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Association Between VEGF-2578 Gene Polymorphism with Clinicopathological Appearance of Year 2021 Patients with Nasopharyngeal Cancer in RSUP Prof. Dr. I.G.N.G Ngoerah Denpasar Bali

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ABSTRACT

Cancer has a close association with aging. Nasopharyngeal cancer (NPC) is the most common head and neck cancer found in Indonesia. One of the factors that plays a role in the development of NPC is VEGF. VEGF is coded in VEGF gene which is very polymorphic. One of the polymorphisms in VEGF gene that influences the expression of the gene itself is VEGF-2578C/A gene polymorphism which has never been studied before in Indonesia. This research aims to study the association between VEGF-2578 gene polymorphism (clinical stadium, T stadium (tumor size), N stadium (local metastasis), M stadium (distant metastasis), and histopathological typing) of year 2021 patients with nasopharyngeal cancer in RSUP Prof. dr. I.G.N.G Ngoerah Denpasar Bali.

This research uses the cross-sectional method and was done in the Biomedical Laboratory in Udayana University Faculty of Medicine from June to December 2022. 43 samples were used in the form of DNA from stored biological specimens of nasopharyngeal cancer patients that were treated in ENT-Oncology polyclinic in RSUP Prof. dr. I.G.N.G Ngoerah in 2021. Secondary data was available from the Biomedical Laboratory in Udayana University Faculty of Medicine. To detect polymorphism from the DNA samples, gene amplification was done using PCR, confirmed using electrophoresis, and sent for sequencing in Genetika Science Jakarta.

From 43 samples, it was found that there were 36 (83,7%) CC genotip and 7 (16,3%) AA genotip. A significant association was found between VEGF-2578C/A gene polymorphism with T stadium (tumor size) with p value = 0,028. Late stage of T stadium (T3-T4) was found significantly more on subjects with CC genotip (75%) compared to AA genotip (28,6%). The effect of confounding variables which are age and gender have been minimalized. It was also found that there was no significant association between VEGF-2578C/A gene polymorphism with clinical stadium, N stadium, M stadium, or histopathological typing on the research subjects.

In conclusion, this research found that there was a significant association between VEGF-2578C/A gene polymorphism with T stadium, but not with clinical stadium, N stadium, M stadium, or histopathological typing of year 2021 patients with nasopharyngeal cancer in RSUP Prof. dr. I.G.N.G Ngoerah Denpasar Bali.

Keywords: Nasopharyngeal cancer, Polymorphism, Tumor size, VEGF-2578C/A, Clinicopathological appearance

INTRODUCTION

Aging and cancer are two biological phenomena closely related to each other, in which, cancer accounts for the highest cause

of death globally. There have been many types of cancer identified throughout the years. One type of cancer commonly found in Indonesia is nasopharyngeal carcinoma (NPC).

Nasopharyngeal carcinoma is a type of cancer originating from the epithelium of nasopharynx. NPC has a low incidence rate, totaling only 86.500 new case (0.6% of all cancer incidence) in the whole world annually. Interestingly, NPC has a demographic tendency towards the eastern part of hemisphere[i], with it being the 5th highest number of malignancies found in Indonesia numbering 19.943 cases (5% of all malignancies found in Indonesia) in the year 2020[ii]. The prognosis for NPC differs significantly based on the stadium of the disease, with early stadium of the disease having very high 5-year survival rate (85-90%) as compared to late stadium of the disease[iii]. One of the factors that play a role in the progression of NPC is vascular endothelial growth factor (VEGF).

Vascular endothelial growth factor is an angiogenic growth factor that binds to heparin and has a high specificity towards endothelial cells. It plays an important role in angiogenesis whether physiologically or pathologically[iv]. Vascular endothelial growth factor is coded in VEGF gene, which is located in chromosome 6p21 and is a very polymorphic gene with more than 30 SNPs identified. One of the SNPs that has been identified to have an effect in the expression of VEGF gene is VEGF-2578C/A polymorphism which is located in the promoter region of the gene[v].

It is believed that CC genotype of VEGF-2578C/A polymorphism will have an increase in NPC progression compared to A-allele carriers which can be seen through the clinicopathological appearance of the disease including overall stadium, tumor size (T stadium), nodal metastasis (N stadium), distant metastasis (M stadium), and histopathological type of the disease[vi].

MATERIALS & METHODS

The study is carried in the Comprehensive Biomedical Laboratory, in The Medical Faculty of University of Udayana, Denpasar, Bali. Stored biological specimens in the form of DNA of 43 subjects with a mean age of 48,33 years were used in this study. Total random sampling was used as the sampling method. Subjects' DNA were amplified using PCR, then confirmation of the results was done with electrophoresis. The samples were then sent for gene sequencing in Genetika Science Jakarta with reverse primer due to the high amount of noise when using forward primer. Hence, the resulting gene sequence must be converted to its complementary base pair.

Secondary data of age, gender, and clinicopathological appearance which includes overall stadium, T, N, and M stadium, and histopathological typing were collected and tabulated in SPSS. Age and gender were used as confounding variables, and data analysis was done using Chi-Square and Fischer's Exact test.

RESULT

From 43 subjects, it was found that the majority of the subject has the CC genotype (83,7%) as compared to the AA genotype (16,3%). There was no heterozygote polymorphic variant found in this research. There is a significant association found between VEGF-2578C/A polymorphisms and T stadium of the subjects, with no significant association found between VEGF-2578C/A polymorphisms and overall stadium, N stadium, M stadium, or histopathological typing.

