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Comparation of Citology Test and Plasma ctDNA for Detection of EGFR Mutation in Lung Adenocarcinoma at Ulin General Hospital Banjarmasin

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Abstract

Introduction: Lung cancer continues to be the leading cause of cancer-related deaths worldwide. In Indonesia the number of lung cancer cases includes the top three. Non-small cell lung cancer (NSCLC) with sub-type Adenocarcinoma is most commonly found. The analysis of epidermal growth factor receptor (EGFR) mutations in lung adenocarcinoma has provided the opportunity for successful treatment with targeted EGFR tyrosin kinase inhibitor. However, insufficient tumor tissue for EGFR mutation testing become problem. Therefore, blood plasma samples for EGFR mutation analysis have been suggested as alternatives.
Methods: The research subjects used were patients with lung adenocarcinoma who had performed cytology and ctDNA examinations simultaneously from October 2017 to October 2018. Then a statistical analysis was performed.
Result: From 55 samples, 22 (40%) patients detected EGFR mutations and predominantly occurred in women at 54.5%. There was no significant difference between the results of cytology examination and plasma ctDNA to diagnose EGFR mutations in adenocarcinoma lung cancer.
Conclusion: Plasma ctDNA can be used as an alternative choice for detecting EGFR mutation status in lung adenocarcinoma if it is difficult to get tumor tissue.
Keywords: Lung adenocarcinoma, EGFR, ctDNA

Abstrak

Latar belakang: Kanker paru terus menjadi penyebab utama kematian terkait kanker diseluruh dunia. Di Indonesia jumlah kasus kanker paru termasuk tiga besar terbanyak. *Non-small cell lung cancer* (NSCLC) dengan sub tipe Adenokarsinoma paling sering ditemukan. Diagnosis mutasi EGFR diperlukan terkait dengan pilihan terapi. Namun, jaringan tumor yang tidak cukup untuk dilakukan pemeriksaan mutasi EGFR sering menjadi masalah. Sehingga, ctDNA menjadi pilihan alternatif jika kesulitan mendapatkan jaringan kanker primer.
Metode: Subjek penelitian yang digunakan adalah pasien dengan adenokarsinoma paru yang sudah dilakukan pemeriksaan sitologi dan ctDNA secara bersamaan pada bulan Oktober 2017 sampai dengan Oktober 2018.
Hasil: Dari 55 sampel didapatkan 22 (40%) pasien terdeteksi mutasi EGFR dan dominan terjadi pada wanita sebesar 54,5%. Tidak ditemukan perbedaan yang signifikan antara hasil biopsi jaringan dengan ctDNA untuk mendiagnosis mutasi EGFR pada kanker paru tipe adenokarsinoma.
Kesimpulan: ctDNA plasma dapat digunakan sebagai pilihan alternatif untuk mendeteksi status mutasi EGFR pada kanker paru tipe adenokarsinoma jika kesulitan mendapatkan jaringan kanker primer.
Kata Kunci: Adenokarsinoma paru, EGFR, ctDNA

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Introduction

In last 100 years, lung cancer has changed from a rare disease to huge global problem. The first time lung cancer mentioned in scientific literature was in 1400s, where 50% of miners in Germany–Czech Republic borders died because of some lung disease called *bergkrankheit*.[1] In 1879, Harting and Hesse did autopsy to 20 patients priorly diagnosed with *bergkrankheit* and they found 75% malignant cells which were classified as squamous cell carcinoma.[1] In 1840s, English researchers still had difficulties to find lung cancer cases, even not more than 22 cases.[2] In 1912, Adler successfully found 374 lung cancer cases.[3] The incidence and mortality of lung cancer drastically increased since 1930s, which were linked to smoking habit in community.[3] Epidemiologically, in 2012 it had been found 1,8 million lung cancer cases with incidence 34,2/100.000 in men and 13,6/100/000 in women.[2] It is estimated that 2,09 million new lung cancer cases were found in 2018 and it has been the highest among other cancers.[2] In United States during 2018, it had been found 234.030 lung cancer cases totally or equal to 641 new cases every day.[2] Those numbers had become the second highest cancer based on sex, after prostate cancer in male and breast cancer in female.[2] In Indonesia, lung cancer is the third most found cancer after breast cancer and servix cancer. The incidence rate of lung cancer has reached 30.023 cases with prevalence 3:1 dominant in male.[4]

