InaJCC The Indonesian Journal of Cancer Control

Original Article

Liquid biopsy and tissue biopsy for epidermal growth factor receptor (EGFR) mutation analysis in lung adenocarcinoma

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e-ISSN 2797-457X
DOI: 10.52830/inajcc.v3i1.74

Received: March 7th, 2023
Accepted: January 13th, 2024

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Abstract

Background: Epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC), especially the type of adenocarcinoma, have emerged as a significant therapeutic target. Obtaining tissue samples from patients with advanced lung cancer has been challenging and the tissue samples often exhibit tumour heterogeneity depending on the location in the lungs. Notably, peripheral blood liquid biopsy exhibits better specificity in detecting EGFR mutations in NSCLC patients, yet tissue biopsy remains necessary for those with negative blood biopsy results due to its lower sensitivity.
Methods: We compared EGFR test from histo/cytopathological samples versus blood of naïve adenocarcinoma NSCLC at Persahabatan Hospital, Jakarta. EGFR mutation test from pathological sample was done using PCR HRM, Fragment Analysis, Direct Sequencing and AmoyDx, whereas EGFR mutation testing from blood was using Therascreen EGFR Plasma RGQ PCR Kit.
Results: Among 100 subject recruited, 89 matched samples were eligible for evaluation. EGFR mutation were detected positive from 39.3% tissue biopsy compare with only 22.5% positive EGFR mutation from blood. Using the tissue biopsy as standard, the sensitivity and positivity of liquid biopsy was 54.29% and 98.15%, with positive predictive value of 95% and negative predictive value of 76.81%.
Conclusion: Liquid biospy has high specificity in detecting EGFR mutation in naive adenocarcinoma NSCLC. However, its use in the clinic cannot replace the role of the PA examination to determine the type of cancer cells.

Keywords: *epidermal growth factor receptor, liquid biopsy, non-small cell lung cancer, tissue biopsy*

Abstrak

Latar belakang: Mutasi gen *epidermal growth factor receptor* (EGFR), terutama pada jenis adenokarsinoma karsinoma paru bukan sel kecil (KPKBSK), memilki peran signifikan karena terkait terapi dan prognosis yang lebih baik. Jaringan biopsi paru merupakan sampel ideal untuk diagnosis dan pemeriksaan mutasi EGFR. Akan tetapi, pengambilan sampel jaringan pada kanker paru relatif kompleks dan jaringan tumor yang didapat terkadang memiliki heterogenitas gen. Sampel alternatif untuk pemeriksaan mutasi EGFR adalah darah, yang relatif lebih mudah terutama pada pasien dengan jaringan biopsi paru negatif.
Metode: Penelitian ini membandingkan pemeriksaan mutasi EGFR pada jaringan sampel histo/sitopatologi dibandingkan dengan pemeriksaan mutasi EGFR dari darah (biopsi cair). Sampel berasal dari pasien KPKBSK jenis adenokarsinoma yang baru terdiagnosis atau belum mendapat terapi di RS Persahabatan, Jakarta. Pemeriksaan mutasi EGFR pada jaringan menggunakan PCR HRM, *fragment analysis, direct sequencing* dan AmoyDx, sedangkan pemeriksaan mutasi EGFR dari darah menggunakan kit Therascreen EGFR Plasma RGQ PCR.
Hasil: Dari 100 subjek yang direkrut, sekitar 89 kasus dapat dianalisis darah dan jaringannya. Mutasi EGFR ditemukan positif pada 39.3% sampel jaringan biopsi, sedangkan hanya 22.5% mutasi EGFR ditemukan positif pada darah atau cairan biopsi. Sensitivitas, spesifisitas, *positive predictive* value (PPV) dan *negative predictive value* (NPV) pemeriksaan biopsi cair pada penelitian ini adalah 54.29%; 98,15%; 95% dan 76.81%.
Kesimpulan: Pemeriksaan EGFR dari darah (biopsi cair) pada KPKBSK jenis adenokarsinoma pada penelitian ini memiliki spesifisitas yang tinggi. Namun penggunaanya di klinik tidak dapat menggantikan peran pemeriksaan PA untuk menentukan jenis sel kanker.

