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The Association of *CYP2E1* Polymorphism and Environmental Factor in Nasopharyngeal Carcinoma Patients

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Abstract

INTRODUCTION: Polymorphism of *CYP2E1* induces nasopharyngeal carcinoma (NPC) by activating pro-carcinogens including nitrosamine. Environmental factor such as salted fish, preserved food, tobacco, and alcohol consumption which contains nitrosamine, join with *CYP2E1* polymorphism leads to an increase of susceptibility for NPC.

OBJECTIVE: The aims of this study were to identify *CYP2E1* polymorphism and the association with other risk factors to NPC in NPC patients.

METHODS: This study was analytic research with the case–control design. The samples were taken based on non-probability consecutive sampling method. The identification of *CYP2E1* polymorphism was done by the PCR-RFLP method. The association of its variable to NPC was analyzed with the Chi-square test and between polymorphism of *CYP2E1* with other risk factors was analyzed with stratified analysis.

RESULT: We found that there was no significant association of *CYP2E1* polymorphism with NPC. However, the joint effect of *CYP2E1* polymorphism with smoking was significant in NPC patients. The risk for NPC in the combination of those two was 4.0-fold.

CONCLUSION: The study showed the capability of genetics and environment in the development of NPC. Further study can be done to find evidence of genetics and environmental influence in the prevention and treatment of NPC.

Introduction

Nasopharyngeal carcinoma (NPC) arising from nasopharynx epithelium and is developed by the relation of genetic, Epstein–Barr virus (EBV) infection, and environment [1], [2]. NPC has a unique geographic distribution which common in South China and Southeast Asia [3], [4]. There were 86.500 cases of NPC and 50.000 deaths due to NPC worldwide [5]. In Indonesia, there are about 13.000 cases of NPC per year [6]. Based on age-standardized rate, there were 8.3/100.000 in males and 3.0/100.000 in females for the incidence of NPC in Indonesia based on GLOBOCAN 2012 [7]. The disease is in the fourth rank for the most common cancer in Indonesia and the most frequent for head and neck cancer [8].

Genetic, EBV infection and the influence of the environment had been known as the etiology of NPC [9]. The distinct pattern for distribution of NPC was thought as the result of genetic and environment interaction [10]. Contribution of genetics in the development of NPC includes alteration of the chromosomal regions and genes and also epigenetic alteration such as DNA methylation [11]. Genetic polymorphism was also known to be associated with NPC. Cytochrome P450 2E1 (*CYP2E1*) is an enzyme works in activating nitrosamine and related carcinogen metabolism. Polymorphism of this enzyme which is homozygote mutant genotype (−/−) is associated with increased risk of NPC [10], [12]. This polymorphism is related to the increased enzyme activity of *CYP2E1* [12].

Clear geographic distribution of NPC is related to the dietary habit of the population in the endemic area including salted fish and preserved food consumption. This diet contains nitrosamine as carcinogen. However, smoking also has a role in the development of NPC [13], [14]. Nitrosamine, besides it is contained in the food, also can be found in tobacco [15]. This carcinogen is a genotoxic carcinogen that induces DNA mutation, leading to malignancy development [16], [17]. We did the study to analyze the role of genetic, polymorphism of *CYP2E1*, and environment as the risk factor for NPC patients in General Hospital Haji Adam Malik Medan. There was no study about this in Medan. The result can be used as the basic data of the further studies ahead in analyzing the interaction of other genetics factors of NPC with the risk factor. Therefore, it can support that intervention of the genetic and risk factor of NPC is important in the treatment and prevention of NPC.

Materials and Methods

Patients and samples

The study was analytic research with a case–control design. The case group was the NPC patients diagnosed based on history taking, physical examination and histopathological evaluation in the Department of Otorhinolaryngology, Head and Neck Surgery, Adam Malik General Hospital Medan. The control group was with the same frequency with the case group and does not have a history of cancer. The inclusion criteria were the NPC patients who agreed to be the participants in the study and the exclusion criteria were the NPC patients who have other malignancies. The interview was done to obtain information on the risk factors of NPC patients.

