ORIGINAL ARTICLE

Accuracy of Circulating Tumor DNA (ctDNA) in EGFR Mutation Detection Among Lung Adenocarcinoma in M Djamil Hospital, Padang

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ABSTRACT

Background: ctDNA is an alternative test for detecting mutation of EGFR in lung cancer type adenocarcinoma if the tissue speciment can not be carried out. Sensitivity, specificity and accuration of ctDNA test is stil varied. This study is aimed to acknowledge sensitivity, specificity and accuration of ctDNA in detecting EGFR mutation in patient with lung cancer type adenocarcinoma in M Djamil Hospital.

Methods: Design this study a diagnostik test comparing ctDNA to tissue speciment in detection EGFR mutation of 42 patients with lung cancer type adenocarcinoma in M Djamil Hospital. Sample was selected through consecutive technique.

Results: Incidence of EGFR mutation in patients with lung cancer type adenocarcinoma from tissue speciment was higher than ctDNA ((42,9% vs 28,6%; p=0,031). There was significant diffierence of EGFR mutation detection between sex, smoking status, and TNM staging based on tissue/cytology examination and ctDNA (p=0,031). EGFR mutation in sitologic test and ctDNA was more likely detected in male patient (66.7% and 58.3%), ex-smoker (50% and 41.7%) and stage IV (88.9% and 91.7%). The results of sensitivity, specificity positive prediction value (PPV) and Negative prediction value (NPV) in ctDNA test to detect EGFR mutation were 66,7%, 100%, 100% dan 80% according to sitology test as gold standard. Furthermore, the ctDNA accuration was measured according to AUC score 0.833 (SE 0,072, CI 95%, 0,693-0,974, p=0.0001).

Conclusion: ctDNA test have a good accuration with sensitiviy 66.7% and specificity 100% in detecting EGFR mutation in patients with lung cancer type adenocarcinoma.

Keywords: EGFR, lung cancer, adenocarcinoma, ctDNA

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INTRODUCTION

Adenocarcinoma is the most common non-small cell carcinoma (KPKBSK) type lung cancer in Indonesia.[1] The treatment of adenocarcinoma lung cancer has undergone many developments including using targeted therapy. Tyrosine kinase inhibitors (TKI) are one of the target therapies given to KPKBSK with epidermal growth factor receptor (EGFR) mutations.[2]

EGFR mutation examination specimens are derived from tissue specimens or cytology, provided that the number of cells is sufficient. A common obstacle is the small number of cells in the specimen and the difficulty of retrieving tissue specimens.[2] Therefore, an alternative examination is required that can detect EGFR mutations with lower invasive rates and good accuracy.[3]

EGFR mutations were first reported in 2004 and EGFR mutations in tyrosine kinase in pulmonary adenocarcinoma patients have been the main focus of research in understanding pathogenesis and current treatment.[4] EGFR mutation screening is a requirement to obtain TKI as a targeted therapy in pulmonary adenocarcinoma.[5]

Multisenter research in Indonesia by Syahruddin et al against 1874 newly diagnosed lung cancer shows the frequency of EGFR mutations 44.5%. The most common obstacle found in the examination of EGFR mutations from this study is the number of tumor cells obtained from too few cytological specimens (<100 cells). The problem is one of the causes of failure in the examination of EGFR mutations which is as much as 95 (5.1%) Specimens.[6]

One alternative EGFR mutation examination other than cytological specimens or tumor tissue is circulating tumor DNA (ctDNA) test using blood specimens.[3] In research conducted by Bettegowda et al., found that patients with pancreatic, ovarian, colorectal, urinary vesical, gastroesophageal, breast, melanoma, hepatovascular and head and neck cancers who have metastatic, ctDNA examination can detect EGFR mutations more than 75% pasien.[7]

In reck et al., research, only 9% of lung cancer patients with EGFR mutation positive from ctDNA screening in several European countries and Japan.[7] In contrast to zaini et al., research at RSUP Persahabatan Jakarta, 41.8% of lung cancer patients with EGFR mutation detected from ctDNA examination. In the study also found that sensitivity was 30-40% and specificity 83%-96% in ctDNA examination in detecting EGFR

mutations of patients before getting treatment.[8]

METHOD

This research is a diagnostic test study comparing ctDNA examination with tissue examination/cytology in 42 KPKBSK patients of adenocarcinoma type in RSUP. DR. M. Djamil Padang from March 2019-February 2020.