Lung cancer continues being the main cause of death related to cancer in the world. The incidence rate has reached 20% of total death related to cancer.[5] In 2018 globally it had been estimated 83.550 deaths in men and 70.500 in women.[2] In United States, lung cancer had become the highest cause of death related to cancer (28%).[1] In South East Asia, the death rate is 17/100.000.[7] High rate of death is caused by lung cancer life expectancy is among the the third lowest of it, with liver cancer and pancreas cancer.[6] Death rate is higher in developing countries since smoking habit is still high, smoking regulation is not as good as in developed countries, and limited access to health facilities that lead to late diagnosis and treatment.[3] This makes death rate is higher compared to developed contries where smoking regulation and access to health facilities are better.[3]

There are two types of main lung cancer, small cell lung cancer (SCLC) which is about 10-15% and non-small cell lung cancer (NSCLC) which is about 80-85%.[8] SCLC is very aggressive lung cancer and has five-year survival only 7%.[9] SCLC is divided to 2 stage, limited stage which means cancer is limited in one lung or the closest lymph nodes, and extensive stage which define as cancer has reached the other lung, chest wall or far located lymph nodes and other organs.[9] Initially, SCLC is more responsive to chemotherapy, but it can relapse quickly and become resistant to next chemotherapy, so that SCLC has bad prognosis.[9] NSCLC is the most highly found and has some subtypes, adenocarcinoma, squamous cell carcinoma and large cell carcinoma.[8] Adenocarcinoma subtype is the most commonly found (40%).[8] This subtype can be found in smokers and non-smokers, in male and female. Usually, the growth of NSCLC is slow and it appears from peripheral area of lung.[8,10] The slow growth of NSCLC if compared to other lung cancers should give more chance in diagnosing before metastasis occured.[8,10] Squamous cell carcinoma is 25-30% of the cases and usually very closely related to smoking habit. Histopatology abnormalities usually come from respiratory tract epithelial cell in bronchial tract that located in the middle of the lung.[8,10] Large cell carcinoma type is also very closely related to smoking habit. This type incidence is 5-10%.[8] Large cell carcinoma type histopatologycally does not show evidence of other epithelia cells and glands, so that become diagnosis in histopatology findings which does not support adenocarcinoma or squamous cell carcinoma.[8,10] In lung cancer, it has been found many biomarkers such as EGFR, ALK, KRAS, ROS1, BRAF V600E and PD-L1. Based on NCCN guidelines, all patients diagnosed with lung cancer should have those biomarkers tested. But not all health centres have facilities to support it, including in

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Indonesia, biomarker tests can only be done on most commonly found types. One of the biomarker test is EGFR mutation in lung adenocarcinoma.[10] Adenocarcinoma is the type of lung cancer which is mostly found mutation of epidermal growth factor receptor (EGFR).[10] EGFR is one of ErbB kinase family from tyrosine kinase-related receptor.[11] In human, ErbB consists of HE1 (EGFR, ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4).[11] Each of ErbB type has important role in cell proliferation.[11] Lack of Erb activity is related with neurodegenerative diseases such as Alzheimer and multiple sclerosis, while excessive ErbB activity is related to many kinds of solid tumor.[11] EGFR is transmembran glycoprotein which binds to extracellular epidermal growth factor.[12] The bond produces autophosphorilation by intrinsic tyrosine kinase activity and triggers cascade of transduction signal to cell proliferation.[12] When mutation occurs in EGFR receptors, it causes agressive cell growth and not compensate with proper apoptosis.[12] In Europe, incidence rate of EGFR mutation in adenocarcinoma is only 10-15%, while in Asia reaches 30%. In Indonesia, EGFR mutation proportion in adenocarcinoma reaches 47,1%.[10] EGFR mutation in adenocarcinoma lung cancer is mostly found in receptor exon 19 and 21, 90% of all EGFR mutation.[3]

