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Association between VEGF Gene Polymorphism -634G>C and Risk of Colorectal Cancer

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Abstract

BACKGROUND: Genetic and environmental factors play an important role in the pathogenesis of colorectal cancer. Angiogenesis is a central process in carcinogenesis and is affected by vascular endothelial growth factor (VEGF). Several genetic variations, such as polymorphism, may alter VEGF expression and influence the risk of colorectal cancer.

AIM: The objective of this study was to determine the association between VEGF gene polymorphism -634G>C and risk of colorectal cancer.

METHODS: A cross-sectional study was conducted at Haji Adam Malik general hospital and its sister hospitals. Subjects were obtained by consecutive sampling. Inclusion criteria for case and control groups were patients with colorectal cancer and healthy subject, respectively, aged 18 years or older and willing to participate in the study. Exclusion criteria were patients with systemic comorbidities or malignancies in other organs. Each subject undergoes an interview, colonoscopy, biopsy, serum VEGF level measurement, and VEGF polymorphism -634G>C evaluation.

RESULTS: Eighty subjects were enrolled and distributed into case and control groups. Males were dominant in both groups, with a mean age of older than 55 years. Most lesions were in the rectum and 45% of subjects had moderately differentiated cancer. The median serum VEGF level in the case group was higher than the control group (2,175.1 pg/mL vs. 253 pg/mL; $p < 0.001$). VEGF gene polymorphism -634G > C was associated with the risk of colorectal cancer ($p = 0.048$). GG and GC genotypes had 1.89 times higher risk of colorectal cancer compared to the CC genotype. GG genotype and G allele had the highest serum VEGF levels.

CONCLUSION: There is an association between VEGF gene polymorphism -634G>C and risk of colorectal cancer in this study.

Introduction

Colorectal cancer is the third most common malignancy worldwide [1] and the most common malignancy in the digestive tract [2]. It is also the second most frequent malignancy-related cause of death in both developed and developing countries and is predicted to surpass mortality from cardiovascular diseases in the future [2], [3], [4]. The incidence of colorectal cancer in the United States in 2015 was 132,700 cases, comprising 8% of total malignancies cases. A total of 49,700 deaths were reported, giving a mortality rate of 8.1/100,000 population [5]. In Indonesia, colorectal cancer was the 4th most prevalent disease in 2020 with 34,189 cases, accounting for 8.6% of total malignancies in the country [6]. There is a shift in the incidence of colorectal cancer from elderly to young adults. Approximately 7% of colorectal cancer incidence occurred in patients younger than 40 years old [7]. A higher rate (over 30%) was even reported in Indonesia [8]. This will give a negative impact on the future economy [9].

Genetic and environmental factors play an important role in the pathogenesis of colorectal cancer. The risk for colorectal cancer doubles in subjects with a first-degree family history of colorectal cancer. The risk increases if the family member had colorectal cancer before the age of 50 and if over one family member is suffering from colorectal cancer [10]. Genetic predispositions which run in the family trigger initial carcinogenesis from normal colonic epithelium to the premalignant lesion and invasive carcinoma. This process takes 10–15 years [11], [12]. Angiogenesis plays an important role in the process since cancer needs new vascularization to grow. New vascularization nurtures the cancer cells and facilitates distant metastases [13], [14].

Vascular endothelial growth factor (VEGF) is an important pro-angiogenic factor. Most solid tumors secrete VEGF [13], [14]. VEGF stimulates the proliferation and migration of endothelial cells and increases vascular permeability [13], [15]. High serum VEGF level is associated with higher incidence and worsened the outcome of colorectal cancer [16]. VEGF gene is located in chromosome 6p21.3 which consists

of eight exons. The gene is highly polymorphic and tends to undergo genetic variations. Single nucleotide polymorphism (SNP) is the most common genetic variation where the sequence of a single nucleotide is rearranged and inherited. SNPs in the VEGF gene are associated with various malignancies [17], [18]. VEGF gene polymorphism -634G>C has been reported to be associated with several malignancies. VEGF gene polymorphism -634C>C may increase VEGF level thus increasing the risk and worsening the outcome of cancers [19], [20]. However, the study regarding VEGF gene polymorphism -634G>C is very limited, particularly in Indonesia. This study aimed to determine the association between VEGF gene polymorphism -634G>C and the risk of colorectal cancer.

