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Association Between BsmI Vitamin D Receptor Gene Polymorphism and Clinicopathological Features of Colorectal Cancer Patients in 2019-2020 at Prof. Dr. I.G.N.G Ngoerah Denpasar Bali

Nadia Carolina Notoprawiro[1], Ni Made Desak Wihandani[2], I Wayan Juli Sumadi[3], Ni Nyoman Ayu Dewi[2]

[1]Magister Program Anti-Aging Medicine, Faculty of Medicine, Udayana University, Bali, Indonesia
[2]Department of Biochemical, Faculty of Medicine, Udayana University, Bali, Indonesia
[3]Department of Pathological Anatomy, Faculty of Medicine, Udayana University, Bali, Indonesia

Corresponding Author: Nadia Carolina Notoprawiro

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ABSTRACT

Aging is related to the occurrence of cancer. Colorectal cancer is one of the most common types of cancer found in Indonesia. The *BsmI* vitamin D receptor polymorphism has been extensively studied before and has been associated with colorectal cancer. This study aims to determine the relationship between the *BsmI* vitamin D receptor polymorphism and the clinicopathological features of colorectal cancer patients in 2019-2020 at Prof. dr. I.G.N.G Ngoerah Denpasar Bali.

This research is a cross-sectional analytical research. The study was conducted at the Biomedical Laboratory Unit of the Faculty of Medicine, Udayana University from June to December 2022. A total of 35 samples were obtained from stored biological materials of colorectal cancer patients who were treated at the Digestive Surgery Polyclinic at Prof. RSUP. dr. I.G.N.G Ngoerah in 2019-2020 in the Udayana University Biochemistry laboratory. To detect the status of the *BsmI* polymorphism, DNA segments were amplified by the polymerase chain reaction (PCR) method and followed by sequencing.

The results showed from 35 samples, 8 samples (22.9%) had the GG allele and 27 samples (77.1%) had the GA allele. A statistically significant association was found between *BsmI* vitamin D receptor polymorphisms and colorectal cancer grading (p=0.016), but no significant association was found between *BsmI* vitamin D receptor polymorphisms and the histopathological type of colorectal cancer (p=0.553).

The conclusion of this study is that there is a significant relationship between *BsmI* vitamin D receptor polymorphism and grading, but not with the histopathological type of colorectal cancer, age and gender of colorectal cancer patients in 2019-2020 at Prof. dr. I.G.N.G Ngoerah Denpasar Bali.

Keywords: Colorectal cancer, Vitamin D receptor, *BsmI* polymorphism, clinicopathology

INTRODUCTION

Cancer is one of the main causes of death in the world. Cancer incidence is affected by increasing age. Colorectal cancer is known to rank third with a percentage of 10% of the total new cases of cancer worldwide after breast cancer and lung cancer. Meanwhile, in Indonesia, colorectal cancer incidence ranks fourth with a percentage of 8.6%[1] of all malignancies found.

Vitamin D is one of the micronutrients needed for body health. Vitamin D plays a role in many ways, including reducing the risk of chronic diseases such as cancer, cardiovascular, neurological, autoimmune and others[2]. Previous studies have shown

that vitamin D deficiency increases the risk of colorectal cancer. Epidemiological data has shown that 25(OH) D2 levels are inversely related to the risk of colorectal cancer and vitamin D deficiency is associated with colorectal cancer mortality[3]. Vitamin D in its active form binds to its receptor, the vitamin D receptor[4]. Abnormalities in the VDR are thought to affect this function. The VDR gene polymorphism causes changes in the activity of the receptor and vitamin D responses[5]. The GG genotype in the BsmI VDR gene shows an association to the occurrence and development of colorectal cancer[6,7], while the AA genotype has protective properties against colorectal cancer[8]. Research on the VDR gene polymorphism and its relation to colorectal cancer is still varied because different conclusions were obtained from different studies. This study aims to determine the relationship between the *BsmI* vitamin D receptor polymorphism and the clinicopathological features of colorectal cancer patients in 2019-2020 at Prof. dr. I.G.N.G Ngoerah Denpasar Bali.

