CYP1A1 rs464903 increased the risk of undifferentiated type nasopharyngeal carcinoma among the Balinese population

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ABSTRACT

Introduction: Nasopharyngeal carcinoma is a tumor that arises from epithelial cells covering the surface of the nasopharynx. In terms of malignancy, NPC is number 1 in the OHNS and number 5 in Indonesia. Certain risk factors that can predispose an individual to NPC include an EBV infection, salted fish consumption, and smoking. Previous studies also show that the polymorphism of CYP1A1 rs464903 affect the risk factor and mechanism of NPC incidence.

Methods: This is a case-control study conducted at Prof. dr. I.G.N.G. Ngoerah General Hospital, Denpasar. The study used 62 samples. The sample then underwent DNA isolation and amplification using PCR and afterward, visualization, DNA sequencing, and analysis.

Results: Bivariate analysis shows that CYP1A1 have a significant relationship with NPC incidence with a p-value of 0.007 (OR: 1.48; 95% CI 4.87 (1.5–15.9)).

Conclusions: The polymorphisms of CYP1A1 rs464903 is proven to be the risk factors in the mechanism of undifferentiated NPC among the Balinese population.

Keywords: Nasopharyngeal cancer, CYP1A1, polymorphism.


INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a tumor that arises from epithelial cells covering the surface of the nasopharynx. It usually originates from the lateral wall of the nasopharynx, specifically the fossa of Rosenmüller. The data of regional classification in GLOBOCAN 2020 reported 133 354 NPC incidences globally and 80.008 deaths in 2020, with the Southeast Asia region recording the highest number of NPC incidences.1 NPC incidences in Indonesia in 2012, which is 5.6/100 000 individuals per year, rank in the top three in Southeast Asia. In terms of malignancy, NPC is number 1 in the OHNS and number 5 in Indonesia. The prevalence of NPC in Indonesia reaches the highest point in decade 4-5, with a male-to-female ratio of 2.3:1. NPC incidence can be found in almost all regions in Indonesia. The Cipto Mangunkusumo Hospital, Jakarta, found more than 100 new cases in a year.2 According to the data of NPC patient register at the OHNS Polyclinic of Prof. dr. I.G.N.G. Ngoerah General Hospital, there were 602 new cases of NPC from January 2016 to December 2020.3

The World Health Organization (WHO) classified NPC into 3 types based on histopathological examination: WHO type I (keratinizing squamous cell carcinoma), WHO type II (non-keratinizing squamous cell carcinoma), and WHO type III (undifferentiated carcinoma). In Indonesia, 12.7% of NPC incidence found is WHO type I, 23% is WHO type II, and 85% is WHO type III. NPC is a multifactorial disease. Infection of the Epstein-Barr virus (EBV), genetics, and environmental and dietary factors are several causes of NPC. WHO type III or undifferentiated carcinoma is the most common histopathological type. Its pathophysiology is closely related to EBV infection, environmental factors as well as dietary and genetic factors.2 Genetic susceptibility is considered an endogenous factor because the majority of NPC patients are Chinese, or of the Mongoloid race, and Asians, particularly Southeast Asians, who are still classified as Malay. The relation between NPC and race can be seen in the Chinese population, particularly the Kanton population, with an age-adjusted rate (AAR) of 30/100 000 for males and 13/100 000 for females. It is higher than the AAR among the Eskimos in the Arctic (10/100 000 for males and 4/100 000 for females) or among the populations in North Africa (3.4/100 000 for males and 1.1/100 000 for females).1

In terms of the susceptibility of a race to NPC, the Mongoloid race is found to be at high risk for NPC. The Negroid race is at a medium risk while the Caucasian race is at a low risk.4

