

Cyp1a1 Gene Polymorphism (6235 T/C) in Head and Neck Cancer of an Indonesian Population

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Abstract

One of the genes that plays a role in the formation of cancer cells is CYP1A1 (cytochrome P450, family 1, subfamily A, polypeptide 1) CYP1A1 codes for enzymes that have an important role in activating or detoxifying carcinogenic elements in tobacco and other compounds. This study aimed to investigate the potential association of CYP1A1 gene polymorphism and the incidence of head and neck cancer (HNC) by comparing the polymorphism distribution in HNC patients and healthy controls of an Indonesian population. Polymerase Chain Reaction – Restriction Fragment length Polymorphism (PCR-RFLP) techniques with the MspI enzyme were used for genotyping of the single nucleotide polymorphisms (SNPs) of CYP1A1 (rs4646903) in HNC patients and healthy controls. The frequencies of the TT genotype were 34% for HNC and 30% for the healthy controls; CT was 56% for HNC and 66% for the healthy controls; homozygote CC was 10% for HNC and 4% for the healthy controls. There is no significant association of CYP1A1 gene polymorphisms (6236 T/C) between patients with HNC and healthy controls.

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Introduction

Cancer is a leading non-communicable diseases that causes death worldwide. Data from the WHO indicate that, in 2012, there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer worldwide.¹ According to data from GLOBOCAN (IARC), a body of international cancer research under the WHO, in 2012, the prevalence of cancer in the Indonesian population of all ages was 1.4%, or 347,792 people.²

One classification of cancer is head and neck cancer (HNC). HNC is the eighth most commonly diagnosed cancer in the United States.³ During the period 1992–2004, more than 48,000 HNCs were diagnosed (43% in the oral cavity, 34% in the larynx, and 24% in the pharynx).⁴ There were an estimated 650,000 new

cases of HNCs globally, 350,000 of which caused death.^{5,6} Two-thirds of these cases occurred in developing countries.⁷

In 2002, there were 420,000 new cases of HNCs in men and 142,000 in women globally. The prevalence of HNC in Indonesia is quite high, ranking fourth in all malignancies found in men and women, and second in all malignancies found in men, with a prevalence of 4.7 per 100,000 population.⁷

There are many factors that can cause HNC. HNC is mostly related to the relative distribution of major risk factors such as tobacco, cigarette smoking, and alcohol consumption.⁸ In the pathogenesis of cancer, genetic elements were also influential. There are several genes that influence the formation of cancer cells. One of them is CYP1A1 (cytochrome P450, family 1, subfamily A, polypeptide 1).

CYP1A1 affects the metabolic activation of polyaromatic hydrocarbons (PAH) by encoding the aryl hydrocarbon hydrolase. One of the PAHs is benzo (a) pyrene. CYP1A1 catalyzes benzo (a) pyrene into an epoxide, which is an ultimate carcinogen.⁹⁻¹¹

CYP1A1 6235 T/C (rs4646903)'s chromosomal location is 15q24.1.¹²

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Several studies on the effect of CYP1A1 with the incidence of HNC have been conducted in Asian and Brazilian populations. Studies in Asian populations showed a significant association between CYP1A1 6235 T/C gene polymorphism (rs4646903) and HNC susceptibilities, but the study in Brazilian populations showed the opposite result.¹¹⁻¹⁴

This study was done to investigate the association between CYP1A1 6235 T/C (rs4646903) gene polymorphism and HNC in an Indonesian population.

Materials and Methods

In total, 50 Indonesian patients with HNC and 50 Indonesian healthy controls were included. This study was approved by the ethical committee of the Faculty of Dentistry, Universitas Indonesia.

Sample

This study is a descriptive study with laboratory analysis. A total of 100 samples were DNA extracted from the blood serum of patients with HNC (50) and healthy controls (50). These samples were stored in a -20°C refrigerator in the oral biology laboratory, Faculty of Dentistry, Universitas Indonesia.

Polymerase Chain Reaction (PCR) Amplification

The polymorphic site was amplified using two primers: forward (5'-TAG GAG TCT TGT CTC ATG CCT-3') and reverse (5'-CAG TGA AGA GGT GTA GCC GCT-3'). The DNA samples were amplified in a 25 μL final volume reaction containing 12.5 μL KAPA taq Ready Mix PCR with dye (Kapa Biosystems), 0.5 μL DNA, 0.75 μL forward primer, 0.75 μL reverse primer, and 10.5 μL ddH₂O.

Initial denaturation was carried out at 94 $^{\circ}\text{C}$ for 5 min, followed by 30 cycles at 94 $^{\circ}\text{C}$ for 1 min, 57 $^{\circ}\text{C}$ for 1 min, 72 $^{\circ}\text{C}$ for 1 min 30 s, and a final extension for 2 min at 72 $^{\circ}\text{C}$. The PCR product was electrophoresed in 1.5% agarose gel (Thermo scientific) set at 70V, 400mA, for about 40 min, with conditions of 100bp DNA

Ladder (Thermo scientific) and 5 μL PCR product. After that, it was visualized using Gel Doc. The PCR product showed the result of 340bp from DNA amplification.

Restriction Fragment Length Polymorphism (RFLP)

Each 340bp PCR product (10 μL) was digested with 0.1U MspI restriction enzyme (Thermo scientific) in a 2 μL buffer (Thermo scientific) and 5.9 μL ddH₂O. Then, to begin the digestion by enzyme activation, it was incubated overnight for 16 h at 37 $^{\circ}\text{C}$. After incubating overnight, inactivation of the enzyme was performed in thermo block at 80 $^{\circ}\text{C}$ for 20 min.