The samples of the study were the DNA extracted from 2 ml of blood taken from the NPC patients and the controls. The identification of *CYP2E1* polymorphism by PCR-RFLP was done in the Integrated Laboratory of the Faculty of Medicine of Universitas Sumatera Utara. The blood was collected in EDTA blood collection tubes and then performed DNA extraction using the Wizard Genomic DNA Purification Kit 100 Isolation® (PROMEGA, United States of America).

PCR-RFLP analysis

DNA isolation product was used for PCR analysis. We used a method from the usage information of the GoTaq® Green Master Mix for amplification. There was 25 µl total reaction volume with each sample consisted of 12.5 µl of the GoTaq® Green Master Mix, 1 µl of forwarding primer, 1 µl of reverse primer, 2 µl of DNA template, and 8.5 µl nuclease-free water. We used the PCR method by Ghania *et al*. to identify *CYP2E1* polymorphism using Rsal digestion [12]. This study used 5'-CCAGTCGAGTCTACATTGTCA-3' as the forward primer and 5'-TTCATTCTGTCTTCTAAC-TGG-3' as the reverse primer. There were 40 cycles in this PCR method with the thermal condition of 92°C for 1 min, 60°C for 1 min, 72°C for 2 min, and final elongation at 72°C for 5 min. Then, it continued with the RFLP method by digestion of 5 µl of samples with 0.2 µl Rsal at 37°C overnight. We continued electrophoresis using agarose gel 2% and visualized the bands under ultraviolet transilluminator.

Statistical analysis and ethics

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) 23.0 computer program. The distributions of the cases and controls were analyzed using the Chi-square test. The association of *CYP2E1* polymorphism with other risk factors to NPC was analyzed using stratification analysis with the value for a risk factor that was mentioned with OR (odds ratio). The result was said to be statistically significant if the two-sided $p < 0.05$. This study has been approved by the Health Research Ethical Committee, Medical Faculty of Universitas Sumatera Utara/Adam Malik General Hospital and the methods did not contradict with the Declaration of Helsinki.

Results

In the study, we found there were 21 (56.8%) patients in the age group of 41–60 years old. It had the highest number than any age group. Male was dominant than the female with a comparison of 2.7:1. We found that WHO type 2, non-keratinizing squamous cell carcinoma was the most common histopathological type of NPC in the study with 23 (62.2%) patients. In control, age group of 41–60 years old had a higher number with 21 (56.8%) persons, and slightly different from the age group of 21 to 40 years old with 16 (43.2%) persons. The data are shown in Table 1.

Table 1: Demographic distribution based on age, gender, and histopathological type

Characteristics	Case (%)	Control (%)
Age (years old)		
<21	0	0
21–40	13 (35.1)	16 (43.2)
41–60	21 (56.8)	21 (56.8)
>60	3 (8.1)	0
Gender		
Male	27 (73.0)	15 (40.5)
Female	10 (27.0)	22 (59.5)
Histopathological type		
WHO type I	5 (13.5)	
WHO type II	23 (62.2)	
WHO type III	9 (24.3)	

Table 2 shows several risk factors of NPC and its distribution in the NPC patients and non-NPC patients. We found that homozygous wild type genotype (+/+) was the most common for *CYP2E1* genotype with 24 (64.9%) patients in case and 26 (70.3%) in control. This genotype had the band which is visualized in the 50 bp and 360 bp. The heterozygous mutant genotype (±) resulted in the presence of band in 50 bp, 360 bp, and 410 bp. We found 3 (8.1%) NPC patients and 5 (13.5%) persons with this type of *CYP2E1* genotype. There were 10 (27.0%) NPC patients and 6 (16.2%) persons carrying homozygous mutant genotype (−/−) in this study. This genotype resulted in the presence of band in 410 bp.

Table 2: Distribution of *CYP2E1* polymorphism and other risk factors in case and control and the risk for NPC

O risk factor	Case (%)	Control (%)	p value	OR (CI 95%)
CYP2E1 genotype				
+/+	24/37 (64.9)	26/37 (70.3)	>0.05	
+/−	3/37 (8.1)	5/37 (13.5)	>0.05	
−/−	10/37 (27.0)	6/37 (16.2)	>0.05	
Smoking				
+	23/37 (62.2)	12/37 (32.4)	<0.05*	3.423 (1.315–8.909)
−	14/37 (37.8)	25/37 (67.6)		
Drinking alcohol				
+	4/37 (10.8)	2/37 (5.4)		
−	33/37 (89.2)	35/37 (94.6)	>0.05	
Salted fish and preserved food consumption				
+	28/37 (75.7)	29/37 (78.4)		
−	9/37 (24.3)	8/37 (21.6)	>0.05	

*Chi-square test. NPC=Nasopharyngeal carcinoma, OR: Odds ratio, CI: Confidence interval.