Methods

This was a cross-sectional study conducted at Haji Adam Malik general hospital and its sister hospitals in Medan, North Sumatera, Indonesia. Subjects were obtained by consecutive sampling method. Inclusion criteria for the case group were patients with colorectal cancer aged 18 years or older and willing to participate in the study, while the control group was healthy subjects aged 18 years or older. Exclusion criteria were patients with systemic comorbidities such as type 2 diabetes mellitus, hypertension, coronary heart disease, heart failure, kidney failure, and other malignancies, patients with metastatic colorectal cancer, and pregnant patients.

Each subject undergoes an interview to obtain demographic data. Colonoscopy and biopsy were conducted to diagnose colorectal cancer. Five biopsy samples were taken from suspected colonic lesions. Samples were fixated with a formalin buffer of 10% and sent to the Pathology Department of Medical Faculty of Universitas Sumatera Utara for a histopathology examination. Serum VEGF level was measured with enzyme-linked immunosorbent assay (ELISA) method using Quantikine ELISA Human VEGF Immunoassay (R&D System Inc., Minneapolis, USA). The sample for ELISA was peripheral blood which was treated according to factory protocol. VEGF gene polymorphism -634G>C was evaluated using deoxyribonucleic acid (DNA) extraction with a High Pure PCR Template Preparation Kit (Roche Applied Science, Penzberg, Germany). The sample for DNA extraction was peripheral whole blood. The extraction process followed the protocol from the manufacturing company.

Qualitative data was presented in frequency and percentage, while quantitative data undergo normality tests. Normally distributed data were presented in mean and standard deviation, but non-normally-distributed one was presented in the median and minimum-maximum value. To determine the association between VEGF gene polymorphism -634G>C and risk of colorectal cancer, a Chi-square test was utilized. Kruskal–Wallis test was used to analyze the difference in serum VEGF level among genotypes and alleles from VEGF gene polymorphism -634G>C. Mann–Whitney U-test was used to determine the difference in serum VEGF level between case and control groups. All statistical analyses were done with a 95% confidence interval and $p < 0.05$ was considered significant. Statistical Package for the Social Sciences software was utilized to support the analysis.

Results

A total of 80 subjects were enrolled in this study. All subjects were distributed into case and control groups equally. Males were dominant in both groups with a mean age of older than 55 years. Most subjects were from Batak ethnic due to the location of the study. In the case group, most lesions were located in the rectum, and from histopathology examination, 45% of subjects had moderately differentiated cancer. There was no association between gender and ethnicity with the risk of colorectal cancer (Table 1).

Table 1: Demographic and clinical characteristics of subjects

Characteristics	Group		p	PR (95% CI)
	Case	Control		
Gender, n (%)				
Male	26 (65.0)	22 (55.0)	0.361	1.24 (0.77–1.98)
Female	14 (35.0)	18 (45.0)		
Mean age, year (SD)	58.43 (9.64)	57.15 (10.22)	0.568	NA
Ethnic, n (%)				
Batak	25 (62.5)	20 (50.0)	0.260	1.296
Non-Batak	15 (37.5)	20 (50.0)		(0.82-2.06)
Location of lesion, n (%)				
Proximal colon	9 (22.5)	NA	NA	NA
Distal colon	14 (35)			
Rectum	17 (42.5)			
Histopathology result, n (%)				
Well-differentiated	12 (30.0)	NA	NA	NA
Moderately differentiated	18 (45.0)			
Poorly differentiated	10 (25.0)			

PR: Prevalence ratio; CI: Confidence interval; SD: Standard deviation; NA: Not available.

The median serum VEGF level in the case group was 2,175.1 pg/mL, while the control group was 253 pg/mL. Based on Mann–Whitney U-test, there was a significant difference in serum VEGF levels between case and control groups ($p < 0.001$). VEGF gene polymorphism -634G>C was significantly associated with the risk of colorectal cancer, particularly GG and GC genotypes compared to the CC genotype. Subjects with GG and GC genotypes had 1.89 times higher risk of contracting colorectal cancer compared to subjects with CC genotype (Table 2).