MATERIALS & METHODS

The research was conducted at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University. Research was conducted in July-December 2022 from making the proposal to preparing research reports. This study used a total sampling of 35 samples. The sample used is stored biological material (BBT), which is a DNA sample of colorectal cancer patients at the Biochemistry Laboratory of Udayana Faculty of Medicine. Data related to clinicopathological characteristics were recorded on a data collection sheet. DNA from the sample was isolated and PCR was performed to amplify the gene. Sequencing was performed at Genetica Science, Jakarta to determine the base sequence of the gene.

STATISTICAL ANALYSIS

The data analysis was carried out using SPSS 25.0

RESULT

As shown in table 1, the average age of all subjects was 60.83 ± 11.08 years with more than half of the subjects are female (57.1%). Colorectal cancer with the histopathological type adenocarcinoma NOS dominated the sample in the study as many as 31 (88.6%) and 4 (11.4%) samples were of the type mucinous adenocarcinoma. From 35 subject, 29 (82.9%) samples have low grade whilst the rest belong to high grade. In terms of tumor location, 62.9% of subjects had cancer located in the rectum and the others spread to the ascending, descending, transverse, and rectosigmoid junction of the colon.

Table 1. Characteristic of the colorectal cancer samples

Variable	Value
Age	60,83±11,08
<60	16 (45,7%)
≥60	19 (54,3%)
Sex	
Female	20 (57,1%)
Male	15 (42,9%)
Location	
Caecum	3 (8,6%)
Ascending Colon	4 (11,4%)
Transversum Colon	1 (2,9%)
Descending Colon	3 (8,6%)
Rectosigmoid junction	2 (5,7%)
Rectum	22 (62,9%)
Grade	
High	6 (17,1%)
Low	29 (82,9%)
Histopathologic Type	
Adenocarcinoma NOS	31 (88,6%)
Mucinous Carcinoma	4 (11,4%)

To detect the type of genotype variant in the sample, electrophoresis and PCR procedures were performed followed by sequencing. The amplicon DNA fragment formed was 215 bp. Blast results consistently show that the amplicon DNA fragment is part of the VDR gene. Polymorphism detection carried out using the Snapgene application (Figure 1). The sequencing results found that the frequency of the GG genotype was 8 samples (22.9%) and the GA genotype was 27 samples (77.1%) as shown in Table 2.

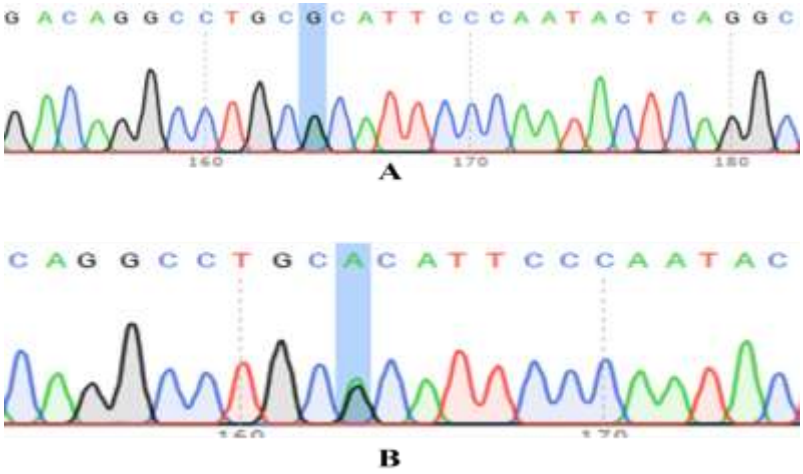

Figure 1. A. Gene sequencing showed GG alel (highlighted blue); B. Gene sequencing showed double peak in GA alel (highlighted blue)

Table 2. The frequency of BsmI polymorphism in colorectal cancer patient

BsmI Sequencing	N= 35 n (%)
(Heterozigot Polimorfism GA)	27 (77,1%)
(Homozigot Wildtype GG)	8 (22,9%)

The results of the Bivariate analysis using Chi-square in table 3 showed that there was a significant association between the BsmI VDR gene polymorphism and colorectal cancer grade ($p < 0.05$), but not with the histopathological type, colorectal cancer location, age and sex of colorectal cancer patients ($p > 0.05$).