Based on the smoking status, in NPC patients, there was a higher number of smokers than non-smokers with 23 (62.2%) smokers and 14 (37.8%) non-smokers. However, in controls, smokers were less than non-smokers with 12 (32.4%) smokers and 25 (67.6%) non-smokers. Alcohol drinkers were less than non-drinkers both in NPC patients and controls. Alcohol drinkers in NPC patients were 4 (10.8%) patients and in controls were 2 (5.4%) persons while non-alcohol drinkers were 33 (89.2%) in NPC patients and 35 (94.6%) persons in controls. The frequent consumption of salted fish and preserved food was found in 28 (75.7%) in NPC patients and 29 (78.4%) in controls. There were 9 (24.3%) NPC patients and 8 (21.6%) persons of controls which were not frequently consuming salted fish and preserved food consumption. We found a significant association of smoking with NPC with a risk of 3.423-fold higher in smokers than non-smokers. There was no significant association of *CYP2E1* polymorphism, drinking alcohol, and salted fish and preserved food consumption with NPC.

We analyzed the joint effect of *CYP2E1* polymorphism with other environmental risk factors in NPC patients which are shown in Table 3. The result was there was a significant association of the joint of homozygote mutant genotype of *CYP2E1* with smoking in NPC patients (OR = 4.000, 95% CI = 1.205–13.283, p = 0.007). It meant that in the person carrying homozygous mutant genotype of *CYP2E1*, the incidence of NPC differed between smokers and non-smokers with the risk in smokers was about four-fold higher than smokers. Above, the risk for NPC in smoking alone was about 3.423 and the combination of smoking and homozygote mutant genotype was higher, about four. We did not find a significant association of the joint of *CYP2E1* polymorphism with drinking alcohol and the consumption of salted fish and preserved food.

Table 3: Association of *CYP2E1* polymorphism with environmental factors in NPC patients

Risk factors	Genetic variance of *CYP2E1*					
	+/+		+/−		−/−	
	Case/ Control	p value	Case/ Control	p value	Case/ Control	p value
Smoking						
Smokers	13/10	>0.05	2/2	>0.05	8/0	<0.05**
Non-smokers	11/16		1/3		2/6	(OR:4.000) CI: 1.205–13.283
Drinking alcohol						
Drinkers	2/1	>0.05	1/1	>0.05	1/0	>0.05
Non-drinkers	22/25		2/4		9/6	
Salted fish and preserved food consumption						
Frequently	18/21	>0.05	3/3		7/5	>0.05
Seldom	6/5		0/2	>0.05	3/1	

**Fisher's exact test. NPC: Nasopharyngeal carcinoma, OR: Odds ratio, CI: Confidence interval.

Discussion

In the study, we found that most NPC patients were frequently had age in the range of 41–60 years old and then followed by the age in the range of 21–40 years old. The result was similar to studies which had been done before. Wei *et al*. in their study of incidence and mortality of NPC in China found that incidence of NPC rose quickly in the age with a range of 25–29 years old with the peak age was in the range of 60–64 years old in males and 75–79 years old in females. The incidence than was decreased in the older range of age [18]. Adham *et al*., in Indonesia, found that the patients were diagnosed with NPC in the range of age 30–59 years old with the peak age was at 40–49 years old [8]. However, in the western area, Finland, Ruuskanen *et al*., found the range of age in the diagnosis of NPC was in 12–85 years old with the average age was 57 years old [19]. The incidences of NPC in the Asian as the high-risk populations rise in adolescences with the peak age at 45–55 years old and then decline. In the low risk and intermediate populations, the western area, the incidences have a bimodal pattern where the first peak at age 15–24 years old and the second peak at 65–79 years old. The bimodality pattern was thought due to the heterogeneous etiology of NPC within the same populations [20].