Further, analysis showed that there was a significant difference in serum VEGF level between genotypes and alleles of VEGF gene polymorphism -634G>C. Kruskal–Wallis test showed that GG genotype had significantly higher serum

Table 2: Association between VEGF polymorphism -634G>C and risk of colorectal cancer

VEGF polymorphism -634G>C	Group, n (%)		p	PR (95%CI)
	Case	Control		
GG	11 (55.0)	9 (45.0)	0.160	NA
GC	24 (55.8)	19 (44.2)		
CC	5 (29.4)	12 (70.6)		
GG+GC	35 (55.6)	28 (44.4)	0.048*	1.89
CC	5 (29.4)	12 (70.6)		(1.28–3.08)
GG	11 (55.0)	9 (45.0)	0.606	1.14
GC+CC	29 (48.3)	31 (51.7)		(0.71–1.83)
G allele	46 (54.8)	38 (45.2)	0.205	1.22
C allele	34 (44.7)	42 (55.3)		(0.89–1.68)

PR: Prevalence ratio; CI: Confidence interval; NA: Not available; *p < 0.05.

VEGF level among all genotypes (p = 0.043), while G allele had higher serum VEGF level compared to C allele (p = 0.034) (Table 3).

Table 3: The difference in serum VEGF level among genotypes and alleles of VEGF polymorphism -634G>C

VEGF polymorphism -634G>C	Median serum VEGF level, pg/ml (min-max)	p
GG genotype	1,579.2 (80.7–3,441.7)[#]	0.043*
GC genotype	737.7 (100.4–3,771)	
CC genotype	310.0 (134.5–3,554.2)	
G allele	1,322.5 (80.7–3,771.0)	0.034*
C allele	405.0 (100.4–3,771.0)	

#Significant compared to CC genotype; *p < 0.05.

Discussion

The incidence of colorectal cancer is higher in males compared to females. Disease outcome is also more favorable in females aged 18–44 years compared to males with corresponding ages [21]. White *et al*., in their study, found that males have a higher incidence and earlier onset of colorectal cancer. They suggested that this condition was due to purely endogen factors [22]. A study from Canada reported that the incidence of colorectal cancer is decreasing but still dominated by males [23]. The presence of estrogen is hypothesized to be a protective factor from colorectal cancer [21], [22]. Our study's result is in line with the previous literature, with males dominating the case group (65.0%).

The incidence of colorectal cancer is increasing with age, but its outcome is inversely related to age. At 50 years or older, the disease outcome is comparable between all genders [21]. After the age of 65, the outcome for females is poorer than four males [22]. Another literature stated that most colorectal cancer cases are diagnosed between the age of 50 and 79 [24]. Recently, there is a shift in colorectal cancer incidence in the younger population [25], [26]. Approximately 9% of newly diagnosed cases occurred in the population under 50 years of age [26]. Cases that occurred before the age of 50 have worse symptoms, but still better outcomes compared to those that occurred later [24], [25], [27]. Our study also found that the mean age of subjects in the case group was 58.43 years, which is by previous literature.

Previous studies reported an association between ethnicity and the incidence of colorectal cancer. Moore *et al*. found that dark-skinned ethnicities have a higher risk for earlier onset colorectal cancer compared to other ethnicities. Conversely, Hispanics suffer from colorectal cancer at a more advanced age [25]. However, Hispanics had earlier ones with the disease compared to Caucasians [27]. Ellis *et al*. also found that dark-skinned ethnic has the highest incidence rate of colorectal cancer. The incidence of disease in Southeast Asia is also increasing [26]. Confirming previous findings, Ollberding *et al*. reported that African-Americans have a higher risk for colorectal cancer compared to Caucasians [28]. In this study, Batak ethnic dominated the case group. This was influenced by the location of the study. There was no significant difference in the incidence of colorectal cancer based on ethnicity in this study.

The primary lesion of colorectal cancer can be found from the proximal colon to the rectum. Based on a study by Loree *et al*., the most common site of the primary lesion was the distal colon (45%), followed by the proximal colon (32%) and rectum (23%) [29]. A retrospective study conducted by Siegel *et al*. also showed that the distal colon is the most common site for primary colorectal cancer lesions. Location of the primary lesion is associated with disease outcome. Lesion located in the distal colon tends to have a better outcome compared to the proximal one [4]. In contrast, our study result was different from previous studies. The most common site for a primary lesion in this study was the rectum (42.5%), followed by distal (35%) and proximal colon (22.5%).