Cancer tissue biopsy is the gold standard to detect EGFR mutation in lung adenocarcinoma.[13] But about 27-31% of NSCLC patients have problem to get those tissues, due to extremely small tumor or high risk invasive procedure.[13] So it is developed other methods to detect EGFR mutation in lung adenocarcinoma and one of them is ctDNA.[13] ctDNA is a method to detect free fragmented tumor DNA in bloodstream.[13] Tumor DNA mutation in NSCLC patient's plasma serum firstly observed in 1998.[13] Innovations in detecting EGFR mutations has been done more after it responds positively to specific therapy.[12] The process of tumor DNA entering bloodstream has not been understood well.[13] Diehl et al concluded that tumor DNA came either from straight out of tumor cell secretion itself or from apoptosis process, necrosis and macrofag fagositosis.[13]

The method to test ctDNA generally divided to 2 types, *Next Generation Sequencing (NGS)* and *PCR based assay*. Those ctDNA methods have been recommended as alternatives in testing EGF mutation status in lung adenocarcinoma. Hence, the researcher feel the need to compare efectivity of ctDNA method to the gold standard of EGFR mutation test using cancer tissue.

Material and Methods

This was a cross sectional study. This study took data from cytology tests which were continued to EGFR mutation tests in adenocarcinoma lung cancer at Anatomic Pathology Laboratory of Ulin General Hospital Banjarmasin, and ctDNA tests data from Prodia Laboratory Banjarmasin. The subjects were patients diagnosed with lung cancer (adenocarcinoma type) who had cytology and ctDNA tests done simultaneously in October 2017 to October 2018.

The variables were cytology test and ctDNA results, which were mutation station on adenocarcinoma lung. The result was positive if there was mutation on exon 19 or 21. The other mutation was called negative. The data would be analyzed using SPSS version 2.0. The data formerly would have homogenecity test using Levene's Test for Equality of Variances method. If the homogenecity test had homogenous data, the test could be continued with T- equal variance assumed test. But if the homogenecity test had non-homogenous data, the test could be continued with T-equal variance not assumed test.

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Result

Table 1. EGFR mutation result in cytology and ctDNA test

	Cytology	**ctDNA**
Total sample	55	55
EGFR with positive mutation		
- Exon 19	17	12
- Exon 21	5	5
EGFR with negative mutation	33	38
EGFR positive to total sample percentage	40%	30%

Table 2. Comparation of EGFR with positive mutation to gender

	Cytology	**ctDNA**
Total EGFR with positive mutation	22	17
Female	12 (54.54%)	11(64.7%)
Male	10 (45.45%)	6 (35.29%)

According to Group Statistics output table, the mean of EGFR was 0,400 and the mean of ctDNA was 0,309. Therefore, statistic descriptively it could be concluded that there was difference in cytology test and ctDNA average results. The next step was to prove if the difference was significant, using T independent test.

Before doing T independent test, the data had its homogenecity tested using Levene's Test for Equality of Variances. From this test, it was found the significant value of Levene's Test for Equality of Variances was 0,057 > 0,05. It was concluded that the cytology tests and ctDNA tests data was homogenous. Therefore we could continue with T equal variance assumed test.

Table 3. The statistic test using SPSS version 2.0

T-Test

Group Statistics

	Result	N	Mean	Std. Deviation	Std. Error Mean
Result	1	55	.400	.4944	.0667
	2	55	.309	.4664	.0629

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Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Results	Equal variances assumed	3.709	.057	.992	108	.323	.0909	.0916	-.0908	.2726
	Equal variances not assumed			.992	107.634	.323	.0909	.0916	-.0908	.2726

According to output table on Equal Variances Assumed we found significant value (2-tailed) was 0,323 > 0,05, then based on decision-making basis in T sample independent test, it was concluded that H0 was accepted and H1 was rejected. It could be concluded that there was no significant difference between cytology test and ctDNA test.

