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Polymorphisms of *CYP2E1* rs2031920 is not Associated with Risk of Nasopharyngeal Carcinoma in Minangkabau Ethnic Group

Sukri Rahman[1*], Eti Yerizel[1], Daan Khambri[1], Djong Hon Tjong[2]

[1]Faculty of Medicine, Andalas University, Padang, Indonesia; [2]Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia

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***Correspondence:** Sukri Rahman. Faculty of Medicine, Andalas University, Padang, Indonesia, E-mail: sukrirahman@med.unand.ac.id

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Abstract

BACKGROUND: Various environmental factors have been suspected to be associated with the risk of developing Nasopharyngeal Carcinoma (NPC). Volatile nitrosamines found in salted fish are thought to be carcinogenic substances for NPC. Nitrosamines are activated by the CYP2E1 enzyme. Several studies investigated the relationship between polymorphism in the *CYP2E1* gene and susceptibility to NPC, but the results obtained were inconsistent.

AIM: This study was conducted to analyze the association of the *CYP2E1* rs2031920 polymorphisms with the incidence of NPC in the Minangkabau ethnic group.

METHODS: The subjects of this study were newly diagnosed NPC Minangkabau ethnic patients, while the controls were healthy people. A total of 23 cases of NPC and 23 aged (± 3 years) and sex-matched controls participated in the study. The method used to identify these polymorphisms is PCR sequencing.

RESULTS: On recent study we found *CYP2E1* rs2031920 gene polymorphism in both the NPC and control groups, in the NPC group there were 8.7% heterozygote mutants while in the control group there were 26.1% heterozygote mutants, and there were no homozygote mutants in the two groups, and statistically none a significant relationship between *CYP2E1* gene polymorphism and the incidence of NPC, with $p > 0.05$.

CONCLUSION: Our study reveals that there is no association of *CYP2E1* gene polymorphism (rs2031920) with the incidence of nasopharyngeal carcinoma in the Minangkabau ethnic group.

Introduction

Nasopharyngeal carcinoma (NPC) is cancer that has a unique distribution pattern both geographically and ethnically. The exact etiology of nasopharyngeal cancer is unknown, but it is thought to be a multifactorial interaction. Epstein-Barr virus (EBV) infection interacts with genetic susceptibility, and environmental factors are the main etiological factors [1].

Various environmental factors have been reported related to the incidence of Nasopharyngeal Carcinoma (NPC), including consumption of salted fish containing nitrosamines [2], [3]. Study on environmental factors as carcinogens has varied reports, but in endemic areas, nitrosamines from salted fish are a factor that is often associated with the incidence of NPC [3]. but clinical study at Dr. RSUP M. Djamil Padang did not get this association [4].

Nitrosamines are activated by the CYP2E1 enzyme, this enzyme also activates light molecules of nitrosamines such as N-nitrosodimethylamine (NDMA) and nitrosamines found in tobacco, N-nitrosonornicotine (NNN) [2]. Activation of nitrosamines can cause the growth of several malignancies. Several studies have found that CYP2E1 is also expressed on the nasal and

nasopharyngeal mucosa, CYP2E1 mutation can cause nasopharyngeal mucosa susceptible to the growth of NPC [2], [5], [6].

Study in Taiwan and Thailand populations found CYP2E1 RsaI (rs2031920) polymorphism in the promoter associated with an increased risk of NPC [7], [8]. while Guo X at al., who conducted studies in the Chinese population did not find an increased risk of NPC in mutant homozygote individuals variants of CYP2E1-RsaI (rs2031920) [9].

This study was conducted to analyze the association of the CYP2E1 rs2031920 polymorphisms with the incidence of NPC in the Minangkabau ethnic group.

Material and Methods

The subjects of this study were newly diagnosed NPC Minangkabau ethnic patients, while the controls were healthy people who were also Minangkabau ethnic. A total of 23 cases of NPC and 23 aged (± 3 years) and sex-matched controls participated in the study. Informed consent was obtained and blood samples were taken. The study was approved by the ethics committee of the Faculty of Medicine, Andalas University, Padang, Indonesia (No.422 / KEP / FK / 2018).

