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The Correlation of Microsomal Epoxide Hydrolase (EPHX1) His139ArgGene Polymorphism and Lung Cancer Incidence in H. Adam Malik General Hospital Medan

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Abstract

Background: *Microsomal epoxide hydrolase 1 (EPHX1) plays an important role in both activation and detoxification of polycyclic aromatic hydrocarbons (PAH) and aromatic amines. Polymorphism of EPHX1 His139Arg on susceptibility to lung cancer has been reported with inconsistent results. The purpose of this study was to analyze the correlation between this gene polymorphism and lung cancer incidence in smokers.*

Method: *This was a case-control study using a consecutive sampling method. Genotyping test was performed by PCR-RFLP assay. The chi-square test with* P *<0.05 was considered significant.*

Results: *Of the 84 subjects, in the case and control groups, His139His wild-type variants were found in 34 subjects (81%) and 30 subjects (71.4%), respectively, while His139Arg heterozygous variants were in 8 (19%) and 12 (28.6%) subjects. No homozygous variants of Arg139Arg were identified (*P*=0.36).*

Conclusion: *The EPHX1 His139His enzyme gene polymorphism was a common polymorphism in both groups of subjects. There was no correlation between EPHX1 His139Arg gene polymorphism of and lung cancer. (**J Respirol Indones 2022; 42 (2): 86–9)***

Keywords: *EPHX1; His139Arg; Lung cancer; PCR-RFLP; Polymorphism*

Hubungan Polimorfisme Gen *Microsomal Epoxide Hydrolase* (EPHX1) His139Arg dengan Kejadian Kanker Paru di RSUP H. Adam Malik Medan

Abstrak

Latar Belakang: Microsomal epoxide hydrolase *(EPHX1) memegang peranan penting dalam aktivasi dan detoksifikasi polisiklik hidrokarbon aromatik (PAH) dan amina aromatik. Polimorfisme gen EPHX1 His139Arg pada kerentanan terhadap terjadinya kanker paru telah dilaporkan dengan hasil yang tidak konsisten. Tujuan penelitian ini adalah untuk menganalisis hubungan antara polimorfisme gen ini dengan kejadian kanker paru pada perokok*

Metode Penelitian: *Penelitian ini merupakan studi kasus kontrol dengan metode pengambilan sampel secara* consecutive sampling*. Uji genotip dilakukan menggunakan PCR-RFLP. Uji* chi-square *dengan P<0,05 dianggap bermakna.*

Hasil: *Dari ke-84 subyek, pada masing-masing kelompok kasus dan kontrol, varian* wild-type *His139His ditemukan pada 34 subjek (81%) dan 30 subjek (71,4%), sedangkan varian heterozigot His139Arg ditemukan pada 8 (19%) dan 12 subjek (28,6%). Tidak ada varian homozigot Arg139Arg yang dijumpai (P=0,36).*

Kesimpulan: *Polimorfisme gen enzim EPHX1 His139His merupakan polimorfisme umum pada kedua kelompok subjek. Tidak terdapat hubungan antara polimorfisme gen EPHX1 His139Arg dengan kanker paru. (**J Respirol Indones 2022; 42 (2): 86–9)***

Kata kunci: *EPHX1; His139Arg; Kanker paru; PCR-RFLP; Polimorfisme*

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INTRODUCTION

Lung cancer is one of the most common cancers worldwide and has the highest mortality rate among all cancer types. Strong evidence suggests that tobacco smoking causes bronchogenic cancer in approximately 85–90% of lung cancer patients.[1]

Although several causes are associated with the development of lung cancer include smoking, history of respiratory disease, and exposure to chemical carcinogens, not all exposed people will develop lung cancer. Other causes such as genetic polymorphisms are also mentioned as a contribution to individual differences in the susceptibility to lung cancer.[2]

Exposure to carcinogens in cigarette smoke, such as polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, and aromatic amines, is a major cause of lung cancer among smokers.[3]