Genetics plays a significant role in the development of NPC. Some gene polymorphisms thought to be endogenous factors of NPC, which also affect its malignancy is the polymorphisms of CYP1A1 rs464903.5,6 The CYP1A1 gene is located on
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chromosome 15q22-q24 and spans 5,810 bp, consisting of 7 exons and 6 introns. Although several polymorphisms have been identified for the CYP1A1 gene, many studies have led to the polymorphisms of CYP1A1*2A or m1 (also known as Msp1), CYP1A1*2C or m2, CYP1A1*3 or m3, and CYP1A1*4 or m4. PAH activation by CYP1A1 is closely associated with tumor pathogenesis. CYP1A1 found on chromosome 15 becomes cytochrome 450 isozyme. To date, two single nucleotide polymorphisms (SNPs) of the CYP1A1, namely rs4646903 T>C and rs1048943 A>G, are known to be associated with cancer. The CYP1A1 rs4646903 T>C (T to C) polymorphism is expressed in the 3'-flanking region of the gene and three genotypes arise, namely TT (wild-type), TC (heterozygous), and CC (homozyzogous). The substitution of isoleucine for valine occurs from the A to G transition at codon 462.9 Research on the CYP1A1 polymorphism associated with nasopharyngeal cancer is abundant. A meta-analysis study on the relationship between the Ile-Val CYP1A1 substitution at codon 462 and head and neck cancer concludes that the Ile/Val and Val/Val genotypes tend to increase the risk of head and neck cancer compared to the Ile/Ile genotype with an odds ratio (OR) = 1.32 and 95% confidence interval (CI) = 0.95–1.82.9 Therefore, it is necessary to conduct a study evaluating the effect of the polymorphism of CYP1A1 rs4646903 as risk factor in the mechanism of undifferentiated NPC among the Balinese population.

METHODS

Study Design
This study used the case-control study design. See Figure 1 for the study design.

Data Collection
The study was conducted at the OHNS Polyclinic of Prof. dr. I.G.N.G. Ngoerah General Hospital, Denpasar. Specimen examination was carried out at the Integrated Biomedical Laboratory Unit of the Faculty of Medicine, Universitas Udayana. The study was conducted from July 2021 to July 2022. The sample in this study is part of the accessible population selected by consecutive sampling and meets the criteria of subject eligibility, which then goes through the matching process on the age and sex variables until the required number of samples is met.

The inclusion criteria in this research are as follows: in the case group, the subjects are patients of undifferentiated NPC at stage I to stage IV, Balinese people who received treatment at the OHNS Polyclinic of Prof. dr. I.G.N.G. Ngoerah General Hospital from July 2021 to July 2022, whose histopathologic diagnosis had been established at the Anatomical Pathology Laboratory by a specialist in Anatomical Pathology. In the control group, the inclusion criteria are non-NPC OHNS patients who received treatment at the OHNS Polyclinic of Prof. dr. I.G.N.G. Ngoerah General Hospital from July 2021 to July 2022 and underwent matching process on the age and sex variables. The exclusion criteria in this research are Balinese OHNS patients receiving treatment at OHNS Polyclinic of Prof. dr. I.G.N.G. Ngoerah General Hospital from July 2021 to July 2022 who refused to participate as research subjects after provided with informed consent, and OHNS patients with cancer or a history of cancer in other organs. There were 31 samples for each group, with a total of 62 samples overall.

The collected samples were divided into two groups: the case group and the control group. Afterward, data on demography were collected and recorded and followed by DNA isolation. Next, the amplification of DNA samples by PCR was done using GoTaq™ Green Master Mix, CYP1A1 SNP primer: 5’ CACCGAAGTGTTCCTATGCTG3’ and 5’ TGTAAGTGGCATGAAACGCC 3’. Sequencing was carried out using a forward primer (Figure 2). Then, DNA samples were visualized using the electrophoresis technique and observation under an ultraviolet (UV) lamp. After that, sequencing was carried out.

Statistical Analysis
The data were analyzed using the Chi-square test, p value < 0.05 was used as the significant level. Computer program SPSS ver. 3.0.1 was used for data processing.

RESULTS
This study is a case-control study with 62 samples in total. 31 samples are undifferentiated NPC cases among the Balinese population and 31 samples are non-NPC cases among the Balinese population. The research was conducted at the OHNS Polyclinic of Prof. dr. I.G.N.G. Ngoerah General Hospital from July 2021 to July 2022, and at the Integrated Biomedical Laboratory of the Faculty of Medicine of Universitas Udayana.

Samples were collected using the consecutive sampling technique. The subjects were selected at the Otolaryngology Polyclinic of Prof. dr. I.G.N.G. Ngoerah General Hospital, Denpasar, based on the inclusion and exclusion criteria. Subjects who met the criteria and were willing to sign the informed consent sheet then underwent a blood drawing procedure, in which 3cc of blood from the cubital vein was drawn.