Table 3. Bivariate analysis between VDR BsmI Polymorphism and Clinicopathology of the Colorectal Cancer

Variable	VDR BsmI Polymorphism		P Value
	GG	GA	
Grade			**0,016***
High	4	2	
Low	4	25	
Histopathologic Type			**0,553**
Adenocarcinoma NOS	8	23	
Mucinous Adenocarcinoma	0	4	
Location	6	21	1,000
left	2	6	
right			
Age	3	13	
<60	5	14	0,700
≥60			
Sex			
Female	3	17	0,246
Male	5	10	

DISCUSSION

In this study, the average age of all subjects was 60.83 ± 11.08 years with 57.1% female predominance. Similar to previous research by Dwijayanthi[9] colorectal cancer in Bali is also dominated by female (92.3%) as compared to male. On the contrary, global data shows that the prevalence of colorectal cancer is dominated by male[10].

Colorectal cancer grade characteristic shows a low grade in most of the study samples. While the histopathological typing found in this study shows a large frequency of Adenocarcinoma NOS, as much as 88.6% as compared to other types. In line with previous studies where most incidence of colorectal cancer was of the NOS Adenocarcinoma type[11]. In terms of tumor location, 62.9% of subjects had cancer located in the rectum whilst the remainder spread to the ascending, descending, transverse, and rectosigmoid junction of the colon.

In this study, the frequency of GA genotype in the BsmI polymorphism was 77%. This frequency is higher compared to the other study who found that the frequency of individuals with BsmI genotype AA and GA polymorphisms was 13% and 39% respectively[12]. In line with previous study, the population in this study was dominated by the GA genotype as compared to the GG genotype (22.9%). Previous study by Pan et al.[13] showed that BsmI was significantly associated with colorectal cancer risk in a meta-analysis that included 39 case-control studies. The hazard ratio of the A allele was reported to be 0.862 which indicated the inhibition of disease progression as

compared to the GG genotype (p = 0.019). Furthermore, a study by Alkhayal et al.[14] also showed a significant relationship between BsmI and ApaI with the risk of colorectal cancer in Arabian. Other researches showed that BsmI polymorphism independently and significantly increased the risk of colorectal cancer[15].

From 35 subject, the GA genotype showed a significant association with the grade of colorectal cancer and was found to be more common in low grade carcinoma. Previous meta-analysis study showed a significant association between reduced risk of colorectal cancer with A-allele carriers. This proves that the A allele in polymorphism has a protective effect on the progression of colorectal cancer. The BsmI polymorphism did not affect amino acid changes, did not affect mRNA, VDR protein levels or active vitamin D levels. However, several meta-analytical studies demonstrated a strong linkage disequilibrium with other VDR polymorphisms including ApaI, TaqI, and microsatellite Poly(A). Based on this evidence, it shows that the BsmI polymorphism affects several biological functions and is most likely related to mRNA stability, regulation of VDR transcription, translation, and RNA formation[7].

On the other hand, patients with the G allele of BsmI are known to have lower blood vitamin D levels and lower receptor activity, which may underlie the pathogenesis of the increased risk of cancer in populations with G alleles[16]. Other studies have shown that the association of the G allele in the BsmI VDR gene increases the expression of the erbB-2 gene, which is a protooncogene. Hence, G allele carriers have an increased risk and development of colorectal cancer The result of this study also showed that there was no significant relationship between the BsmI polymorphism and the histopathological type of colorectal cancer, age and sex of colorectal cancer patients. In line with previous studies where the stage and histopathological type were not associated with the BsmI VDR polymorphism or other VDR polymorphisms[7]. Whereas the absence of a relationship between location, age, sex of colorectal cancer patients and the BsmI VDR gene polymorphism is not known with certainty because there have been no similar studies previously and the BsmI VDR gene polymorphism is inherited randomly in both males and females and is not affected by increasing age.

Further research needs to be carried out with a more comprehensive design and with the addition of variables in the form of vitamin D levels and external factors to increase the validity of the research findings.

CONCLUSION

The majority of the research subjects had the GA genotype. There was no AA genotype in the research samples examined. There is a significant association between the BsmI VDR gene polymorphism and grade, but not the histopathological type of colorectal cancer, age and sex of colorectal cancer patients in 2019-2020 at Prof. Hospital. dr. I.G.N.G Ngoerah Denpasar Bali

Declaration by Authors

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