COMPARISON OF CYTHOCROME P450 FAMILY 2 SUBFAMILY A POLYPEPTYDE 6 (CYP2A6) GENE POLYMORPHISM PROPORTION BETWEEN EARLY AND ADVANCED STAGE OF UNDIFFERENTIATED TYPE NASOPHARYNGEAL CARCINOMA IN BALINESE

I Gde Ardika Nuaba^{1*}, Komang Andi Dwi Saputra¹, I Ketut Suanda¹, I Gusti Ayu Trisna Dewi¹

¹Department of Otorhinolaryngology, Medical Faculty of Udayana University, Sanglah Hospital, Denpasar, Indonesia

Abstract

Introduction: Nasopharyngeal carcinoma (NPC) is a malignancy derived from epithelial cells lining the nasopharynx. The etiology of nasopharyngeal carcinoma is multifactorial. One of the risk factors is CYP2A6 gene polymorphism which causes nitrosamines are not metabolized, leading to DNA change that could trigger cancer.

Objective: The purpose of this study is to know the association of CYP2A6 gene polymorphism and clinical stage of undifferentiated type of NPC.

Method: This is a cross sectional analytic study. The sample in this study were 80 nasopharyngeal carcinoma patients whose treated in ENT-HN department of Sanglah General Hospital between 2017-2018. The collected data consist of subject's characteristic and CYP2A6 gene polymorphisms identified by the PCR-RFLP technique.

Result: The probability of CYP2A6 gene polymorphism in the undifferentiated type of NPC in the Balinese tribe is 3.125 times greater in advanced stage than early stage. Based on multivariate analysis, there was a statistically significant association between CYP2A6 gene polymorphism and clinical stage of undifferentiated type NPC in Balinese with p value =0.0048 (p <0,05).

Conclusion: There is association between CYP2A6 gene polymorphism and clinical stage of undifferentiated type NPC in Balinese tribe.

Article Info

Keywords:

Nasopharyngeal carcinoma, Balinese, Polymorphism, CYP2A6 gene

*Corresponding author:

Address: Jl. P.B. Sudirman, Dangin Puri Klot, Kec. Denpasar Bar., Kota Denpasar, Bali 80232

e-mail: ardika.tht@gmail.com

1. INTRODUCTION

Nasopharyngeal carcinoma (NPC) is the most common head and neck malignancy in the world and in Indonesia. The incidence of NPC in the world is 1.2/100,000 individuals per year with diverse distribution based on geography [1]. The incidence of NPC is almost evenly distributed in every region in Indonesia. In RSCM Jakarta, more than 100 cases were found in a year and based on data from the NPC patient register at the ENT outpatient clinic at Sanglah Hospital from September 2015 to September 2017 there were 265 NPC patients.

The main cause of NPC still unknown. The cause is multifactorial and is often related to Epstein-Barr virus infection, genetic, and the environment factors [2]. Genetic susceptibility as a predisposing factor for NPC is based on the fact that there are many sufferers from the Chinese race, Mongoloid race, including the nations of Asia, especially Southeast Asia, which are still classified as Malay.

One of the genes associated with NPC is the CYP2A6 gene. This gene codes for the CYP2A6 enzyme, which is a first phase enzyme that plays a role in the metabolism of nicotine and some nitrosamine compounds found in tobacco. Polymorphisms in genes that encode the CYP2A6 enzyme are associated with individual metabolic variations in a xenobiotic [3]. CYP2A6 gene polymorphism is found around 1% in Caucasian race and its frequency is quite high, reaching 15-25% in Asian nations. Some mutations in CYP2A6 will reduce enzymatic activity and even eliminate enzyme activity. CYP2A6 gene polymorphism as one of the biomarkers in NPC is interesting to study because there is a strong association between CYP2A6 gene polymorphisms and the incidence of NPC. Research on CYP2A6 polymorphisms in people with NPC in Indonesia is still very little while in Bali has never been studied. The Balinese are still classified as a Malay family, where there are many NPC incidents.