Samples are taken on a nonprobable way with consecutive techniques. Inclusion criteria are Patients with previous chemotherapy-naive (never had chemotherapy before), have no other inorgan malignancy, willing to participate in research and sign informed concerns. While the exclusion criteria is the number of tumor cells in tissue specimens/cytology less than 100 cells.

The research sample was then examined for EGFR mutations through tissue/cytology and ctDNA plasma in a patient at the same time. Examination of EGFR mutations through tissue /cytology is carried out by sending slides of cytological anatomical pathology or pulmonary adenocarcinoma tissue to CITO clinical laboratory Yogyakarta. CtDNA examination is carried out through a 5 ml venous blood specimen taken by prodia jakarta clinical laboratory staff.

The research has been approved by the ethics committee of RSUP Dr. M. Djamil Padang. Statistical analysis used is diagnostic test (sensitivity, specificity, positive prediction value (NPP), negative prediction value (NPN)) using test performance table 2x2. In this study, EGFR mutation examination of tissue/cytology as diagnostic reference /gold standart and EGFR mutation examination of ctDNA as diagnostic index. To assess the accuracy of ctDNA checks used ROC curve analysis (receiver operating characteristic) with output of Area Under Curve (AUC).

RESULT

This research is a diagnostic test study that includes patients as a sample of 42 people who are treated to poly pulmonary or treated in the pulmonary ward of RSUP Dr. M. Djamil Padang. The results showed that the majority of patients were male (81.0%). The average age of patients in the study was 58.9±10.07 years, with the youngest age being 34 years old and oldest 79 years. More than half (54.8%) patients are former smokers and have TNM staging IV (73.8%). A total of 52.4% of patient specimen retrievals were carried out by transthoracic needle aspiration (TTNA) method, as seen in Table 1.

The incidence rate of EGFR mutations in KPKBSK patients of adenocarcinoma was assessed based on more tissue specimens/cytology compared to ctDNA (42.9% vs. 28.6%; P=0.031). The most mutations were found in exon 19 insertions/deliesthesia, both in tissue specimens/cytology (77.8%) ctDNA (66.7%) (Table 2).

The results of the analysis showed that there were differences in detection of EGFR mutations in gender, smoking status, and TNM staging based on tissue/cytology and ctDNA (P=0.031) (Table 3). In the results of the study also found that EGFR mutations were widely detected in former smokers at tissue/cytological examination (50.0%). While in ctDNA examination, EGFR mutations were widely detected in former smokers (41.7%) and non-smoking (41.7%) (Table 3). Positive EGFR mutation detection was found in stage IV patients from both tissue/cytological examinations (88.9%) ctDNA (91.7%).

Table 1. Characteristics of KPKBSK patients with adenocarcinoma at RSUP DR. M. Djamil Padang

Characteristics	**n**	**(%)**
Gender		
Male	34	81,0
Female	8	19,0
Age (Mean±SD)	58,90±10,07	
Smoking Status		
Smokers	11	26,2
Ex-smokers	23	54,8
No smokers	8	19,0
TNM staging		
I + II	0	0,0
IIIa	5	11,9
IIIb	6	14,3
IV	31	73,8
Specimen Retrieval Method		
Cytological sputum	1	2,4
Pleural fluid cytology	4	9,5
Fine needle aspiration biopsy (BJAH)	2	4,8
Transthoracic needle aspiration (TTNA)	22	52,4
Core Biopsy	4	9,5
Rinse the bronchial	3	7,1
Bronchial sikatan	5	11,9
Bronchial forcep biopsy	1	2,4

Table 2. Incidence of EGFR mutation of KBKBSK patients of adenocarcinoma type in RSUP. Dr. M. Djamil Padang

Mutasi EGFR	**Tissue specimens/cytology**	**ctDNA**	**P**
Positive	18 (42,9%)	12 (28,6%)	0,031
Common mutation			
Exon 19 insertion/deletion	14 (77,8%)	8 (66,7%)	
Exon 21 (L858R)	2 (11,2%)	3 (25,0%)	
Uncommon mutation			
Exon 18 (G719X)	0 (0,0%)	0 (0,0%)	
Exon 20 (T790M)	0 (0,0%)	0 (0,0%)	
Mix mutation			
Exon 21, Exon 20 (T790M)	2 (11,2%)	1 (8,3%)	
Negative (wild type)	24 (57,1%)	30 (71,4%)	

Table 3. Differences in positiveness of EGFR mutations based on gender, smoking status, and TNM staging.

