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

Differences in Levels of Human 1,3-β-D-Glucan from Bronchoalveolar Lavage (BAL) Fluid between The Immunocompromised and Immunocompetent Groups Patients with Suspected Lung Cancer

Association Between CEA Serum Level on NSCLC Patients with EGFR Mutation from Tissue and Plasma Sample

Comparison of Eutectic Mixture of Local Anesthesia Cream and Subcutaneous Lidocaine to Reduce Chest Tube Removal Pain and Willingness to Repeat Procedure

Risk Factors for Mortality of Patients with COVID-19 in RSJPD Harapan Kita, Jakarta

An Evaluation of Short-Acting β2-Agonist Prescriptions and Associated Clinical Outcomes in Asthma Management in Indonesia – The SABINA Indonesia Study

Increased Serum SP-D Level, Neutrophils and Lymphocytes Sputum in Malang Splendid Bird Market Workers

Expression of Immune Checkpoint Marker PD-L1 in Surgical Lung Cancer Specimens

The Effect of Roflumilast on Absolute Neutrophil Count, MMP-9 Serum, %VEP1 Value, and CAT Scores in Stable COPD Patients

The Surfactant Protein D (SP-D) Serum Levels in Limestone Mining Worker

Gastro-Eosophageal Reflux Is Not a Common Cause of Chronic Cough: A Singapore Case Series

Impact of Underweight on the Unsuccessful Treatment Outcome Among Adults with Drug-Resistant Tuberculosis: A Systematic Review
Editorial Advisory Board
M. Arifin Nawas
Faisal Yunus
Agus Dwi Susanto

Editorial-in-Chief
Fanny Fachrucha

Editorial Board
Feni Fitriani Taufik
Noni Novisari Soeroso
Tutik Kusmiati
A. Farih Raharjo
Ginanjar Arum Desianti
Irandi Putra Pratomo
Jamal Zaini
Mia Elhidsi

International Editorial Board
Guido Vagheggini
Mayank Vats
Motoyasu Kato
Ira Paula Wardono

Secretariat
Shalzaviera Azniatinesa
Suwondo
SST: Surat Keputusan Menteri Penerangan RI

Editorial Office
PDPI Jl. Cipinang Bunder, No. 19, Cipinang Pulo Gadung
Jakarta Timur 13240 Telp: 02122474845
Email : editor@jurnalrespirologi.org
Website : http://www.jurnalrespirologi.org

Publisher
The Indonesia Society of Respirology (ISR)
Published every 3 months (January, April, July & October)

Jurnal Respirologi Indonesia
2nd Rank Accreditation
According to the Decree of the Minister of Research and Technology/Head of the National Research and Innovation Agency of the Republic of Indonesia Number: 200/M/KPT/2020
December 23, 2020
TABLE OF CONTENT

Original Article

The Correlation of Microsomal Epoxide Hydrolase (EPHX1) His139Arg Gene Polymorphism and Lung Cancer Incidence in H. Adam Malik General Hospital Medan

Rosidah Hanum Hasibuan, Noni Novisari Soeroso, Setia Putra Tarigan, Yahwardiah Siregar, Erna Mutiara, Lucia Aktalina

Differences in Levels of Human 1,3-β-D-Glucan from Bronchoalveolar Lavage (BAL) Fluid between The Immunocompromised and Immunocompetent Groups Patients with Suspected Lung Cancer

Asih Trimurtini, Ngakan Putu Parsama Putra, Teguh Rahayu Sartono, Harun Al Rasyid

Association Between CEA Serum Level on NSCLC Patients with EGFR Mutation from Tissue and Plasma Sample

Frenky Hardiyanto Hendro Sampurno, Suryanti Dwi Pratiwi, Ngakan Putu Parsama Putra

Comparison of Eutectic Mixture of Local Anesthesia Cream and Subcutaneous Lidocaine to Reduce Chest Tube Removal Pain and Willingness to Repeat Procedure

Roman Diaz, Yusup Subagio Sutanto, Ahmad Faridh Raharjo

Risk Factors for Mortality of Patients with COVID-19 in RSJPD Harapan Kita, Jakarta