VEGF is an important growth factor in angiogenesis [30], [31]. Serum VEGF level is significantly higher in patients with colorectal cancer compared to healthy subjects. Several factors such as geographic location and ethnicities affect the serum VEGF level [32]. Celen *et al*. studied serum VEGF and carcinoembryonic antigen (CEA) levels in colorectal cancer patients and healthy controls. Their results showed that serum VEGF and CEA levels are higher in colorectal cancer patients and even higher in patients with progressive disease. They also mentioned that serum VEGF level is more sensitive in diagnosing colorectal cancer compared to serum CEA level [33]. The study is confirmed by Werther *et al*., but they used plasma instead of serum VEGF level [34]. Another study stated that increased expression of VEGF is positively associated with the severity of colorectal cancer and serum interleukin (IL)-23 levels [35]. Serum VEGF level is also associated with therapeutic response in colorectal cancer. Decreased serum VEGF level is in line with a positive response toward chemotherapy and a more favorable outcome [36]. Chen *et al*. also reported that high VEGF expression will hamper patients' response toward chemotherapy and impair survival [37]. A similar phenomenon is observed in operation procedures for colorectal cancer management. Post-operative serum VEGF level decrease is associated with better disease outcomes [34]. Our result was in conjunction

with previous literature. Statistically, there was a higher serum VEGF level in the case group compared to the control group ($p < 0.001$).

Polymorphism the in VEGF gene is hypothesized to be associated with elevated expression of VEGF, increased angiogenesis activity, and higher risk for colorectal cancer. Januzzi *et al*. reported that VEGF gene polymorphism -2578A>C is related to the risk of colorectal cancer. Subjects with the A allele had 1.81 times higher risk for colorectal cancer compared to subjects with the C allele [38]. Wang *et al.* in their meta-analysis found similar results. The presence of A allele and AA genotype from polymorphism -2578C>A in VEGF gene increased the risk for colorectal cancer compared to C allele and CA and CC genotypes. Unfortunately, the study was conducted only on Caucasians [39]. Different findings were submitted by Park *et al*. They denied the association between VEGF gene polymorphism -2578C>A and risk of colorectal cancer. In addition, the presence of the A allele even played a role as a protective factor for colorectal cancer [40].

In Iran, VEGF gene polymorphism rs833061T/C was reported to be associated with the risk of colorectal cancer. TG genotype holds a higher risk compared to TT and GG genotypes [41]. Furthermore, in Iran, VEGF gene polymorphism +936C>T acted as a protective factor for colorectal cancer, particularly T allele and TT genotype [42]. VEGF gene polymorphism -460T>C increased VEGF expression and was correlated with the incidence of colorectal cancer. TC and CC genotypes were the associated risk factor according to Chen *et al*. [37]. Another study reported contradictive results. Yang *et al*. did not find any significant relationship between VEGF gene polymorphisms rs3025039C/T, rs3025040C/T, rs3025053G/A, and rs10434A/G with risk of colorectal cancer in the Han ethnic [43].

There is no published study regarding the association between VEGF gene polymorphism -634G>C and risk of colorectal cancer yet. From our study, subjects with GG and GC genotypes had 1.89 times higher risk of colorectal cancer compared to those with CC genotype. This was associated with increased serum VEGF levels in the corresponding genotypes. However, there was no significant association between alleles and the risk of colorectal cancer in this study.

Our study gives crucial information regarding the possibility of VEGF gene polymorphism -634G>C utilization as a predictor for incidence of colorectal cancer besides previously studied polymorphisms. The limitation of our study lies in the relatively small sample size and homogenous ethnicity. Further, the study is mandatory to elaborate the role of VEGF gene polymorphism -634G > C in evaluating therapeutic response and to determine factors affecting the polymorphism in subjects with colorectal cancer.

Conclusion

A statistically significant association is observed between VEGF gene polymorphism -634G>C and the risk of colorectal cancer. Subjects with GG and GC genotypes have a higher risk for contracting disease compared to subjects with CC genotypes. This is due to increased serum VEGF levels in GG and GC genotypes.

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