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.

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.

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) |
|----------------------------------|-------------|-------|-------------|
| Gender, n (%)                    |             |       |             |
| Male                             | 26 (65.0)   | 22 (55.0) | 0.361 | 1.24 (0.77–1.98) |
| Female                           | 14 (35.0)   | 18 (45.0) | 0.568 | NA |
| Mean age, year (SD)              | 56.43 (8.64) | 57.15 (10.22) |       |               |
| Ethnic, n (%)                    |             |       |             |
| Batak                           | 25 (62.5)   | 20 (50.0) | 0.260 | 1.296 (0.82–2.06) |
| Non-Batak                        | 15 (37.5)   | 20 (50.0) |       |               |
| Location of lesion, n (%)        |             |       |             |
| Proximal colon                   | 9 (22.5)    | NA    | NA          |
| Distal colon                     | 14 (35)     | NA    | NA          |
| Rectum                           | 17 (42.5)   | NA    | NA          |
| Histopathology result, n (%)     |             |       |             |
| Well-differentiated              | 12 (30.0)   | NA    | NA          |
| Moderately differentiated        | 16 (40.0)   | NA    | NA          |
| Poorly differentiated            | 10 (25.0)   | NA    | NA          |

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)      |
|---------------------------|-------------------|------|-----------------|
| GG                        | 11 (55.5)         | 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            |
| CG                        | 5 (29.4)          | 12 (70.6) | 1.28–3.08 |                 |
| GC                        | 11 (55.0)         | 9 (45.0) | 0.606 | 1.14            |
| GG+CC                     | 29 (48.3)         | 31 (51.7) | 0.71–1.83 |                 |
| G allele                  | 46 (54.6)         | 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               | 1,759.2 (80.7–3,441.7)                   | 0.043*|
| GC genotype               | 737.7 (100.4–3,771)                      |      |
| CC genotype               | 310.0 (134.5–3,584.2)                    |      |
| 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 studies.
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 had 1.89 times higher risk of colorectal cancer compared to those with CC genotype. This was observed between VEGF gene polymorphism -634G>C and 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.

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 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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