Figure 1. Study design.
using an aseptic technique. Subjects were also requested to fill in a questionnaire to find out their basic characteristics. The samples were grouped into case and control groups. The case group consisted of Balinese patients with undifferentiated NPC. The control group was Balinese, patients of the OHNS Polyclinic with no NPC, no cancer, and no history of cancer in other organs. Specimen examination for DNA PCR was carried out at the Integrated Biomedical Laboratory Unit of the Faculty of Medicine, Universitas Udayana. The result was then sent to the laboratory of Genetika Science, Jakarta, for sequencing. This study has received ethical clearance from the Research Ethics Commission of the Faculty of Medicine, Universitas Udayana/Prof. dr. I.G.N.G. Ngoerah General Hospital, Denpasar, number: 2269/UU14.2.2.VII.14/LT/2021 dated August 18, 2021. Research results are presented in tables and narratives. The DNA samples were visualized using the electrophoresis technique and observation under an ultraviolet (UV) lamp (Figure 3). Shows that the result of alignment with the NCBI database is 99% similar.

### Study Characteristics

The results of the analysis of the case and control groups reveal the average age equation of both groups is 48 years old. Both groups also have the same proportion of male and female subjects. The dominant occupation in the control group is civil servants. As for the case groups, the majority work as farmers. Subjects who work as self-employed workers, university students, and garment workers are all in the control group. Subjects working as painters, construction workers, and housewives are in the case group. In the control group, most were diagnosed with chronic suppurative otitis media (CSOM), sinusitis, rhinosinusitis, and conductive hearing loss (CHL). In the case group, the majority of research subjects were diagnosed with NPC at stage III and IV. Table 1 shows characteristics of the subjects.

See Table 2 for the bivariate analysis of the relationship of risk factors for undifferentiated NPC among the Balinese population and the polymorphism of CYP1A1 rs4646903. In the case group, 26 or 83.9% experienced the CYP1A1 rs4646903 polymorphism, while in the control group, there are 16, or 51.6%. These results indicate that the CYP1A1 rs4646903 polymorphism is a risk factor for undifferentiated NPC among the Balinese population with an OR of 4.87, which is statistically significant.

### DISCUSSION

The CYP1A1 gene is a gene that produces an enzyme from the cytochrome P450 family 1 subfamily A member 1 (CYP1A1) that involves the metabolism of xenobiotics and environmental carcinogens. The polymorphism of CYP1A1 rs4646903 can affect the function of this enzyme, thereby influencing the risk and occurrence of NPC. Therefore, it is necessary to further research on the role of CYP1A1 rs4646903 polymorphism in undifferentiated NPC among the Balinese population.
Table 2. Relationship between the polymorphisms of CYP1A1 rs4646903 and undifferentiated NPC among the Balinese population.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (n=31)</td>
<td>Control (n=31)</td>
<td></td>
</tr>
<tr>
<td>CYP1A1 rs4646903</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, CC</td>
<td>26 (83.9%)</td>
<td>16 (51.6%)</td>
<td>4.87 (1.5-15.9)</td>
</tr>
<tr>
<td>TT</td>
<td>5 (16.1%)</td>
<td>15 (48.4%)</td>
<td></td>
</tr>
</tbody>
</table>