Kata kunci: *biopsi cair, biopsi jaringan, epidermal growth factor receptor, karsinoma paru bukan sel kecil*

Introduction

Lung cancer exhibits the highest incidence rate among all types of cancer and ranks second in terms of mortality globally. The affliction accounts for as many as 154.050 cancer-related in male patients in the United States, with an estimated 234.030 new cases in 2018.[1] The elevated mortality rates associated with lung cancer primarily stem from its belated detection and diagnosis, leading to an unfortunate decline in overall survival rates.[2,3] Even patients diagnosed during the early stages and at the contained phase of the disease, the five-year overall survival rate is distressingly low, standing below 60% when compared to other types of cancer. Regrettably, the disease progression is a key factor contributing to the poor prognosis even in cases where early detection has been achieved. [4]

The etiology of lung cancer involves intricate biological processes resulting in highly heterogenous tumor formations.[5] Histologically lung cancer is categorized into two principal subtypes; small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC).[6] Among the NSCLC variants, Adenocarcinoma stands out as the most prevalent type worldwide. Empirical evidence from Persahabatan Central General Hospital during the period of 2004-2006 demonstrated that Adenocarcinoma was the predominant from of NSCLC, accounting for a substantial 56.3% of all diagnosed lung cancer cases.[7,8]

In the past decade, Epidermal Growth Factor Receptor Mutations (EGFR) in NSCLC, especially the type of adenocarcinoma, have emerged as a significant therapeutic target. Obtaining tissue samples from patients with advanced lung cancer has been challenging and the tissue samples often exhibit tumor heterogeneity depending on the location in the lungs. Notably, peripheral blood liquid biopsy exhibits better specificity in detecting EGFR mutations in NSCLC patients, yet tissue biopsy remains necessary for those with negative blood biopsy results due to its lower sensitivity. Regardless of whether EGFR mutations are detected through tissue or liquid biopsy, they have been linked to improved Progression Free Survival (PFS) compared to wild-type NSCLC patients.[9,10]

Methods

This study was a cross-sectional study involving patients diagnosed with lung adenocarcinoma. The sampling method used was a concecutive sampling, where all subjects meeting the research's criteria were included in the study until the required number of participants was reached. The subject were selected based on their diagnosis obtained from histopathological and/or cytologic exmination and the had also undergone routine gene mutation testing using existing specimens. EGFR gene mutation from tissue was examined using DNA-extraction with the Qiagen QIAamp DNA Micro kit, mutation analysis by PCR HRM, Fragment Analysis, Direct Sequencing and AmoyDx, 100% specificity, mutant allele could be detected in at least 25% of tumor cells.

Patients will be informed that there are additional tests besides routine examinations for EGFR gene mutations using 2 x 9cc of venous blood, approximately 2 x 1 teaspoon by laboratory staff or nurses. The patient will sign informed consent as a research subject by providing the required information and agreeing to have venous blood taken from a vein at the elbow or another location that is easier and more comfortable for the patient.

DNA was isolated from plasma samples using 2 methods: (1) the automatic silica beads method using the QIASymphony Circulating DNA Kit (Qiagen) and, (2) the manual spin column method using the QIAamp Circulating Nucleic Acid Kit (Qiagen) and the QIAVac 24 system. DNA was amplified with the EGFR Plasma RGQ PCR Kit (Qiagen) therascreen, using the RotorGene Q tool. The interpretation of the results was determined based on the calculation results from ΔCt between the mutation assay and control assay. Therascreen EGFR Plasma RGQ PCR Kit uses 2 technologies: ARMS and Scorpions, to detect mutations with the real-time PCR method.

This study included 100 blood and tissue samples from patients histopathologically diagnosed with lung adenocarcinoma. Of the 100 samples, 11 were excluded because the samples did not meet the requirements to be examined, so the total samples included in the analysis were 89. The data obtained is processed using SPSS.