Zhara Juliane, Asri C Adisasmita, Yoga Yuniadi

An Evaluation of Short-Acting β2-Agonist Prescriptions and Associated Clinical Outcomes in Asthma Management in Indonesia – The SABINA Indonesia Study

Wiwin Heru Wiyono, Muhammad Amin, Susanthi Djajalaksana, Amira Permatahari Tarigan, Febrina Susanti, Hisham Farouk, Helyanna

Increased Serum SP-D Level, Neutrophils and Lymphocytes Sputum in Malang Splendid Bird Market Workers

Ratih Dwi Ary Merdekaawi, Tri Wahju Astuti, Garinda Alma Duta

Expression of Immune Checkpoint Marker PD-L1 in Surgical Lung Cancer Specimens

Elisna Syahruddin, Jamal Zaini, Lisnawati, Yayi DB Susanto, Sarah Fitriani, Shanty R. Kusumawardani, Romi Baginta

The Effect of Rufumilast on Absolute Neutrophil Count, MMP-9 Serum, %VEP1 Value, and CAT Scores in Stable COPD Patients

Ratna Andhika, Suradi, Yusup Subagio Sutanto

The Surfactant Protein D (SP-D) Serum Levels in Limestone Mining Worker

Sita Andarini, Anna Yusrika, Sri Wening Pamungkasningisih, Farhan Hilmi Taufikulhakim, Ahmad Hudoyo, Widhy Yudistira Nalapraya, Agus Dwi Susanto

Case Report

Gastro-Esophageal Reflux Is Not a Common Cause of Chronic Cough: A Singapore Case Series

Vijo Poulse

Literature Review

Impact of Underweight on the Unsuccessful Treatment Outcome Among Adults with Drug-Resistant Tuberculosis: A Systematic Review

Kemas Rakhmat Notariza, Jaka Pradipta
The Correlation of Microsomal Epoxide Hydrolase (EPHX1) His139Arg Gene Polymorphism and Lung Cancer Incidence in H. Adam Malik General Hospital Medan

Rosidah Hanum Hasibuan¹, Noni Novisari Soeroso¹, Setia Putra Tarigan¹, Yahwardiah Siregar², Erna Mutiar³, Lucia Aktalin²

¹Department of Pulmonology and Respiratory Medicine Faculty of Medicine, Universitas Sumatera Utara, Medan
²Department of Biomedical Sciences Faculty of Medicine, Universitas Sumatera Utara, Medan
³Department of Biostatistics Faculty of Public Health, Universitas Sumatera Utara, Medan

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 and lung cancer.

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 1 (EPHX1) memegang peranan penting dalam aktivasi dan detoksifikasi polisiklik hidrocarbon 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 subjek, 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 homozygot 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.

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

Correspondence: Rosidah Hanum H
Email: rosidahhanum10@gmail.com
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 MgCl₂, 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 94°C for 5 minutes. Thirty-five amplification cycles began with denaturation at 94°C for 30 seconds, primary annealing at 62°C for 30 seconds, and extension at 72°C for 45 seconds, followed by a final 5-minute extension step at 72°C. 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>
<thead>
<tr>
<th>Characteristics</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40–59 years</td>
<td>23</td>
<td>54.8</td>
</tr>
<tr>
<td>≥60 years</td>
<td>19</td>
<td>45.2</td>
</tr>
<tr>
<td>Brinkman Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moderate</td>
<td>6</td>
<td>14.3</td>
</tr>
<tr>
<td>Severe</td>
<td>36</td>
<td>85.7</td>
</tr>
<tr>
<td>Cigarette Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clove</td>
<td>23</td>
<td>54.8</td>
</tr>
<tr>
<td>White</td>
<td>11</td>
<td>26.2</td>
</tr>
<tr>
<td>Mixture</td>
<td>8</td>
<td>19.0</td>
</tr>
<tr>
<td>Polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG (His/Arg)</td>
<td>8</td>
<td>19.0</td>
</tr>
<tr>
<td>AA (His/His)</td>
<td>34</td>
<td>81.0</td>
</tr>
</tbody>
</table>

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 ($P > 0.05$).

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 carcinogetic 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.\(^9\)

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.\(^8\)

**CONCLUSION**

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

**REFERENCES**