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Profile of Adenocarcinoma in Cohort of Female Lung Cancer Related Second Hand Smoker

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Abstract: ***Background**: Environmental tobacco smoker is the major significant factor in developing lung cancer in females never smokers. There are two products of cigarette smokes; mainstream and side stream smoke that both of these smoke will be metabolyzed by CYP2A6 in both active and passive smoker. Further, adenocarcinoma is the most common histology in lung cancer related to female never smoker and strongly related to nicotine metabolism in the carcinogenesis. **Objectives**: This study aimed to assess the genetic polymorphism of CYP2A6 and cytology characteristics in female second - hand smokers who suffered from lung cancer among the North Sumatera population. **Methods**: A cross sectional study was carried out in three centers of cancer hospital in Medan with a total of 53 subjects enrolled in this study from purposive sampling. The diagnosis of lung cancer was confirmed by clinical, radiology, cytology, and histopathology preparation. The CYP2A6 gene was examine by PCR - RFLP from blood. Data were analyzed with the Conditional Logistic Regression test using Epi info 7.0 software. **Results**: A total 53 subjects diagnosed with lung cancer, with 51 participants had adenocarcinoma type. The most common genotype is wild type, including *1A/*1A (31.3%), *1A/*1B (27.4%), *1B/*1B (39.2%). Meanwhile, only 1 subject had mutant type *4A/*4A (1.9%). For allele, only 1 subject had mutant type *4A (1.9%), while the wild type were counted as *1A (58.8%), *1B (39.2%). There was no significant associations between genotype of CYP2A6 and the incidence of lung adenocarcinoma (p = 0.61). Neither was the alelle of CYP2A6 and the incidence of lung adenocarcinoma (p = 0.25). **Conclusion**: Adenocarcinoma is the most common histology in female never smoker. Yet, there was just 1 subject had the mutant type of CYP2A6, so this study cannot associate between allele and genotype of lung cancer and the incidence of lung adenocarcinoma.*

Keywords: Polymorphism, CYP2A6, Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR - RFLP), Female Second Hand Smokers, Lung Cancer, Cytology of Lung Cancer

1. Introduction

The incidence of lung cancer among females increased in the last decades with total 112.520 women diagnosed with lung cancer in 2020 (1) in line with the increasing of lung cancer incidence in non - smoking populations around 10 - 25% in 2019 (2). Although they do not smoke, but the inhalations of both mainstream and side stream smoke in never smoker populations who had partner or being around the smoker had the same metabolic nicotine activity with smokers. So, the polymorphism of CYP2A6 as one of the main enzymes in nicotine metabolism had the impact in the lung carcinogenesis.

As the main enzymes in nicotine metabolism, CYP2A6 converts nicotine to its active substrate, cotinine that will affect the DNA alterations in lung carcinogenesis (3, 4). Several studies reported a significant association between CYP2A6 polymorphisms and the incidence of lung cancer, while other studies reported no association (5, 6, 7, 8). Based on this, researchers are interested in examining the associations between CYP2A6 and the incidence of lung adenocarcinoma in female never smokers in North Sumatera.

2. Methods

This research is an observational analytic study with a case - control study, which analyzes the relationship of CYP2A6 genetic polymorphisms, analyzes the relationship of CYP2A6 genetic polymorphisms to the histopathology of lung cancer in secondhand smoker women with the incidence of lung cancer. Examination of lung cancer diagnostic measures was carried out at the Central General Hospital (RSUP) H. Adam Malik, Medan (a type A hospital and a referral center from Sumatra Island), and Santa Elizabeth Hospital Medan as a cancer referral center hospital in the city of Medan. The patient's blood collection was carried out at the same hospital. DNA isolation and Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR – RFLP) examinations were carried out at the USU Medical Faculty Integrated Laboratory. The research was carried out within a period of 6 months, starting with library research, title consultation, proposal preparation, proposal seminar, data collection (January to June 2021).

The population in this study were female secondhand smoker patients with a diagnosis of lung cancer, who had

been diagnosed based on cytology/histopathology examinations at H. Adam Malik Hospital and Santa Elizabeth Hospital Medan. The inclusion criteria for this case sample are; a) diagnosed with lung cancer based on clinical, radiology, cytology, and histopathology findings; b) passive smokers with a history of exposure > 10 years; c) Age >17 years; d) Provide a written statement of willingness to participate in the research, after obtaining a detailed explanation of this research (informed consent). The exclusion criteria were a) samples who were taking phenobarbital, methoxsalen (8 - methoxy psoralen), tranylcypromine, tryptamine, coumarin, and neomathylthiol. Those drugs can alter the activity of CYP2A6 enzymes; b) Samples that experience errors or damage during inspection starting from isolation and PCR.

Based on the calculation of the sample, the minimum subject of this study was 44 research subjects, but because researchers were afraid of errors during examination and analysis errors, the researchers decided to take a sample of 53 subjects. This is done to increase the level of significance in the results of further data processing.

3. Results

Based on age, the majority of the ages in this study were in the age range of 50 - 59 years (37.7%), housewives (75.5%). The source of exposure to cigarette smoke was from home environment (56.6%) with duration of exposure > 10 years, respectively 37.7%.

