6.5
Acquired resistance		
Negative	21	67.7
Positive	10	32.3
Total	31	100.0

Table 3. Relationship of Exon 20 T790M Mutation (Acquired resistance) with RECIST 1.1

Exon 20 T790M Mutation (Acquired resistance)	RECIST 1.1 RESULT				P
	Pr (%)	S (%)	Pa (%)	Co (%)	
(+)	6 (35.2)	1 (11.1)	1 (33.4)	2 (100)	0.93
(-)	11 (64.8)	8 (88.9)	2 (66.6)	0 (0)	

Note: *Kurskal Wallis Test; Pr=Progressive; S=Stable; Pa=Partial; Co=Complete

This means that the occurrence of acquired resistance cannot be said to be the only factor that determines the occurrence of progressive disease in lung adenocarcinoma patients receiving tyrosine kinase inhibitor therapy. This is evidenced by the results of statistical analysis using Fisher's exact test, which showed that there was no relationship between the patient's RECIST 1.1 results and the incidence of acquired resistance EGFR exon 20 T790M (P>0.05).

DISCUSSION

Lung adenocarcinoma at a young age with the proportion of women who are not smokers obtained in this study can be a separate lung cancer entity. Several studies have reported the role of gene mutations in young lung cancer.[14] The PIONEER study reported the proportion of positive EGFR mutations for lung adenocarcinoma in 7 Asian countries ranged from 22-64%. This study found a high proportion of positive EGFR mutations in the young age group (70.8%), and this is higher than the old age (51.6%). This confirms the association of EGFR mutations as oncogene activators in young-onset 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.[15]

The ddPCR method is the second difference, based on research showing that ddPCR and ARMS-PCR have high specificity with practical sensitivity for detecting EGFR mutations in cfDNA, which supports their application as a supplement or conditional alternative to tissue biopsy in clinical practice for genotyping. It seems that ddPCR has a higher sensitivity than ARMS-PCR, especially in the early stages.[16]

First-line therapy is given to patients who have never received previous treatment. If it turns out that the EGFR mutation is known early on, then EGFR-TKI therapy immediately becomes the first-line therapy option. On the other hand, if the EGFR mutation is negative, then platinum-based chemotherapy is the treatment option. Furthermore, after the administration of EGFR-TKI, progress was observed. If there is a worsening of the disease caused by the T790M mutation, the next treatment option can be given as second-line EGFR-TKI (Osimertinib).[17]

Adenocarcinoma patients with EGFR mutations will initially respond very well to EGFR-TKI therapy, but subsequently develop secondary resistance or resistance to the drug for an average of 9-14 months.[17,18] In the trial of the AURA3 study, using DNA testing without cells, it was found that about 51.2% of patients had the T790M mutation. This is why 60% of patients who have received EGFR-TKI eventually develop an EGFR T790M mutation at exon 20, which has been investigated as being associated with molecular changes.[19]

There are several mechanisms for the occurrence of resistance to EGFR-TKI, such as secondary mutation (T790M), activation of alternative pathways (c-Met, HGF, AXL), abnormalities at the end of the pathway (K-RAS mutation, loss of PTEN), and

disruption of the EGFR-mediated apoptotic pathway, TKI (polymorphisms in the form of deletion of the 11/BIM gene such as BCL2), histologic changes, ATP-binding transporter site (ABC) fusion, and others.[19,20] This mutation causes tridimensional alterations in the tyrosine kinase domain structure, preventing erlotinib and gefitinib from binding to the EGFR.[12]

Osimertinib is a potent and irreversible EGFR-TKI, selective for EGFR-TKI mutations and resistance T790M mutations, but has minimal effect on wild-type EGFR. The phase 2 study of the AURA trial demonstrated the efficacy and safety required for obtaining approval from the US Food and Drug Administration in the treatment of patients with EGFR T790M-NSCLC positive who had progressed during or after TKI therapy by demonstrating an objective response rate of 62%, and survival. Progression-free survival (PFS) ranges from 12.3 months.[21]

This study found that 10 people experienced acquired resistance. There were 6 people whose RECIST 1.1 results showed progressive disease. In other words, it can be interpreted that the majority of patients who experience acquired resistance after giving tyrosine kinase inhibitor therapy experience worsening of the disease based on the results of the RECIST 1.1 evaluation. However, at the same time, of the 21 people who did not experience acquired resistance, 11 of them also experienced progressive disease. This means that the occurrence of acquired resistance cannot be said to be the only factor that determines the occurrence of progressive disease in lung adenocarcinoma patients receiving tyrosine kinase inhibitor therapy.

However, the researcher admits that further studies are needed to detect the incidence of acquired resistance using specimens from rebiopsy tissue so that more accurate results can be obtained. In addition, the detection of acquired resistance events should be made a routine procedure for all adenocarcinoma patients after 9-12 months of receiving tyrosine kinase inhibitor therapy.[21]

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CONCLUSION

The incidence of acquired resistance in lung adenocarcinoma patients receiving tyrosine kinase inhibitor therapy was 32.3%. There is no association between the incidence of acquired resistance and RECIST 1.1 results in lung adenocarcinoma patients receiving tyrosine kinase inhibitor therapy.

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