2. MATERIAL AND METHODS

The research design used in this study is a cross-sectional design. The sample was selected by consecutive sampling until the required number of samples were met which meet the inclusion criteria. The inclusion criteria were new patients undergoing nasopharyngeal biopsy and histopathological examination at the Anatomy Pathology Laboratory of the Faculty of Medicine, Udayana University/Sanglah Hospital Denpasar and were diagnosed with undifferentiated type NPC, and were Balinese tribe. The subject were excluded from this study if they did not underwent biopsy in Sanglah Hospital or their biopsy preparations are unreadable, damaged and not found.

The data was analyzed by univariate, bivariate, multivariate analysis. Univariate analysis was used to describe the subjects studied according to age, sex, clinical stage of undifferentiated type NPC, and CYP2A6 gene polymorphism. Bivariate analysis was used to determine the association of CYP2A6 gene polymorphism with clinical stage of undifferentiated type NPC in Balinese.

3. RESULT

This study is using 80 samples who meets inclusion criteria. Researcher use genomic DNA measurements obtained from isolation using the PCR (Polymerase Chain Reaction) technique, genomic DNA fragments that was analyzed can be increased in quantity by in vitro amplification in a short time using primary pairs of synthetic oligonucleotides that limit the area which will be reproduced.

Table 1. Distribution of Undifferentiated NPC based on the clinical stage

Variable	n=80	n%
Clinical stage of undifferentiated NPC		
Stage I	0	0%
Stage II	16	20%
Stage III	5	6.25%
Stage IV A	25	31.3%
Stage IV B	34	42.5%
Early stage	16	20%
Advanced stage	64	80%

Table 1 shows the distribution of undifferentiated NPC based on clinical stage were stage II as many as 16 patients (11.8%), stage III as many as 5 patients (7.50%), stage IVA as many as 25 patients (31.3%), and stage IVB as many as 34 patients (42.5%). Distribution of early stage NPC patients was 16 patients (20%) and advanced stage 64 patients (80%).

Table 2. The distribution of CYP2A6 gene genotype

Genotype Group	n=80	n%
*1A/*1A	53	66.3%
*1A/*non 1A	2	2.5%
*non1A/*non1A	25	31.3%

The most common genotypes found were wild type homozygous (*1A/*1A) as many as 53 patients (65%). The mutant heterozygote group (*1A/*non 1A) were 2 patients (2.5%) and the mutant homozygote group (*non1A/*non 1A) were 25 patients (31.3%).

Table 3. The distribution of CYP2A6 gene genotype in clinical stage undifferentiated type NPC

Clinical Stage	*1A/*1A	*1A/*non 1A	*non1A/*non1A	Total
Early stage	14 (87.5%)	1 (6.3%)	1 (6.3%)	16 (100%)
Advanced stage	39 (60.9%)	1 (1.6%)	24 (37.5%)	64 (100%)

The most common distribution of genotypes in the early-stage NPC group was homozygous wild type (*1A/*1A) genotype, as many as 14 patients (87.5%). The most common genotype of advanced stage group was wild type (*1A/*1A) as many as 39 patients (60.9%).

Table 4. The distribution of CYP2A6 gene genotype

Genotype	Early stage	Advanced stage
CYP2A6 *1A/ CYP2A6 *1A	14 (87.5%)	39 (60.9%)
CYP2A6 *1A/ CYP2A6 *1B	0 (0%)	0 (0%)
CYP2A6 *1A/ CYP2A6 *4C	1 (6.3%)	1 (1.6%)
CYP2A6 *1B/ CYP2A6 *4C	0 (0%)	0 (0%)
CYP2A6 *1B/ CYP2A6 *1B	0 (0%)	0 (0%)
CYP2A6 *4C/ CYP2A6 *4C	1 (6.3%)	24 (37.5%)
Total	16 (20%)	64 (80%)

After obtaining the results of stage 1 RFLP, then samples with *non 1A mutant alleles were analyzed by stage 2 RFLP with the Bsu36I enzyme to distinguish *4C and *1B alleles. After the second phase of RFLP, the highest genotype of the undifferentiated type NPC in Balinese was *1A/*1A which was found in 53 samples (66.3%) while the mutant genotype group as many as 27 samples (33.8%).