superfamily, this enzyme is not commonly found in the liver and is more often found in tissues outside the liver such as the lungs, placenta and gastrointestinal tract. The enzyme produced from the CYP1A1 gene generally works as a xenobiotic metabolizing enzyme in the placenta, as an aryl hydrocarbon hydroxylase, and plays a role in phase I liver metabolism. However, CYP1A1 also has an important role in turning procarcinogens into carcinogens, procarcinogen molecules that become substrates of These enzymes are polycyclic aromatic hydrocarbons (benzo[a]pyrene (B[a]P), heterocyclic aromatic amines/amides, and mycotoxins (aflatoxin B1 (AFB1))). One of the CYP1A1 substrates that is often found is Benzo[a]pyrene. When CYP1A1 meets B[a]P, two different reactions can occur, producing either B[a] P 7,8-epoxide or B[a]P 4,5-epoxide. B[a]P 7,8-epoxide then undergoes hydroxylation to become B[a]P 7,8-dihydrodiol with the help of the epoxide hydroxylase enzyme and here CYP1A1 again plays a role in converting B[a]P 7,8-dihydrodiol to B[a]P 7,8-dihydrodiol -9,10-epoxide which is carcinogenic, and one of the 4 isomers of 7,8-diol-9,10-epoxide which is carcinogenic. If this procarcinogen activation process is followed by the activity of the glutathione S-transferase enzyme, it can inactivate carcinogens or carcinogen intermediates formed by CYP1A1, the inactivation process that is carried out is conjugation with glutathione. Expression of the CYP1A1 rs4646903 polymorphism gene is also induced by PAHs by activating aryl hydrocarbon receptors (AhR)-dependent pathways. This creates a feedback loop that encourages more carcinogens to form. The mechanism described above is a function of the CYP1A1 enzyme which is produced from the wild type CYP1A1 gene, over time many polymorphisms of this gene are formed so that several aspects of the enzyme change, one of which is the inducibility of the CYP1A1 enzyme. When this enzyme can be induced more easily, the results of carcinogen intermediates and the carcinogen itself can increase, polycyclic aromatic hydrocarbon molecules are one of the molecules that can induce the activity of CYP1A1.

In this study, it was found that the CYP1A1 rs4646903 polymorphism was proven to be a risk factor for undifferentiated NPC in the Balinese. The polymorphism of CYP1A1 that has high inducibility is CYP1A1 rs4646903 (CYP1A1-MspI/CYP1A1*2A/T3801C) and because of the extra-hepatic site of action of this enzyme, it can be a candidate risk factor for nasopharyngeal carcinoma. A number of studies of CYP1A1 gene polymorphisms associated with NPC have been carried out. Studies conducted in other countries such as Algeria also showed similar findings, finding that the CYP1A1*2A gene polymorphism was associated with an increased risk of NPC in individuals who also had smoking habits by 1.41 times. This study states that this finding is present in smoking individuals who have at least one copy of the CYP1A1*2A mutant allele compared to the non-smoker population. This case-control study reported that there was a statistically significant association between the homozygous mutant CYP1A1*2C allele genotype polymorphism CYP1A1*2C (mt/mt) and an increased risk of NPC (OR=2.5, 95% CI [1.72-2.30]). Another study also reported a significant association between the combination of CYP1A1 and the risk for NPC. The study found that the combination of the CYP1A1 T3801 T+CC genotype and the GSTM1-null genotype has a significant association with the increased risk for nasopharyngeal cancer (p=0.001), in which the risk increased by 3.22 times. A study of 457 Cantonese families revealed that the CYP1A1 M2 polymorphism has a statistically significant association with the risk for nasopharyngeal cancer (p=0.045). A study concerning the risk factors for this gene polymorphism in north-east India found that this gene polymorphism had no significant effect in homozygous (ORs = 1.03; 95% CI = 0.48–2.21) and heterozygous (ORs = 1.44; 95% CI) individuals = 0.79–2.59). This study also observed a significant association in individuals with a genetic combination of GSTM1 null, GSTT1 null, and CYP1A2 T3801C with the TC + CC genotype with an increased risk of NPC by 5.71 times. Another study in the same area also found that the CYP1A1 rs4646903 polymorphism had a small effect on
NPC risk factors, both heterozygous (ORs = 1.08; 95% CI = 0.45 - 1.89) and homozygous (ORs = 0.74; 95% CI = 0.62 - 2.93). In contrast, patients who have the Glutathione S-Transferase Mu 1 (GSTM1) null genotype and have the CYP1A1 rs4646903 polymorphism have 2.76 times the risk of developing NPC (95% CI: 1.61 - 4.71; p < 0.0001). 33

**CONCLUSIONS**

The research found that the polymorphism of CYP1A1 rs4646903 proved to be a risk factor for undifferentiated NPC among the Balinese population.

**DISCLOSURES**

**Author Contribution**

All authors have contributed to this research process, including conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and collection and assembly of data.

**Funding**

None.

**Conflict of Interest**

The author declares there is no conflict of interest.

**Ethical Approval**

Ethical approval was granted by the Research Ethics Committee of Prof. dr. I.G.N.G. Ngoerah General Hospital with number 124/EC/KEPK-RSDK/2019.

**REFERENCES**