Characteristic	**EGFR mutation detection**		**P**
	Tissue/cytology (n=18)	**ctDNA (n=12)**	
Gender			
Male	12 (66,7%)	7 (58,3%)	0,031
Female	6 (33,3%)	5 (41,7%)	
Smoking Status			
Smokers	3 (16,7%)	2 (16,7%)	
Ex-smokers	9 (50,0%)	5 (41,7%)	0,031
No smokers	6 (33,3%)	5 (41,7%)	
TNM staging			
I + II	0 (0,0%)	0 (0,0%)	
IIIa	2 (11,1%)	1 (8,3%)	0,031
IIIb	0 (0,0%)	0 (0,0%)	
IV	16 (88,9%)	11 (91,7%)	

The results showed that the examination had a sensitivity of 66.7%, meaning that ctDNA's ability to detect positive EGFR mutations was 66.7%. While the result of specificity is 100%, it means that ctDNA's ability to detect negative EGFR mutations is 100% (Table 4). The proportion of patients with positive ctDNA results (positive prediction value/NPP) is 100% and negative prediction value (NPN) is 80%.

ROC curve analysis shows that the area value below the AUC curve is 0.833, standart error (SE) 0.072, confidence interval 95% (CI 95%) 0.693-0.974 with P=0.0001. This value can be interpreted as ctDNA's ability to tissue/cytology to accurately detect

EGFR mutations classifying positive and negative EGFR mutation detection as 0.833. Because the value is large enough can be interpreted that the level of accuracy of the examination is good. Statistical test results also show that the P<0.05, it can be concluded that ctDNA examination is proven to have the ability to distinguish the detection of positive and negative EGFR mutations (Figure 1).

Table 4. ctDNA Diagnostic Test Results on Tissue/Cytology in Detecting EGFR Mutations

ctDNA	Tissue/cytology		Total
	Positive	Negative	
Positive	12 (66,7%)	0 (0%)	12 (28,6%)
Negative	6 (33,3%)	24 (100%)	30 (71,4%)
Total	18 (100%)	24 (100%)	42 (100%)
Sensitivity=66,7%		NPP=100%	
Spesificity=100%		NPN=80%	

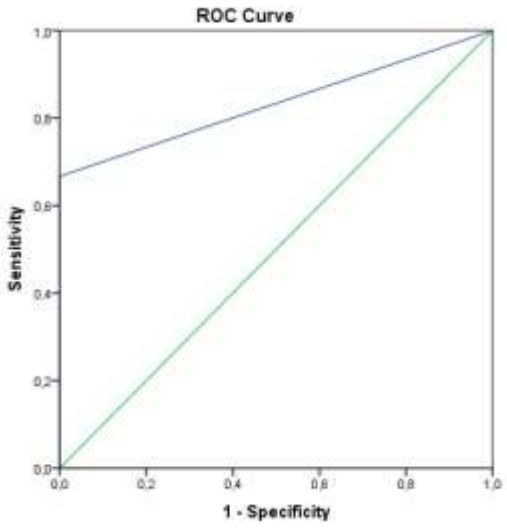

Figure 1. Accuracy of ctDNA against tissue / cytology in detecting EGFR mutations

DISCUSSION

This study found that the mean age was 58.90±10.07 and was common in men (81.0%). %). Many studies have found that people with lung cancer are more than 40 years old.[9] Research by Siegel et al. Found that the average age of lung cancer patients in the United States is 55-74 years.[10] The risk of lung cancer increases with age. Previous cell damage can take years to develop into cancer.[11]

The older you get, the longer you are likely to be exposed to various risk factors for lung cancer, followed by a decrease in the ability to repair cells.[11] Male gender is also a risk factor for lung cancer.[12] Based on data from the American Cancer Society, the number of lung cancer cases continued to increase in 2018, especially in men, as many as 121,680 instances from 234,030 total lung cancer cases.[9]

This study also found that lung cancer patients were still smokers (26.2%) and former smokers 54.8%, all of whom were male. Secondhand smoke exposure is one of the main risk factors for lung cancer in men and women.[13] This data is also supported by other studies that found that nearly 90% of lung cancer incidence was caused by cigarette smoke exposure.[9] The risk of smokers who have lung cancer is 10-30 times higher than

nonsmokers.[14] The incidence of lung cancer in smokers is influenced by age at initiation of smoking, the number of cigarettes smoked per day, length of the smoking habit, and smoking cessation duration.