Table 5. The distribution of CYP2A6 mutant gene genotype

Genotype	Early stage n=16	Advance stage n=64
CYP2A6 *1A/ CYP2A6 *4C	1 (6.3 %)	1 (1.6 %)
CYP2A6 *4C/ CYP2A6 *4C	1 (6.3 %)	24 (37.5 %)

This study only found allele mutants *1A/*4C and *4C/*4C. After the stage 1 RFLP examination which was later identified with stage 2 RFLP no other mutant alleles were found.

Table 6. Comparison of CYP2A6 gene polymorphism in early and advanced stage of NPC patients

Variable	Polymorphism		PR 95% CI	P
	Found	Not Found		
Advanced Stage	25 (39.1%)	39 (60.9%)	3.125	0.046
Early Stage	2 (12.5%)	14 (87.5%)		

Based on the cross tabulation above, it was found there were 2 patients (12.5%) in the early stages, while in the advanced stage 25 patients (39.1%) had polymorphisms in the CYP2A6 gene allele. while there are as many as

14 patients (87.5%) in the early stages and 39 patients (60.9%) in advanced stage who did not experience polymorphism in the CYP2A6 (wild type) gene. To find out the proportion comparison of CYP2A6 gene polymorphisms between advanced stage and early stage of undifferentiated type NPC in Balinese, chi square test was used and the value of $p=0.046$ ($p<0.05$) was obtained. So there is a statistically significant relationship.

To find out the relationship between CYP2A6 gene polymorphism and clinical stage of undifferentiated type NPC in Balinese, a multivariate analysis with logistic regression test was performed. Selection of this test to rule out confounding factors such as age and sex in the analysis. In this study, a significant relationship was found between the CYP2A6 gene polymorphism and the clinical stage of undifferentiated type NPC Bali, with a p value $=0.048$ ($p<0.05$)

4. DISCUSSION

The distribution of subjects based on the clinical stage of the undifferentiated type NPC in Balinese were 16 patients (20%) in early stage and 64 patients (80%) in advanced stage. This is consistent with research by Cheng et al (2014) that found 39.1% of patients in early-stage and 60.9% of patients in advanced stage NPC. This shows that patients usually came in advance stages. The diagnosis of NPC is often made too late because the location of the nasopharynx is hidden behind the nasal cavity and under the base of the skull so NPC patients generally come for treatment at an advanced stage [4,5].

CYP2A6 is a first phase enzyme which plays a role in the metabolism of nicotine and some nitrosamine compounds found in tobacco. NNK and NNN are among the major toxic components found in tobacco cigarettes [6].

Polymorphisms in genes that encode the CYP2A6 enzyme are associated with variations in the metabolic activity of an individual against a xenobiotic therefore two subgroups with different metabolic abilities will be found in the population. Subgroups with less metabolic ability are referred to as poor metabolizers or PM phenotypes, while subgroups with normal metabolic ability are referred to as extensive metabolizers or EM phenotypes [7].

The CYP2A6 gene polymorphism was found to be quite high in Asian nations. The proportion based on the CYP2A6 gene allele polymorphism in the undifferentiated type of NPC patient in Balinese was the mutant genotype group as many as 27 samples (33.8%). The mutant genotypes group consisted of heterozygotes mutants (*1A/*non 1A) in 2 patients (2.5%) and homozygotes mutant (*non 1A/*non 1A) groups of 25 patients (31.3%).

This study only found allele mutants *1A/*4C and *4C/*4C. After the phase I RFLP examination which was later identified with phase II RFLP, no other mutant alleles were found. This is in accordance with research conducted by Savitri (2014) where the most polymorphisms found in patients with NPC were on allele *4C of 24 subjects (25%) [8].

In general, the genotype frequency of the CYP2A6 gene was highly dependent on ethnic or ethnic background. For example, the frequency of the CYP2A6 *4/*4 genotype is 3.2-5% in the Japanese population, 1.9% in the Korean population and 1.5-3.0% in the Chinese population and 0% in the Caucasian. In contrast, genotypes CYP2A6 *2/*2 and CYP2A6 *3/*3 are often found in Caucasian than in Asian. Therefore, the CYP2A6 *4/*4 genotype is often becoming a field of study particularly in Asia [9].