Discussion

This study was done with 55 samples which were patients with lung cancer (adenocarcinoma type) who had cytology test and ctDNA test done simultaneously October 2017 until October 2018. Based on Table 1, on cytology test, there was 22 samples with EGFR positive mutation and 33 samples with EGFR negative mutation, or the percentage of EGFR with mutation positive was 40%. This data was suitable with Anita et al study in 2015 which stated that the percentage of EGFR with positive mutation in NSCLC in Asia-Pasific was 47% in average.[14] And specifically, in countries around Indonesia, which were Malaysia and Singapore, the percentage lied between 40-45%.[14] Hadisantoso study in 2014 which were done in Indonesia was about EGFR mutation in adenocarcinoma group had mean percentage 47,1%.[10]

Based on Table 2, in terms of gender on EGFR with positive mutation we found dominance in female (54,5%). This result was suitable with study by Ika in 2015 which stated that the percentage of EGFR with positive mutation was dominance in female (53,8%).[15] It was supported by Nicolas et al study in 2018 which stated that incidence rate of EGFR with positive mutation in female was 57%.[16] This data was suitable also with Anita et al study in 2015 which stated that the number of EGFR with positive mutation in NSCLC in Asia-Pasific was dominance in female with 60% in average.[14]

In table 3, based on Independent T test we had conclusion that there were no significiant difference between cytology test and ctDNA test in diagnosing EGFR mutation status in adenocarcinoma type lung cancer. The result from statistic test was supported by Arriola et al's study in 2018 which stated that EGFR test result using ctDNA was no different from biopsy of cancer tissue, therefore ctDNA could be an option when it was hard to collect cancer tissue sample.[17]

Zhang et al in 2018 found that from 2581 samples had similarity results between tissue biopsy and plasma biopsy. The number was 80,5%. There were many factors that could influence the effectiveness of ctDNA method, among them were the time of sampling, the stage and metastasis of cancer, and the technique.

The time of sampling was so important because it could affect the result. Time recommended from sampling to plasma isolation must not more that four hours, because it could be cell degradation. In four hours there was no significant difference between samples saving in room temperature and in temperature 4^0 C. For saving media, it was enough with EDTA cylinder if the process was not more than 4 hours. But if more time is needed, then it neesds cylinder with some blood stabilizer substance, or even more than seven days without cell degradation process.[18] Currently there are some media to

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save samples for long time, one of them is PAXgene. Using PAXgene, the samples could survive three days in room temperature (15-25°C), five days in temperature 2-8°C and until eight years in temperature
-20°C or -70°C. PAXgene is one of sample fixation system using two reagents, the first uses methanol and acetic acid solution, then stabilize in ethanol solution.[22] According to Mathieson et al in 2016, PAXgene was very promising method and significantly surpass other non-formaldehyde fixation like Z7 and HOPE in maintaining RNA integrity.[22]

Based on currently known theories, the more severe the cancer in terms of the stage and the metastasis, the higher cancer DNA concentration soluted in blood plasma.[18] Cancer DNA concentration in blood plasma will be higher in M1b if compared to M1a, 13% to 7%. Mou et al in 2015 recommended ctDNA is most effective in stage III and IV. If it is tested in stage I and II, the sensitivity will be affected.[19] Other data also said that the type of metastasis could give significant effects too. The further the metastasis, the higher cancer DNA concentration in plasma. Multiple metastasis is the highest, followed by single metastasis outside of thorax. The lowest cancer DNA concentration was found in metastasis inside thorax.[18]