Male was common suffering NPC than females in this study with the ratio 2.7:1. Xiao *et al*. found 213 (71.2%) males and 86 (28.8%) females with NPC [21]. Xie *et al*. found that the overall ratio of male to female for NPC was ranged in 2.2–3.1. Male is dominant for NPC was thought related to a delayed development of cancer in females, especially before menopause. The protective effect of estrogen was thought has a role in that suggestion. Environmental factors such as smoking and salted fish and preserved food consumption are mainly found in males. It also thought of as the reason for the predominantly male in NPC [22].

In this study, non-keratinizing squamous cell carcinoma was found mostly from other histopathology types. This type is the most common in the endemic area about more than 95% cases [23], [24]. It is associated with EBV infection and more radiosensitive than other types [23], [25]. Keratinizing squamous cell carcinoma is common in the west area with low EBV, DNA is detected in patients with this histopathology type. This type is associated with smoking and has a poorer prognosis than non-keratinizing squamous cell carcinoma [26], [27]. Pan *et al*., in Singapore, found 103 of 111 NPC patients with non-keratinizing squamous cell carcinoma [28]. A study by Ji *et al*., in China, also found most histopathology type of NPC in their study was non-keratinizing squamous cell carcinoma with 932 of 1044 NPC patients [29].

Tobacco contains carcinogens, nitrosamines, and an active carcinogen metabolite that damaging DNA and induces chronic inflammation in nasopharyngeal mucosa [30]. Tobacco- specific nitrosamine is the highly carcinogenic substances in tobacco which is the product of nitrosation of nicotine that is contained in tobacco. Nitrosamine ketone (NNK) is one of the active carcinogens of tobacco-specific nitrosamine.

It causes a mutation of oncogenes such as K-Ras and p53. Besides, NNK is also responsible for the modulation of signal-transducing networks such as the PI3K-Akt pathway. The pathway increases cell growth, proliferation, survival, migration, and metabolism [31].

In this study, the risk for NPC in smokers was 3.423 fold higher than non-smokers. A study by Ekburanawat *et al*. found that a significant association of smoking with NPC with the risk was 2.41-fold higher than non-smokers. The study also was similar to our findings that there was no significant association between alcohol and salted fish consumption with NPC [32]. A meta-analysis study by Xue *et al*. found an increased risk of NPC about 1.6-fold for smokers than non-smokers. Smokers with a cumulative exposure of ≥30 pack-years had a higher risk for NPC, 2.93-fold than smokers with cumulative exposure <30 pack-years which had the risk for NPC about 1.29-fold than non-smokers [33].

CYP2E1 acts in the activation of pro-carcinogens including nitrosamines which are contained in tobacco, meat, and salted and preserved food [34], [35], [36]. This enzyme metabolizes the xenobiotic substances into toxic substances which induces carcinogens [37]. *CYP2E1* also can induce ROS formation that can damaging DNA, leading to mutation and increased cell growth [38], [39]. Polymorphism of *CYP2E1* had known to be associated with cancers including NPC [40], [41], [42], [43]. The polymorphism increases the transcription and activity of *CYP2E1*. Therefore, the activation of pro-carcinogens such as nitrosamine also increased [42], [43]. The type of *CYP2E1* polymorphism which has higher enzyme activity is homozygous mutant genotype (−/−) [12]. In our study, there was no association of *CYP2E1* polymorphism to NPC while the joint effect of this genetic variance with smoking was found to be significantly associated with NPC with the risk 4.0-fold higher in smokers than non-smokers. A similar result was found by Ghania *et al*. in their study that showed an increased risk for NPC which 3.3-fold higher in smokers than non-smokers in patients carrying homozygous mutant genotype [12].

Conclusion

We found that NPC patients were mostly male in the age group of 41–60 years old. The most common histopathology type was non-keratinizing squamous cell carcinoma. Among the risk factors, smoking was found associated with NPC while others were not related to NPC. However, the combination of the homozygous mutant genotype of *CYP2E1* with smoking had a significant association with NPC that there was increased the risk for NPC in smokers which carrying homozygous mutant genotype.

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