Research by Tiwawech et al. (2006) in a Thai population revealed that individuals with *1B and/or *4C mutant alleles were twice as likely to suffer from NPC as wild-type allele individuals [9]. Wassenaar et al. (2011) also stated the same thing that CYP2A6 gene polymorphisms increase the risk of lung cancer. While the research of Cao et al. (2011) concluded that there was no significant relationship between CYP2A6 polymorphisms compared to wild type individuals with NPC and other types of cancer [9].

CYP2A6 gene polymorphisms are associated with individual metabolic variations of a xenobiotic. Variation of the CYP2A6 gene allele not only causes variations in genotype level, but also in phenotype level in the form of increasing or decreasing and even resulting in the loss of enzyme activity altogether (null mutation) as in the CYP2A6 *4 allele [10]. CYP2A6 *4C gene polymorphism is a type of base number variation genetic polymorphism (copy number variation) involving a broader segment of DNA in the form of a comprehensive deletion of the CYP2A6 gene that causes a complete loss of enzyme activity (null mutation) [10].

A defect in the CYP2A6 gene will increase the risk of cancer, this is related to the first-pass clearance of nitrosamines as a carcinogen. CYP2A6 has an important role in the pathway of nitrosamine metabolism in the liver. Loss of this

enzyme activity due to total deletion will inhibit the first pass clearance of nitrosamines so that the level of this carcinogen increases in other organs (post-liver) such as the esophagus and airway. The defects on CYP2A6 in increasing cancer risk mainly occurs in tobacco induced cancer. Individuals with complete deletion of the CYP2A6 gene have lower levels of cotinine in their urine, which is only 15% compared to individuals without the CYP2A6 gene polymorphism who consume cigarettes in the same amount and time span. This shows that the level of pro-carcinogen in the body is still very high therefore the risk of smokers is very high for malignancy [6].

Based on the cross tabulation in table 6, in the early stages of the undifferentiated type of NPC there were 2 subjects (7.4%) and 25 subjects (92.6%) in the advanced stage had polymorphisms in the CYP2A6 gene allele. In the early stages as many as 14 patients (26.4%) and in the advanced stage as many as 39 (73.6%) who did not experience polymorphism in the CYP2A6 gene (wild type). In this study, a total of 27 patients (33.8%) had CYP2A6 gene polymorphisms. To find the comparison of the proportion CYP2A6 gene polymorphisms between the advanced stage and the early stage of undifferentiated NPC type in Balinese, the chi square test was used and the proportion of CYP2A6 gene polymorphisms in patients with advanced stage undifferentiated type NPC was higher than the initial stage, which was 3.125 times with a p value = 0.046 ($p < 0.05$).

To find the relationship between CYP2A6 gene polymorphism and clinical stage of undifferentiated type NPC in Balinese, a multivariate analysis with logistic regression test was performed. Selection of this test to rule out confounding factors such as age and sex in the analysis. A significant relationship was found between the CYP2A6 gene polymorphism and the clinical stage of undifferentiated type NPC in Balinese, with a p value = 0.048 ($p < 0.05$). Age and gender did not show a significant relationship with the clinical stage of NPC sufferers.

Examination of the CYP2A6 gene polymorphism can be used as one of the supporting modalities in assessing tumor growth, progression, and prognosis of the disease. This is consistent with Ren's (2018) regarding the relationship of CYP2A6 gene defects with overall survival and recurrence free survival in hepatocellular carcinoma found that CYP2A6 gene defects are associated with worse overall survival and recurrence free survival in patients with hepatocellular carcinoma. In also found CYP2A6 gene polymorphisms associated with metastases that include vascular invasion, porta major venous invasion, intrahepatic spread, and elevated levels of AFP. [11,12] Research by Tiwawech et al. showed relationship between the CYP2A6 gene polymorphism and the clinical stage of NPC patients [9,13].

The etiology of NPC is often associated with the presence of Epstein-Barr virus infection, genetic, and the environment factors (exposure to carcinogens). Gene polymorphisms related to carcinogenic metabolism are one of the risk factors for NPC. One of them is the cytochrome P450 gene family 2 subfamily A polypeptide 6 (CYP2A6) which codes for the CYP2A6 enzyme for activation of pro-carcinogens [14,15].