Table 1: Characteristics of research subjects

Variable	CYP2A6		p - value
	n	%	
Age			
< 40 years old	0	0.0	<0.01
40 - 49 years old	12	22.6	
50 - 59 years old	20	37.7	
60 - 69 years old	17	32.1	
⩾70 years	4	7.5	
Occupation			
Cleaning service	0	0.0	<0.01
Teacher	2	3.9	
Housewife	40	75.5	
Nurse	0	0.0	
Farmer	1	1.9	
Government employees	3	5.7	
Entrepreneur	7	13.2	
Passive Smokers			
In the home environment	30	56.6	0.47
In the work environment	7	13.2	
Long exposure to cigarette smoke			
< 10 years	11	20.8	0.05
⩾10 years	20	37.7	

In the genotypic characteristics of the CYP2A6 genetic polymorphism, Table 2 shows the frequency distribution of the CYP2A6 genetic polymorphism for both genotypes and histopathological types of lung cancer. In the table above, it can be seen that the highest genotype frequency is the *1B/*1B genotype with 20 subjects with Adenocarcinoma cancer cells and 1 subject in SCC itself. As for the mutant genotype variant, there was only 1 subject and it was found in Adenocarcinoma cancer cell types.

Table 2: CYP2A6 genotype frequencies by cancer cell type cell

Genotypes	Adenocarcinoma	SCC
*1A/*1A	16	1
*1A/*1B	14	1
*1B/*1B	20	0
*4A/*4A	1	0

Table 3: CYP2A6 allele frequencies by cancer cell type cell

Allele	Adenocarcinoma	SCC
*1A	30	2
*1B	20	0
*4A	1	0

Table 3 shows that most subjects with the *1A allele have adenocarcinoma cell types and 2 of them have squamous cell carcinoma cells. As for the mutant type itself, in this study, we found only one patient with adenocarcinoma cell type and no squamous cell carcinoma.

4. Discussion

Smoking is the main dominant factor contributing to the development of lung cancer (9), but the incidence of lung cancer in the non - smoker population, particularly women, increases significantly based on geographic location (10). Recent articles have also shown that environmental tobacco smoke is one of the major risk factors for lung cancer in non - smokers. This shows indirectly shows that both active smokers and passive smokers have a risk of developing lung cancer. In the non - smoking population in Europe, women tend to have a greater risk of lung cancer than the male population because of several biological aspects including hormonal, expression of oncogenic drivers, and mutations of several epigenetic factors, and tumor suppressor genes (11). And a recent study conducted by the International for Research on Cancer (IARC) concluded that exposure to cigarette smoke is one of the carcinogenic substances for humans, with a risk rate of 20% in women and 30% in men who are not smokers who are exposed to cigarette smoke. their partner. Based on 37 epidemiological analyses, Hacksaw et. al. reported that lung cancer patients who were not smokers but lived with smokers were 26% more likely to develop lung cancer than those who did not live with smokers (12).

Squamous cell carcinoma is the most common type of cancer found in smokers, while we recognized adenocarcinoma as the dominant type of lung cancer in smokers in recent years. We report this shift to result from a decrease in the amount of polycyclic aromatic hydrocarbons and an increase in the relative content of nitrosamines in the smoke inhaled from filter cigarettes. Thus, the increase in adenocarcinoma is thought to be associated with nitrosamines. The epithelium in the main bronchus is the area where squamous cell carcinoma predominates, while the periphery of the lung is the area where adenocarcinoma is most common. Due to the amount of CYP2A6 mRNA expressed in humans in bronchial epithelial cells, CYP2A6 present can activate nitrosamines in tobacco smoke in the bronchi. This mechanism may explain the significant association between CYP2A6 polymorphisms and the risk of squamous cell carcinoma. The presence of functional

CYP2A6 in the pulmonary periphery is still a matter of controversy. This may be the reason for the less pronounced effect of the *4/*4 genotype on adenocarcinoma compared with small cell or squamous carcinoma. Because the difference is simple and suggestive, we have confirmed it in a larger cohort study. Alternatively, the risk of adenocarcinoma may be more closely related to the activity of CYP2A13, which is reported to have a higher metabolic capacity to activate 4 - (methylnitrosamino) - 1 - (3 - pyridyl) - 1 - butanone and is expressed higher in peripheral lung tissues compared to CYP2A6.

This study has several limitations, including the minimal sample size and the limitations of the PCR method. RT - PCR with subsequent sequencing analysis can be carried out to analyze whether the polymorphism is associated with CYP2A6 mutation types in certain geographic areas, especially Indonesia. In the study of this polymorphism, it did not prove the CYP2A6 gene in the carcinogenesis process. This proves the existence of other factors such as genetic factors and environmental factors that play a role in the incidence of lung cancer.

5. Conclusion

In this study, we found that the risk factor for lung cancer in women who smoke passively is exposure to cigarette smoke, in this case, environmental tobacco smoke. It was also found that the risk factor for exposure to cigarette smoke was in the home environment, namely the husband of a female lung cancer patient with an exposure duration of > 10 years. The most commonn histology of lung cancer is never smoker. In this study, we found that the highest frequency of CYP2A6 genotype in lung cancer patients in passive smoking women was the allele*1B frequency (37.7%). There is no proven associations between CYP2A6 polymorphisms in the incidence of lung cancer because of the influence of genes or genetic variations associated with cancer. Here, there are environmental factors or environmental gene interactions that can influence lung cancer, which is multifactorial and polygenic, so further research is needed on other genes related to the carcinogenesis process.

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