Results

In this study, there were 89 patients, 59 males (66.3%) and 30 females (33.7%). The average age of the subjects of this study was 56.07 years, ranging from 30 to 80 years old. The ethnic distribution included Javanese 46 Javanese patients (51.7%), 13 Sundanese patients (14.6%), 9 Batak patients (10.1%), 11 Betawi patients

(12.4%), 4 Minangkabau patients (4.5%), 2 Ambon patients (2.2%), 2 Bugis patients (2.2%), 1 Malay patient (1.1%), and 1 Flores patient (1.1%). Among the subjects, 60 smokers (67.4%) with 7 having a light Brinkman Index (11.7%), 23 having moderate Brinkman Index (38.3%), and 30 having a heavy Brinkman Index (50.0%). Table 1 provides further details on the characteristics of the patients.

Table 1. Patient characteristics (n=89)

Characteristics	n (%)
Age	
<45 years	13 (14.6)
45-65 years	56 (62.9)
>65 years	20 (22.5)
Gender	
Female	30 (33.7)
Male	59 (66.3)
Smoking Habit	
Nonsmoker	29 (32.6)
Smoker	60 (67.4)
Brinkman Index (BI)	
Light	7 (11.7)
Moderate	23 (38.3)
Heavy	30 (50.0)
Brain Metastasis	
Yes	13 (14.6)
No	76 (85.4)

In this study, 89 patients were examined, and EGFR mutation was detected in 20 patients through liquid biopsy representing 22.5% of the participants. Among these, 16 patients (18%) had DelEx19 mutation, and 4 patients (4.5%) had L858R mutation. The prevalence of EGFR mutations was found in 13,5% of males and 9.0% of females. On the other hand, an in-frame EGFR mutation was obtained from tissue biopsy in 35 patients (39.3%) out of the total. Among these, 18 patients (20.2%) had DelEx19 mutation, 13 patients (14.6%) had L858R mutation, 2 patients (2.2%) had L861Q mutation, 1 patient (1.1%) had T790M mutation, and 1 patient had mixed DelEx19 and L858R mutation. It was found in 25.8% of males and 15.7% of females. The distribution of EGFR mutations from tissue biopsy and liquid biopsy is presented in Table 2, and the distribution of EGFR mutations by gender can be seen in Table 3.

Table 2. Distribution of EGFR Mutation from liquid biopsy and tissue biopsy

No	EGFR Mutation	Blood	Tissue
1	WildType	69 (77.5 %)	54 (60.7 %)
2	DelEx19	16 (18 %)	18 (20.2 %)
3	L858R	4 (4.5 %)	13 (14.6%)
4	DelEx19 and L858R		1 (1.1 %)
5	L861Q		2 (2.2 %)
6	T790M		1 (1.1 %)

Table 3. Gender distribution of EGFR mutations

Mutation Type	Gender		Total
	Male	Female	
Tissue biopsy			
Positive	23 (25.8%)	14 (15.7%)	37 (41.5%)
Negative	36 (40.5%)	16 (18.0%)	52 (58,5%)
Liquid biopsy			
Positive	12 (13.5%)	8 (9.0%)	20 (22.5%)
Negative	47 (52.8%)	22 (24.7%)	69 (77.5%)

Out of the 13 patients in this study who had brain metastases, 3 patients (23%) had mutations in the EGFR gene; 2 patients (66.7%) had the exon 19 mutation, while a point mutation in exon 21 was detected in the remaining 1 patient (33.3%). Further details regarding the distribution of brain metastasis are provided in Table 4.

Table 4. Brain metastasis distribution related to liquid biopsy mutations

Brain Metastasis	Liquid Biopsy	
	Wild type	Mutation
Yes	10 (8.9%)	3 (3.4%)
No	59 (66.3%)	17 (19.1%)

This study also evaluated the accuracy of detecting EGFR mutations using ctDNA and tissue samples. The result showed that ctDNA had a sensitivity rate of 54.29% and a specificity rate of 98.15%. This means that ctDNA could identify 54.29% of patients with positive EGFR mutation correctly, while 45.71% of cases would be incorrectly identified as negative for EGFR mutations. The positive predictive value of ctDNA was found to be 95%, indicating that out of all patients detected with positive EGFR mutations using ctDNA, only 95% of them indeed had positive EGFR mutations at the time of sampling. Further details on the diagnostic accuracy value of ctDNA plasma can be found in Table 5