Microsomal epoxide hydrolase (EPHX1) is very important in various detoxification processes and metabolism of endogenous and exogenous compounds. The EPHX1 plays a dual role in the detoxification and bioactivation of PAHs and other environmental pollutants depending on their substrates. Reactive compounds such as sugar palm, alkene, and aliphatic epoxide are hydrolyzed by EPHX1, which is produced by cytochrome P450 and other phase 1 enzymes to the corresponding dihydrodiol upon addition of trans water. On the other hand, dihydrodiols which are less reactive than PAHs can be substrates for further transformation into dihydrodiol-epoxides such as benzo[a]pyrene-7,8-diol-9,10 epoxide, the most mutagenic and carcinogenic metabolites.[4]

A polymorphic form in the EPHX1 gene, amino acid residues 139 (Arg/His), has been identified. The amino acid substitution from Histidine to Arginine can cause changes in protein stability. Changes in exon 4 were associated with a 25% increase in EPHX1 activity.[5]

Many studies have investigated the association between the EPHX1 His139Arg gene polymorphism and lung cancer risk, but the impact of this polymorphism on lung cancer risk were reported with inconsistent results. This study aimed to identify the EPHX1 His139Arg gene polymorphism and its association with lung cancer in smokers.

METHODS

Eighty-four male subjects with smoking history were recruited into the study: 42 subjects with lung cancer at Adam Malik General Hospital compared with 42 healthy subjects. All subjects gave written *informed consent* and were interviewed regarding age, smoking status, history of cancer, and family history of cancer. Only individuals without a history of cancer and chronic respiratory disease were eligible for controls. Histological outcome information was searched manually in the pathology result. The Ethics Committee had approved this study protocol of the Faculty of Medicine, Universitas Sumatera Utara.[4]

Three milliliters of venous blood were collected from the median cubital vein, put into a sterile tube containing EDTA, and stored at 4°C in the refrigerator.[4]

The EPHX1 gene was amplified using a forward 5'- GGG GTA CCA GAG CCT GAC CGT-3' primer and a reverse 5'- AAC ACC GGG CCC ACC CTT GGC-3' primer (MBI Fermentas). PCR was carried out with a final volume of 25 l containing 2 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 0.2 mM dNTP (MBI Fermentas), and 1.5-unit Taq polymerase (MBI Fermentas). The DNA was denatured at 94ºC for 5 minutes. Thirty-five amplification cycles began with denaturation at 94ºC for 30 seconds, primary annealing at 62ºC for 30 seconds, and extension at 72ºC for 45 seconds, followed by a final 5-minute extension step at 72ºC. The PCR product produced a band at 357 bp. After one hour of digestion of the 15-µL PCR product with 10 U RSaI (MBI Fermentas), this product was visualized by electrophoresis on a 3% agarose gel.[4]

A Chi-square test was used to compare genotype frequencies between groups with P <0.05, which was considered significant. Odds ratio and

95%CI were calculated to determine the correlation between the variables and the risk of lung cancer in the EPHX1 His139Arg gene polymorphism. Statistical analysis was carried out using SPSS 17.0 statistical software for computer devices.

RESULT

The characteristics of the subjects based on age, Brinkman index, type of cigarette, and the distribution of EPHX1 gene polymorphism can be seen in Table 1.

Table 1. Characteristics of Study Subjects

Characteristics	Case		Control	
	n	%	n	%
Age				
<40 years	0	0	26	61.9
40–59 years	23	54.8	14	33.3
≥60 years	19	45.2	2	4.8
Brinkman Index				
Mild	-	-	4	9.5
Moderate	6	14.3	22	52.4
Severe	36	85.7	16	38.1
Cigarette Type				
Clove	23	54.8	35	83.3
White	11	26.2	4	9.5
Mixture	8	19.0	3	7.2
Polymorphism				
AG (His/Arg)	8	19.0	12	28.6
AA (His/His)	34	81.0	30	71.4

Based on Table 1, the majority of the case group were aged 40–59 years (54.8%), while the control group was mostly <40 years old (61.90%). The most common Brinkman index found in the case group was the severe category as observed in 36 subjects (85.7%), while in the control group was the moderate category as found in 22 subjects (52.4%). The mostly used cigarettes in both case and control groups was cloves as observed in 23 subjects (54.80%) and 35 subjects (83.30%), respectively.