5. CONCLUSION

The proportion of CYP2A6 gene polymorphisms in patients with advanced stage of undifferentiated type NPC was higher than the early stage, which was 3.125 times with $p = 0.046$ ($p < 0.05$). Based on a multivariate analysis test, a statistically significant relationship was found between the polymorphism of the CYP2A6 gene and the clinical stage of undifferentiated type NPC in Balinese, with a p value = 0.048 ($p < 0.05$).

ADVICE

Optimizing data distribution nasopharyngeal carcinoma patients at the Ear Nose Throat-Head Neck Surgery Bangli Hospital. Further research needs to be conducted with a sample of more as a reference to determine the distribution of patients with nasopharyngeal carcinoma development in Indonesia.

REFERENCE

- [1] Adham, M., Kurniawan, A.N., Muhtadi, A.I., Roezin, A., Hermani, B., Gondhowiadjo, S., dkk. 2012. Nasopharyngeal carcinoma in indonesia. Chinese Journal of Cancer. 31(4): 185–96.
- [2] William, I.W. 2012. Nasopharyngeal Cancer. Bailey Head and Surgery Otolaryngology. 4(4): 213–224.
- [3] Pelkonen, O., Rautio, A., Raunio, H., Pasanen, M.2009. CYP2A6 : a human coumarin 7-hydroxylase. Toxicology, 144: 139–147.
- [4] Chen, M., Mai, H., Sun, R., Guo, X. Zhao, Hua, Y. 2013. Clinical Finding and Imaging features of Nasopharyngeal Carcinoma with Postradiation Nasopharyngeal Necrosis. J Chin Anti-Ca Associa.32: 533–538.
- [5] Faiza, S., Rahman, S., Asri, A. 2015. Karakteristik klinis dan patologis karsinoma nasofaring di bagian THT-KL Dr M Djamil Padang. Jurnal Kesehatan Andalas. 5 (1): 90 - 95.
- [6] Rao, Y., Hoffman, E., Zia, M., Bodin, L., Zeman, M., Sellers, E., dkk. 2008. Duplications and Defects in the CYP2A6 Gene : Identification, Genotyping, and In Vivo Effects on Smoking. Mol Pharmacol.58 (4): 747–755.
- [7] Saleh, M.I. 2010. Penentuan genotip gen CYP2A6 sebagai biomarker keberhasilan terapi obat yang dimetabolisme melalui enzim CYP2A6. Universitas Sriwijaya. Tidak dipublikasikan
- [8] Savitri, B. 2015. Hubungan Polimorfisme Gen CYP2A6*4c Dengan Karsinoma Nasofaring Di Rumah Sakit Dr. Mohammad Hoesin Palembang Periode 2013-2014.
- [9] Tiwawech, D., Srivatanakul, P., Karalak, A., Ishida, T. 2006. Cytochrome P450 2A6 polymorphism in nasopharyngeal carcinoma. Cancer Letter. 241: 135–141.
- [10] Wijaya, Hendy. 2011. Gen CYP2A6 meningkatkan risiko ketergantungan fisik perokok terhadap nikotin. Thesis Universitas Udayana, Bali
- [11] Ren, Liua, W.S., Qina, H.D., Xua, Y.F., Yua, D.D. 2017. Effect of family history of cancers and environmental factors on risk of nasopharyngeal carcinoma in Guangdong. Cancer Epidemiology Biomarkers and Prevention, 34(4): 201-207.
- [12] American joint committee on cancer. 2018. AJCC cancer staging form supplemen (8th ed). American College of Surgeon. Chichago ; 1 - 520
- [13] Adhikari, A., Madhusnata. 2015. Association of Cytochrome P450 with Cancer Induced. American Journal of Medicine. 1(1): 1–14.
- [14] Tang, K., Li, X., Xing, Q., Li, W., Feng, G., He, L., dkk. 2010. Genetic polymorphism analysis of cytochrome P4502E1 (CYP2E1) in Chinese Han populations from four different geographic areas of Mainland China. Genomics. 95:224-229.
- [15] Akrodou, Y. 2015. CYP2A6 Polymorphisms May Strengthen Individualized. Hindawi Publishing Corporation Scientific.1: 1–7.