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

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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.

Keywords: Nasopharyngeal carcinoma, Balinese, Polymorphism, CYP2A6 gene

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1 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.

2. 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 paires 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  | %  |
|---------------------------------|-------|----|
| 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 2. The distribution of CYP2A6 gene genotype

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

The most common genotypes found were wild type homozygous (*1A/*1A) as many as 52 patients (65%). The mutant heterozygote group (*1A/*non 1A) were 2 patients (2.5%) and the mutant homozygote group (*non 1A/*non 1A) were 26 patients (32.5%).

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

| Clinical Stage | *1A/*1A | *1A/*non 1A | *non 1A/*non 1A | Total |
|----------------|--------|------------|----------------|-------|
| Early stage    | 14 (87.5%) | 1 (6.3%) | 1 (6.3%) | 16 (100%) |
| Advanced stage | 39 (60.9%) | 1 (1.6) | 1 (6.3%) | 41 (66.7%) |

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 genotypes 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  | Advanced |
|----------|--------|----------|
| CYP2A6*1A/ CYP2A6*1A | 14 (87.5%) | 39 (60.9%) |
| CYP2A6*1A/ CYP2A6*4C | 0 (0%) | 0 (0%) |
| CYP2A6*1B/ CYP2A6*4C | 0 (0%) | 24 (37.5%) |

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 | Advanced stage |
|----------|-------------|----------------|
| CYP2A6*1A/ CYP2A6*4C | 1 (6.3%) | 24 (37.5%) |
| 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  |
|----------|--------------|-----------|----|
| 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).
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 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 s

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 P4A50 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.

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