Primers for recognizing CYP2E1 gene polymorphism (rs2031920) are constructed using software Geneious 11.1.2. The method used to identify these polymorphisms is PCR sequencing. Primers used for CYP2E1 amplification are forward: 5 '- CAGTCGAGTCTACATTGTCAGT - 3' and reverse: 5 '-CTTGATGTCTGATGAGGAGGTTTG - 3', to amplify DNA with amplicon size 931bp. After DNA isolation and primer construction, PCR is then carried out.

All PCR product samples were sequenced at 1stBASE, Singapore. The results of sequencing data are then processed with Genious 11.1.2 software. In individuals who have CYP2E1 rs2031920 that do not experience polymorphism, no change in CC base is called a wild type, while those that change from CC to CT are called heterozygote mutants and those that change from CC to TT are called homozygote mutants. Sequencing results are then aligned with the reference genes (NG_055447.1).

Results

CYP2E1 rs2031920 gene polymorphism was found in both the NPC and control group, in the NPC group there were 8.7% heterozygote mutants while in the control group there were 26.1% heterozygote mutants, and there were no homozygote mutants in the two groups, and statistically none a significant relationship between CYP2E1 gene polymorphism and the incidence of NPC, with $p > 0.05$ (Table 1).

Table 1: Association of CYP2E1 Polymorphism (rs2031920) with NPC

Polymorphisms	Group		p
	NPC f (%)	Control f (%)	
Wild type (CC)	21 (91.3)	17 (73.9)	0.243
Mutant Heterozygote (CT)	2 (8.7)	6 (26.1)	
Mutant Homozigote (TT)	0 (0)	0 (0)	
Total	23(100	23(100)	

The presence of heterozygote mutant CYP2E1 rs2031920 gene polymorphism is indicated by the changes in CC base to CT. In the case of NPC, there were only heterozygote mutations in the two samples (K07 and K09), there were no homozygote mutations, whereas in the control group there were six samples also in the form of heterozygote mutants (S09, S10, S11, S17, S18, and S24) (Figure 1 and Figure 2).

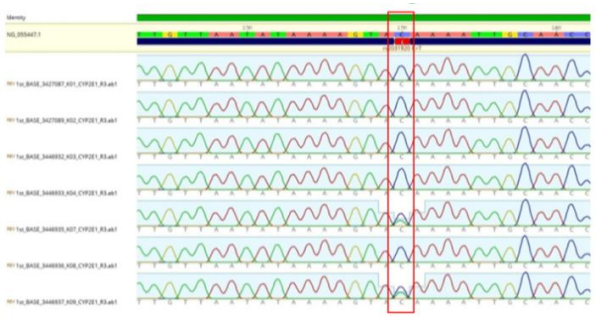

Figure 1: Alignment of sequencing results of NPC case group, samples; K01, K02, K03, K04, K07, K08 and K09 with reference genes CYP2E1

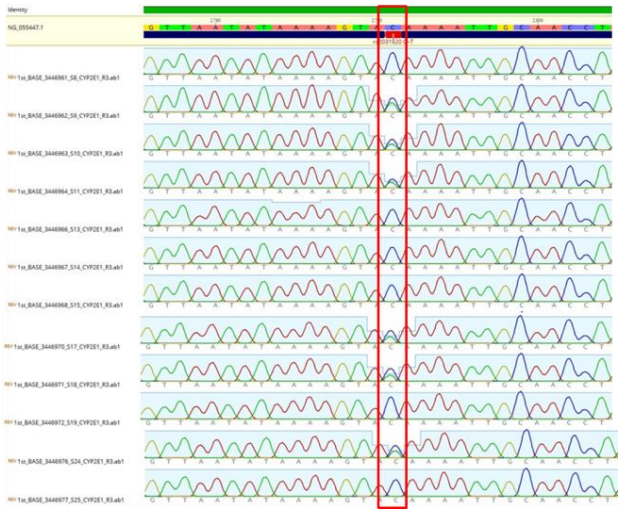

Figure 2: Alignment of sequencing results of several control group samples with the CYP2E1 reference gene

Discussion

This study shows that the CYP2E1 rs2031920 gene polymorphism was found in the NPC group and controls. The form of polymorphism found was only heterozygote (c1c2) mutants in both groups, and no homozygote mutants (c2c2) were found. Polymorphism is more common in the control group, but no significant differences were found between the two groups.