In table 4, some test techniques would give more accurate results. ctDNA test methods is generally divided to two type, Next Generation Sequencing (NGS) and PCR based assay. NGS is the latest method and has most trusted result. Generally, it isolates DNA fragment, then NGS sequences it automatically to millions of small DNA fragments in parallel until it can detect mutation. One of NGS is Tagged-amplicon deep sequencing (TAM-Seq) which has sensitivity and specifity up to 97%. In NGS, there is also a test called Cancer-personalized profiling by deep sequencing (CAPP-Seq) which is proven effectively if used in earlier stage (I and II). With this method, we can detect EGFR mutation in lung adenocarcinoma stage I and II with specifity 96%. If this method is enhanced in combination with integrated digital error suppression (iDES) significantly can detect 93% of cancer DNA in stage I patients.[20]

Table 4. Sensitivity and specifity of NGS and PCR based assay[20]

Examination Techniques		Sensitivity	Specifity
NGS	MiSeq	93% (T790M)	94% (T790M)
		100% (L858R)	100% (L858R)
		87% (19del)	96% (19del)
	Ion Torrent	58%	87%
	TAM-seq	97%	97%
	iDES-CAPP-seq	90%	96%
	TEC-seq	97,4%	>99,99%
PCR-Based Assay	BEAMing	81% (T790M)	58% (T790M)
		87% (L858R)	97% (L858R)
		82% (19del)	97% (19del)
	Cobas EGFR Mutation Test	73% (T790M)	67% (T790M)
		87% (L858R)	97% (L858R)
		82% (19del)	97% (19del)
	ddPCR	77% (T790M)	63% (T790M)
		69% (L858R)	100% (L858R)
		86% (19del)	100% (19del)

In this study, the method to detect cancer DNA at Prodia Laboratory Banjarmasin was PCR based assay with Droplet *digital PCR* (ddPCR) technique . If it is compared to NGS, PRC based assay has lower sensitivity and specifity. ddPCR itself was developed PCR technique, multiplying DNA copies in forms of microdroplets, therefore it could detect DNA mutation even if to small pieces. But the sensitivity was still about 69-86% and the specifity was up to 97%. It was said that the specifity was enough to find positive result in plasma.

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Sample collecting process was also been done according to the standard. In this study, because the samples were sent to Centre Prodia Laboratory in Jakarta where it took more than four hours of delivery, therefore the blood samples collecting and saving process used PAXgene cylinders which could save samples up to 8 years without damages. The samples in this study were patients from advanced stage (stage III and IV) in average. Based on previous theory, cancer DNA concentration was high enough in blood plasma. Therefore, though the method used in this study has sensitivity which not as good as NGS method, we believed that our method still could give reliable result and not much different from cancer tissue biopsy.
Meanwhile, the method of cytology test in detect EGFR mutation at Anatomic Pathology Laboratory of Ulin General Hospital is Amplification refractory mutation system (ARMS PCR). ARMS PCR has 100% in sensitivity and 98% in specifity, which is a reliable method.[21]

Conclusion

Based on this study, we conclude that plasma ctDNA can be used as alternative to detect EGFR mutation status in lung adenocarcinoma if cancer tissue is hard to be found. Even if ctDNA result is negative to EGFR mutation, it cannot be concluded as absolute negative. More confirmation with cancer tissue biopsy is needed. This study is lack of sample and did not give details about stage of the disease to test the effectiveness of ctDNA method in diagnosing lung adenocarcinoma EGFR mutation.