Table 2. Correlation of Gene Polymorphisms with Lung Cancer Incidence

Gene Polymorphisms	Case		Control		*P*
	n	%	n	%	
AG (His/Arg)	8	19.0	12	28.6	0.30
AA (His/His)	34	81.0	30	71.4	

The genotype frequency of AA (His/His) was a common polymorphism in both groups of subjects. No GG (Arg/Arg) genotype was found in this study. The chi-square test stated that there were no correlation between the EPHX1 His139Arg gene polymorphism and the incidence of lung cancer in the case and control groups (*P*>0.05).

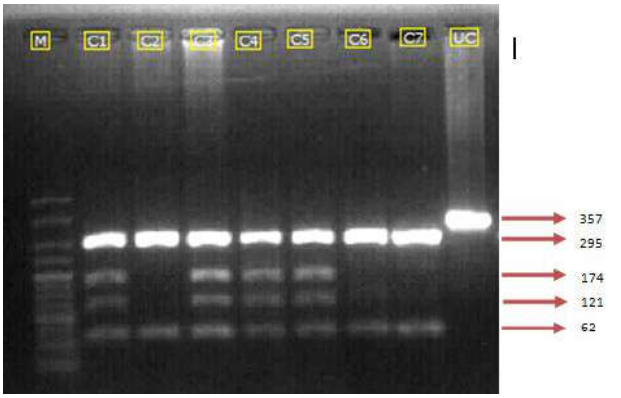

Figure 1. PCR-RFLP analysis for His139Arg polymorphism with RSaI. M: DNA markers, pathways C1, C3, C4, and C5 were genotyped with His/Arg at 295, 174, 121, and 62 bp; pathways C2, C6, and C7 were genotyped with His/His at 295 and 62 bp, respectively. UC: PCR product 357 bp.

DISCUSSION

In this study, lung cancer patients were mostly in the 40–59 years age group, which was 54.76%. These results were in line with several studies, such as the study from Erkisi et al. on 2010 in Turkey, which pointed out that the mean age of lung cancer patients was >50 years. The similar result was also obtained by Soeroso et al. in 2014, who stated that lung cancer patients who visited H. Adam Malik General Hospital Medan were mostly 51–60 years old.

Increasing age could lead to the accumulation of carcinogenic substances in the body and also genetic disorders. Increasing age also causes a decline in immunity, DNA repair and induces a loss of cell regulation that facilitates carcinogenesis.

This study noticed that severe Brinkman index was the most common category in the lung cancer group while moderate Brinkman index was the most common in the control group. Hoffman in 1997 reported that lung cancer risk factors were directly proportional to the Brinkman index. Cigarette consumption in large quantities will lead to long-term exposure to carcinogens; this condition can escalate the risk of lung cancer.[6]

Clove cigarettes were the most widely used type of cigarette by both the lung cancer group and the control group, with 54.8% and 83.3%, respectively. Clove cigarettes are the most popular and well-known type of cigarette in Indonesia. This type of cigarette is a cigarette whose raw material is tobacco added with cloves and other flavors to get a certain effect and aroma. Cloves have a pleasant aroma and secrete eugenol, which can affect the sensory effects to trigger deeper cigarette smoking.[7]

Although smoking increases a person's risk of developing lung cancer and other smoking-related malignancies, not all individuals who smoke experience lung cancer. It is thought that genetic differences or polymorphisms in genes encoding xenobiotic metabolic enzymes may influence individual susceptibility to potential carcinogens.[8]

In this study, we found no significant difference between the distribution of the EPHX1 enzyme genotype and lung cancer risk. Zhou et al. analyzed 974 Caucasian lung cancer patients and 1142 controls, in which no association was observed between EPHX1 enzyme gene polymorphisms and lung cancer risk.

The lower affinity of the enzyme could not effectively remove the epoxide compounds causing the accumulation of intermediate metabolites in lung tissue. This metabolite is lipophilic, which is very easy to react with DNA to form DNA-Adducts. The formation of these DNA-adducts can cause mismatches in DNA replication, methyl replacement, and promoter changes, resulting in inherited DNA mutations or abnormal gene expression, and ultimately the process of carcinogenesis. Reactive metabolism can also induce the formation of Protein-Adducts in cells, which affects the normal activity of these proteins. Metabolites can also trigger an increment in reactive oxygen species (ROS), that directly affect DNA, lipids, or proteins and initiate carcinogenesis.[9]

CONCLUSION

There were no significant differences between the EPHX1 His139Arg gene polymorphism and the risk of lung cancer.

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