DISCUSSION

In this study, the majority of the population is male (76,7%) which is in accordance with previous research that found that male is more prone to NPCs as compared to female[vii]. However, risk could not be calculated due to the nature of this study. Non-keratinizing squamous cell carcinoma (SCC) was also found in 86% of the whole population, which is in accordance with

previous study where area that is endemic in NPCs such as Eastern Asia and Southeastern Asia has a higher proportion of non-keratinizing SCC as compared to other types of NPCs[viii].

Tumor size (measured by T stadium) was also found to be associated with VEGF-2578C/A polymorphism, with subjects that have a larger tumor size (T3-T4) are more likely to be found in CC genotype (75%) as compared to AA genotype (28,6%). This finding is in accordance with the previous study, where CC genotype was found to be closely associated with an increase in stadium and larger tumor size compared to A-allele carriers[vi]. Although the mechanism of how VEGF-2578C/A polymorphism could be associated with tumor size is not completely known, but it can be inferred that subjects with A-allele are more likely to have a reduced in VEGF gene expression due to its location in the promoter region. It was proposed that because VEGF-2578 is located in the GATA-2 transcription factor binding site, A-allele carriers are more likely to have a reduced specificity towards GATA-2 transcription factor, hence resulting in the reduced expression of VEGF gene as compared to the CC genotype with relatively higher specificity towards GATA-2 transcription factor[ix]. Control of confounding variables which are age and gender was also done with no association found between the confounding variables and tumor size.

In contrast, there was no significant association found between VEGF-2578C/A polymorphisms with other clinicopathological appearance such as overall stadium, N stadium, M stadium, and histopathological typing. Overall stadium of the disease is closely related to the T, N, and M stadium; hence the result might be inaccurate as the majority of the people in this study is in the IV stadium (65,1%) as compared to II and III stadium (34,9%). The IV stadium is closely related to the N and M stadium, and while VEGF plays a direct role in increasing tumor size due to its angiogenetic nature, it does not play a direct role in metastasis, whether it is local or distant[x], hence an association between VEGF-2578C/A gene polymorphism and overall stadium, N stadium, and M stadium were not found.

Although the mechanism was not known, histopathological typing was found in previous study to be associated with VEGF-2578 gene polymorphism[vi]. In this study, contrasting result was produced, where there was no significant association between histopathological typing and VEGF-2578 gene polymorphism. This result might be due to the proportion of some groups having very low count; hence it might affect the result of this study.

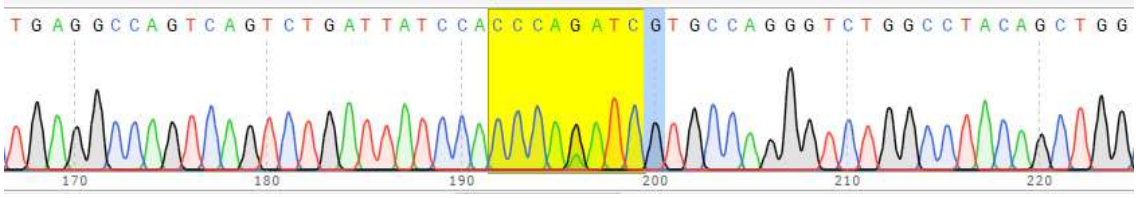

Figure 1. Gene Sequencing Result with Reverse Primer Showing GG Base Pair (highlighted blue)

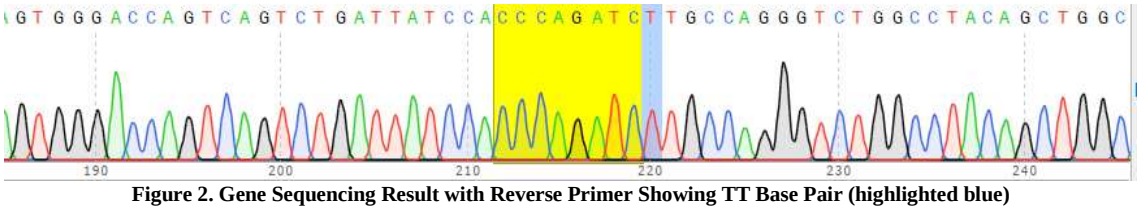

Figure 2. Gene Sequencing Result with Reverse Primer Showing TT Base Pair (highlighted blue)

Table 1. Bivariate Analysis of The Association between VEGF-2578C/A Gene Polymorphism and Clinicopathological Appearance of NPC Patients

Variable	VEGF-2578C/A Gene Polymorphism		p value
	AA n (%)	CC n (%)	
Stadium			1,000
IV	5 (71,4)	23 (63,9)	
II dan III	2 (28,6)	13 (36,1)	
Tumor size (T)			0,028*
T3-T4	2 (28,6)	27 (75)	
T1-T2	5 (71,4)	9 (25)	
Regional lymph node metastasis (N)			0,315
N3	0 (0)	8 (22,2)	
N1-N2	7 (100)	28 (77,8)	
Distant metastasis (M)			0,685
Yes	5 (71,4)	21 (58,3)	
No	2 (28,6)	15 (41,7)	
Histopathological typing			1,000
Non-keratinizing SCC	6 (85,7)	31 (86,1)	
Other types	1 (14,3)	5 (13,9)	

***: All analytical result is done with 5% type I margin of error**

CONCLUSION

There is a significant association between VEGF-2578C/A gene polymorphism and tumor size (T stadium) in patients with NPC with CC genotype having higher proportions of subjects with larger tumor size (T3-T4) as compared to AA genotype. The effect of confounding variables such as age and gender have been minimalized. No significant association was found between VEGF-2578C/A gene polymorphism and overall stadium, N stadium, M stadium, or histopathological typing. Further research is needed with different study method such as case control or cohort to increase the validity of these findings.

Declaration by Authors

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