The CYP2E1 enzyme is involved in the metabolic activation of several lights and pro-carcinogenic molecules such as nitrosamines. Based on previous epidemiological studies, it was found that salted fish containing nitrosamines and nitrosamine precursors were associated with the incidence of NPC, so CYP2E1 gene polymorphisms were thought to cause the susceptible nasopharyngeal epithelium to NPC growth [6], [10].

Several studies have reported in several ethnic groups that have inconsistent results. The meta-analysis study that combined several studies in various ethnicities found a correlation between CYP2E1 rs2031920 gene polymorphism and the incidence of NPC, but also no association with the incidence of NPC if it was only heterozygote mutant polymorphisms [6].

In this study, Minangkabau ethnicity in both NPC and controls group found only heterozygote mutants (c1c2), and not related to the incidence of NPC. The same was reported by Lourembam et al. who conducted a study in India in areas with a high incidence of NPC also found only heterozygote polymorphisms and also did not get a significant relationship with the incidence of NPC [3]. Hildesheim et al., [2]. who conducted a study on NPC patients in Taiwan also, did not get an increase in the incidence of NPC in the heterozygote (c1c2) mutant CYP2E1 gene polymorphisms. Guo X et al., also found no increased risk of NPC in individuals both homozygote TT (c2c2) mutants and heterozygote CT (c1c2) CYP2E1-RsaI (rs2031920) in Han populations in South China [9].

The Kongruttanachok et al., [8]. report, which conducted a study on NPC patients in Thailand, only found an increase of incidence of NPC in homozygote variant polymorphisms both Thai and Chinese ethnic [8]. The same report in NPC in Tunisia has increased the incidence of NPC (OR = 8.39; CI95% [0.99 – 388.1]) in the c2c2 polymorphism and did not get in the c1c2 polymorphism.

Various reports concluding that there is a relationship between CYP2E1 gene polymorphism and the incidence of NPC generally combining homozygote and heterozygote mutants or calculating the frequency of c2 allele, whereas if only looking at heterozygote mutants, there are no reports that have a significant relationship with the incidence of NPC [6], [11], [13].

The RsaI position in the 5-Flanking region −1053 C > T is important in the difference in transcription activity. In vitro studies have shown that homozygote (mutant) c2c2 genotypes are associated with an increase of 10 times the CYP2E1 gene transcription. Further studies with liver sample microsomes showed that the level of mRNA subjects with c1c2 genotype was higher than those with c1c1 (wild type) genotype, thus also indicating that the transcription activity of c2 allele was stronger than that of c1. However, other in vitro studies did not find a significant increase in CYP2E1 activity in c2 carriers, compared to carrier c1 [14].

Increased expression in homozygote variants will cause more procarcinogens to turn into carcinogens, which will then cause DNA damage. The effect of differences in metabolic gene expression will be reduced if someone who has abnormal genotypes is not exposed to the substrate. So that mutations will not cause phenotype without interaction with environmental factors. The role of CYP2E1 polymorphisms is also likely to vary according to the amount of food consumed containing nitrosamines and precursors of nitrosamines (Kongruttanachok et al., 2001). Epidemiological studies in NPC patients in West Sumatra did not get a relationship between the consumption of salted fish and the increased incidence of NPC [4].

In conclusion, our study reveals that there is no association of CYP2E1 gene polymorphism (rs2031920) with nasopharyngeal carcinoma in the Minangkabau ethnic group.

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