The most common stage found in this study was stage IV (73.8%). Research conducted by Wang et al. in East China in 2011-2015 found that the most stage lung cancer found was stage IV (59.4%), and 62.2% were adenocarcinoma types. In the research conducted by Zaini et al. At Persahabatan Hospital, Jakarta, 42.45% of patients with stage IIIB and IV adenocarcinoma lung cancer were reported. Delay in diagnosis is one of the most common factors causing the high incidence of lung cancer patients with stage III and IV when the diagnosis is made.[15]

In this study, obtaining TTNA (52.4%) took the most specimens to get the most adenocarcinoma cell types with a cell count> 100. TTNA is a method of collecting cytological specimens that is most commonly used in lung cancer patients.[16] In general, Indonesia is the country with the highest detection of EGFR mutations through the cytological specimen method (98.0%).[17] One of cytology specimens' problems in detecting EGFR mutations is the insufficient number of tumor cells (<100 cells).[6]

The EGFR mutations detected from tissue/cytology, and ctDNA examinations were 42.9% and 28.6%, with the most exon 19 insertions/cell lesions mutations found (66.7%). This study's results were higher compared to studies conducted by Reck et al. In patients in Europe and Japan, where the incidence of EGFR mutations in adenocarcinoma from tissue/cytology and ctDNA was 20% and 11%.[7]

Based on Indonesia's multicenter research, the EGFR mutations detected were no different from this study, namely as much as 44.5%.[6] However, in Zaini et al.'s study at the Friendship Hospital, 62.7% and 41.8% of EGFR mutations from tissue/cytology and ctDNA examinations were obtained, which were higher than this study.[8]

Besides, many exon 19 mutations were found in previous studies, namely as much as 85% to 90%, so that mutations in exon 19 and L858R on exon 21 are called classic mutations or common mutations of EGFR in adenocarcinoma lung cancer.[5] Research by Zaini et al. at the Friendship Hospital in 2016 found EGFR mutations on exon 19 and L858R on exon 21, getting 15.45% and 21.8% (tissue/cytology) and 5.45% and 13.6% (ctDNA).[8] EGFR mutations in lung cancer were found on average in

women, Asian races, and non-smoking with adenocarcinoma type (60%).[18] In our study, men had a higher detection of EGFR mutations through tissue/ cytology examination (66.7%) and ctDNA (58.3%). The level of EGFR mutation positivity in tissue/cytology and ctDNA examinations also differed by sex. Li et al.'s study found the detection of EGFR mutations associated with patient sex and smoking history.[19]

This study also found that EGFR mutations were mostly detected in former smokers through tissue/cytology (50.0%), and through ctDNA examination, they were found in former smokers (41.7%) and non-smokers (41.7%). The results of our study analysis also found that there was a difference between the detection of EGFR mutations on tissue/cytology and ctDNA based on smoking status. Research by Tseng et al. (2017) reported that the average EGFR mutation in smokers and non-smokers was 41.9% and 70.0%. In that study, age, gender, smoking status were also predictors of the low mean level of EGFR mutation positivity. Smoking is known to be a negative predictor of the detection of EGFR mutations.[19,20]

A meta-analysis study by Ren et al. confirmed that non-smokers were significantly associated with high rates of detection of EGFR mutations.[20] Pham et al.'s study assessed smoking degrees by the pack and smoke-free years on the prevalence of detection of EGFR mutations. The study found that the EGFR mutation was not much different between non-smokers and patients who had smoked <15 pack-years or the length of time they quit smoking. Tseng's study also found that smoking at a young age had a low EGFR mutation.[19,20]

The study by Wiencke et al. Found that the level of abduct DNA in the carcinogenesis process was inversely related to the age at initiation of smoking among former smokers.[19] This suggests that young smokers are more susceptible to DNA damage and persistent genetic changes than patients who start smoking at an older age.[20] The high EGFR mutations in former smokers from the current study were likely influenced by the number of cigarettes consumed, age at initiation, and length of time to quit smoking.