References

1. Ridge CA, McErlean AM and Ginsberd MS. Epidemiology of lung cancer. Seminar in Interventional Radiology. 2013:30 93-98
2. Groot PM, Wu CC, Carter BW and Munden RF. The epidemiology of lung cancer. Translation lung cancer research. 2018:7(3) 220-233
3. Barta JA, Powell CA and Wisnivesky JP. Global Epidemiology of Lung Cancer. Annals of Global Health. 2019: 85(1) 1-16
4. WHO. 2019. International Agency for Research on Cancer. Available at: https://gco.iarc.fr/today/home (cited : April, 30th 2019)
5. Siegel RL, Miller KD and Jemal A. Cancer Statistics. Cancer Journal for Clinicians. 2019:69 7-34
6. Cheng TYD, Cramb SM, Baade PT, Youlden DR, Nwogu C and Reid ME. The international epidemiology of lung cancer : latest trend, disparities, and tumor characteritics. Journal Thoracic Onkology. 2016:11(10) 1653-1671
7. Rafiemanesh H, Mehtarpour M, Khani F, Hesami SM, Shamlou R, towhidi F et al. Epidemiology, incidence and mortality oflung cancer and their relationship with the development index in the world. Journal of Thoracic Disease. 2016:8(6) 1094-1102
8. Zappa C and Mousa SA. Non small cell lung carcinoma : current treatment and future advances. Translation Lung Cancer Research. 2016:5(3) 288-300
9. Gazdar AF, Bunn PA and Minna JD. Small cell lung cancer: what we know, what we need to know and the path forward. Nature review cancer. 2017:17 725-737
10. Hadisantoso DW. Asosiasi Jenis Kelamin dan Status Merokok dengan Mutasi Epidermal Growth Factor Receptor pada Pasien Adenokarsinoma Paru Stadium Lanjut. Program Pendidikan Dokter Spesialis Ilmu Penyakit Dalam Fakultas Kedokteran Universitas Indonesia. 2014
11. Hirsh V. Turning *EGFR* mutation-positive non-small-cell lung cancer into a chronic disease: optimal sequential therapy with EGFR tyrosine kinase inhibitors. Therapeutic Advances in Medical Oncology. 2018:10 1-12
12. Bethune G, Bethune D, Rudgway N and Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer : an overview and update. Journal of Thoracic Diseases. 2010:2 48-51
13. Normanno N, Denis MG, Thress KS, Ratcliffe and Reck M. Guide to detecting epidermal growth factor receptor (*EGFR*) mutations in ctDNA of patients with advanced non-small-cell lung cancer. Impact Journal. 2017:8(7) 12501-12516

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14. Midha A, Dearden S and McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapll). Am J Cancer Res. 2015:5(9) 2892-2911
15. Oktayanti IK. Mutasi EGFR pemeriksaan sitologi adenokarsinoma paru. Berkala Kedokteran. 2015:11 213-219
16. Marcoux N, Gettinger SN, O’Kane G, Arbour KC, Neal JW, Husain H, et al. EGFR-mutant adenocarcinomas that transform to small cell lung cancer and other neuroendocrine carcinomas: Clinical Outcomes. Journal of Clinical Oncology. 2019:37 278-289
17. Arriola E, Lario AP, Gomez RG, Tain PD, Constenla M, Giron CG et al. Comparison of plasma ctDNA and tissue/cytology-based techniques for the detection of EGFR mutation status in advance NSCLC: Spanish data subset from ASSESS. Clinical and Translation Oncology. 2018; 20 1261-1267.
18. Zhang S, Zhu L, Cheng X, Zhang X, Chen E, Fang H et al. ctDNA assessment of EGFR mutation status in Chinese patients with advanced non small cell lung cancer in real world setting. Journal of Thoracic Disease. 2018:10(7) 4169-4177
19. Mao C, Yuan JQ, Yang ZY, Fu XH, Wu XY, Tang JL et al. Blood as a substitute for tumor tissue in detecting EGFR mutations for guiding EGFR TKIs treatment of non small cell lung cancer. Medicine Journal. 2015:94 1-10
20. Lim M, Kim CJ, Sunkara V, Kim MH and Cho YK. Liquid biopsi in lung cancer: clinical applications of circulating biomarkers (CTCs and ctDNA). MDPI Journal. 2018:9 1-18
21. Zhu Y, Guo Z, Liu Y, Zheng X, Yang G and Zheng G. A novel ARMS-based assay for the quantification of EGFR mutations in patient with lung adenocarcinoma. Oncology Letter. 2018:15 2905-2912.
22. Mathieson W, Marcon N, Antunes L, Ashford DA, Betsou F, Frasquilho SG et al. A critical evaluation of the PAXgene tissue fixation system. American Journal Clinical Pathology. 2016: 146 25-40