The RFLP product was then subjected to electrophoresis in 1.5% agarose gel (Thermo scientific) set at 70V, 400mA, for about 40 min, with conditions of 100 bp DNA ladder (Thermo scientific) and 5 μL RFLP product. It was then visualized using Gel Doc. The presence of the MspI restriction site polymorphism was performed by the result of TT (340 bp), CC (200 bp and 140 bp), and CT (340 bp, 200 bp, and 140 bp).

Statistical Analysis

The distribution of genotypes and alleles was analyzed using the Hardy Weinberg equilibrium and Fisher exact tests to determine the significance of the genotypes in the population.

Results

The PCR reaction produced a 340 bp PCR product and digested an MspI restriction enzyme to identify 340bp, 200bp, and 140bp bands. In this study, there were 17 (34%) cases of HNC and 15 (30%) healthy controls for the wild-type TT with a single band (340bp). For the heterozygote (CT) with three bands, there were 28 (56%) HNC patients and 33 (66%) healthy controls. For the CC type with two bands, there were five (10%) HNC patients and two (4%) healthy controls. Therefore, the most common type of CYP1A1 (6235 T/C) both in HNC patients and healthy controls is the heterozygote CT with three bands. The result is summarized in Table 1.

	HNC (n = 50)	Healthy Controls (n = 50)	P-value
Genotype			
TT	17 (34%)	15 (30%)	1.0
CT	28 (56%)	33 (66%)	
CC	5 (10%)	2 (4%)	
Allele			
T	62 (62%)	63 (63%)	0.675
C	38 (38%)	37 (37%)	

Table 1: Distribution of CYP1A1 (6236 T/C) gene polymorphism in HNC in our Indonesian population.

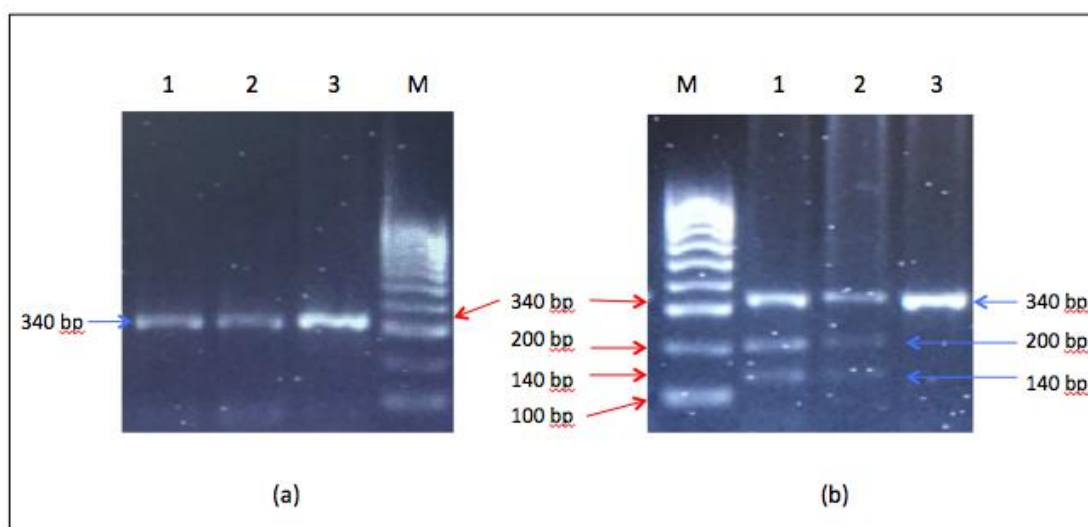


Figure 1: Results of PCR-RFLP. (a) Result of PCR amplified target fragment at 340bp; (b) Fragments after MspI enzyme cutting; M = marker.

Both *p*-values for genotype and alleles from the Fisher exact calculation were higher than 0.05 (1.0 for HNC patients and 0.675 for healthy controls). This shows that there is no significance association between CYP1A1 (6235 T/C) gene polymorphism in HNC patients in the Indonesian population.

Discussion

This study was done objectively to investigate the association between CYP1A1 (6235 T/C) gene polymorphism and HNC in Indonesia. It shows that the T alleles (62%) are higher than C alleles (38%) in HNC. The most common genotype HNC is the heterozygote one with three bands (CT).

The genetic polymorphism of CYP1A1 (6235 T/C) was investigated earlier in Asian and Brazilian populations (Table 2).¹¹⁻¹⁴ In the study conducted by Liu et al., it was shown that CYP1A1 (rs4646903) gene polymorphism contributed to increased PAH activation, which was verified to increase the risk of HNC.¹¹ However, the studies by Gattas et al. and Lourenço et al. showed that there were no significant associations between CYP1A1 (rs4646903) gene polymorphism and HNC.^{13,14}

In this study, the *p*-value from the Fisher exact calculation was higher than 0.05 in both genotype and alleles in HNC, which indicates that there is no significant association between CYP1A1 6235 T/C (rs4646903) gene polymorphism and HNC in our Indonesian population.

Study	Population	Significance Level
Liu et al., 2015	Asia	Significant
Gattas et al., 2006	Brazil	Insignificant
Lourenço et al., 2011	Brazil	Insignificant
Olivieri et al., 2009	Brazil	Insignificant

Table 2: Comparison of some studies of significant-level association between CYP1A1 6235 T/C gene polymorphism and HNC.

Conclusion

In this study, we found that there are three different genotypes in CYP1A1 6235 T/C gene polymorphism: TT (340bp), CT (340bp, 200 bp, and 140bp), and CC (200bp and 140bp). There is also no significant association between CYP1A16235 T/C (rs4646903) gene polymorphism and HNC in our Indonesian population.

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Declaration of Interest

The authors report no conflict of interest.

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