This study showed the highest EGFR mutations in stage IV patients, both from tissue/cytology examination (88.9%) and ctDNA (91.7%). There are also differences in the detection of EGFR mutations in TNM staging characteristics based on tissue/cytology and ctDNA examinations. The results of Oh et al.'s study in Korea (2019) found that

positive EGFR mutations were also the highest in stage IV, both from tissue/cytology (53.4%) and ctDNA (32.0%). The high EGFR mutation in stage IV lung cancer patients is probably due to the tumor's spread into the circulation during the metastasis process.[21] In daily practice, differences in metastatic stage and status can significantly influence the detection of EGFR mutations in plasma, considering that ctDNA release is influenced by tumor size and metastatic processes.[22]

Identifying EGFR mutations in the adenocarcinoma type of KPKBSK is an essential guide for clinicians as a basis for providing targeted therapy. Several previous studies have suggested ctDNA plasma examination as an alternative to detect EGFR mutations in lung cancer.[23] Our research got a moderate sensitivity of 66.7% but with a specificity of 100%, NPP 100%, and NPN 80%. The ASSESS study on 1162 patients found that plasma ctDNA was a good test for detecting EGFR mutations in lung cancer with a 46% sensitivity, 97% specificity, NPP of 78%, and NPN of 90%.[7,23] The results were not much different from Zaini's study. et al. at the Friendship Hospital obtained the sensitivity of plasma ctDNA in detecting EGFR mutations, namely 30%-40% with high specificity (83-96%).[8]

Liquid biopsy, especially plasma ctDNA, is an examination that has been recommended by the IASLC, CAP, and AMP into the guideline for molecular analysis in KPKBSK patients.[24] CtDNA examination is not recommended as a substitute for tissue/cytology examination, possibly because previous studies' sensitivity results still vary between low to moderate.[8,24] However, ctDNA examination is recommended in patients with CPBC with fewer cell counts than tissue/cytology specimens or in patients where tissue/cytology specimens are difficult to do, especially in advanced stages.[24]

The results of the positive predictive value (NPP) of ctDNA in this test were very high, namely 100%. These results indicate that a positive test result can indicate that patients with adenocarcinoma type KPKBSK have very high EGFR mutations detected. Meanwhile, the negative predictive value (NPN) of ctDNA in this test was relatively high, namely 80%. These results indicate a negative test result can predict that patients are not detected with high EGFR mutations. Thus, all lung cancer patients with positive EGFR mutations from ctDNA testing did have EGFR mutations. Based on the above studies' results, the detection of EGFR mutations in plasma is very likely to have a high predictive

value identical to mutations in tumors.[25]

There were no false-positive results in this study, where negative results from ctDNA tests were also negative on tissue/cytology examinations. In contrast, the results of this study showed 6 out of 42 patients with false-negative results, where the results of the ctDNA test were negative. Still, the tissue/cytology examination was positive for the detection of EGFR mutations. The absence of false-positive results is probably due to the lack of a tumor's heterogeneity. There is no difference in EGFR mutations between the tumor and the patient's plasma, or the tumor does not have a mutation (wild type).[23] The use of blood specimens as a source for examining EGFR mutations is limited by circulating cfDNA in the circulation. Doing so can result in a false-negative test result. Also, the volume of mutated DNA in the plasma is below the detection limit of the method used, leading to false negatives.[19]

Molecular examination to determine mutation status (including in the provision of targeted therapy) has become a routine examination in clinicians' daily practice.[26] Another advantage of ctDNA testing is that all DNA mutations from all tumors (including metastases) in the patient can be used as examination samples. This can reduce the risk of undetected EGFR mutations due to heterogeneity or an insufficient number of tumor cells.[7]

In the ASSESS study, it was found that ctDNA examination could be used to detect EGFR mutations that might be missed from tissue/cytology due to inadequate specimens obtained. However, ctDNA testing may not always detect EGFR mutations present in tumors even when the latest technology is susceptible.[7] Therefore, tumor tissue models remain the gold standard for detecting EGFR mutations before administering targeted therapy.[26]

Analysis of circulating tumor cells can only be performed on fresh blood specimens.[26,27] Several observational studies have found that the half-life of cfDNA in the circulation is between 16 minutes and 2.5 hours.[27] Ideally, the blood samples that have been taken should be checked for EGFR mutation detection in the laboratory. However, in our study, blood samples that have been taken by laboratory personnel are then sent out of town because the Real-Time PCR Scorpion-AMRS examination tool is not available, which is a weakness in our study.

CONCLUSION

The ctDNA examination has good accuracy with a sensitivity of 66.7% and a specificity of 100% in detecting EGFR mutations in adenocarcinoma lung cancer. The use of ctDNA is a promising alternative diagnostic examination if the tumor cell count in the tissue/cytology specimen cannot be used to detect EGFR mutations.

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