Table 5. Diagnostic accuracy of liquid biopsy and tissue biopsy

EGFR mutations		Tissue Biopsy		Kappa value
		Mutation	WildType	
Liquid Biopsy	Mutation	19	1	0.574 (p < 0.000)
	WildType	16	53	
Sensitivity of EGFR mutations from liquid biopsy			54.29 %	
Specificity of EGFR mutations from liquid biopsy			98.15%	
Positive predictive value (PPV)			95%	
Negative predictive value (NPV)			76.81%	

Discussion

The patients in this study consisted of 59 males (66.3%) and 30 females (33.7%). This study is following lung cancer data in Indonesia based on Syahruddin's research from 1874 research samples, with the demographic distribution of patients being 61.0% male and 39% female. Lung cancer studies from several countries also found that the prevalence of lung cancer in males is higher than in females, and is following lung cancer incidence data in the United States of America (USA) in 2018. The frequency of EGFR mutations in this study was higher in males (66.3%) than in females (33.7%), and the results were different from Syahruddin's research that EGFR mutation is higher in women (52.9%) than in men (39.1%). [1,11]

Approximately 10% of newly diagnosed patients with NSCLC have brain metastases. A retrospective study by Noronha V et al. showed that 13.9% of 101 patients with EGFR mutated had brain metastases, with 61.3% exon 19 deletion mutations and 28.7% exon 21 L8585R mutations.[12] Retrospective analysis of the 69 patients with brain metastases by Porta R et al., 24.6% harbored mutations in the EGFR gene, with an in-frame deletion in exon 19 found in 70.6% of patients, while a mutation in exon 21 L858R was detected in 29.4% patients.[13] The result of the two studies were in line with the results of this study that of the 13 patients in this study with brain metastases, 23% had mutations in the EGFR gene, and the highest prevalence of mutations that occurred was exon 19 mutation as this study result was 66.7% patients and followed by a mutation in exon 21 was detected in the remaining 33.3%.

But this study result was different from Shin D et al which showed a significant association between EGFR mutation and risk of brain metastases, 43.9% of the 314 patients had EGFR mutations as the frequency of EGFR mutation was statistically higher for patients with brain metastases as 64.7%.[14] In this study, 85% of patients had EGFR mutation without brain metastases, while 15% of patients with brain metastasis.

The proportion of EGFR mutation from ctDNA in this study was found in 20 patients (22.5%) using a therascreen EGFR Plasma RGQ PCR Kit. The result of this study are similar to a study conducted by Reck et al using several ctDNA detection tools in 291 Japanese people, the results of mutations were found in 37 patients (13.0%), while in European ethnicity, 82 mutations cases (8.4%) from ctDNA were found in 903 patients. The result of a mutation in Japan and Europe were lower compared with the result in this study. The difference in the results could be due to differences in the EGFR mutation rates for European ethnicities (14.1%) and Asians (38.4%).[15,16]

The study of Ariola et al in Spanish about EGFR mutation obtained the sensitivity, specificity, PPV, and NPV values of ctDNA examination were 45.5%, 96.7%, 71.4%, and 90.7%. Ariola et al concluded that ctDNA could be used when tumor tissue is not available but has limitations in testing for EGFR mutations. Standardization and trained laboratories are needed in addition to the various available testing techniques and the different levels of sensitivity of the testing techniques. The result of sensitivity (54.29%), specificity (98.15%), PPV (95%), and NPV (76.81%) from this study were almost the same as the result of Ariolla's. The difference in the ctDNA detection tool used can affect the result of sensitivity and specificity, because each method and tool have different approach to detecting EGFR mutations in ctDNA.[17-19]

Conclusion

Liquid biopsy has demonstrated heightened specificity in discerning EGFR mutation within NSCLC patients, nevertheless, tissue biopsy remains imperative owing

to its superior sensitivity when liquid biopsy is confronted with negative results. Employing plasma ctDNA as the gold standard for scrutinizing EGFR mutations remains contingent upon additional research endeavors.

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