Association Between VEGF + 936 C> T Gene Polymorphism with Degrees of Neutrophils and Lymphocytes Infiltration in Gastritis

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Abstract

Introduction: Activation of angiogenesis stimulated by Vascular endothelial growth factor (VEGF) in host cells play a role in response to damaged gastric mucosal in gastritis patient with Helicobacter pylori (*H. pylori*) infection. The study showed that presence of polymorphisms in VEGF gene is associated with an increased risk of several disorders like gastric cancer. Infiltration of neutrophils in the gastric mucosa characterized acute gastritis. It can become chronic inflammation characterized by lymphocyte infiltration. This condition will complicate glandular atrophy and intestinal metaplasia in the gastric mucosal epithelium and subsequently cause gastric malignancy. The aim of this study to analyze association between VEGF +936 C>T polymorphism gene with degree of neutrophils and lymphocytes infiltration in gastritis patients with *H. pylori*.

Methods: Samples were obtained through consecutive sampling in April-August 2019. Gastritis was ensured by endoscopy while histological feature was defined by Sydney system. *H. pylori* was examined by Campylobacter Like Organism test (CLO) and VEGF + 936 C> T gene polymorphism was ensured using PCR TaqMan SNP Genotyping Assay rs2010963. Chi-square analysis was used in this study to determine the association between VEGF + 936 C>T gene polymorphism with degree of neutrophils and lymphocytes infiltration.

Results: Of 60 gastritis patients, there were CT genotype (37.5%), followed by CC genotypes (36.7%), and TT genotypes (35%). Patients with CC genotype increased the risk of 18 times moderate and severe neutrophil infiltration compared to CT+TT genotypes (p=0.001). There was no relationship between VEGF + 936 C>T polymorphism and the degree of lymphocytes infiltration (p=0.293)

Conclusion: There was a significant association between VEGF + 936 C>T polymorphism and the degree of neutrophil infiltration but there was no association between VEGF + 936 C>T polymorphism and the degree of neutrophil infiltration.

Keywords: VEGF+936 C>T polymorphism, gastritis, h. pylori

Introduction

Helicobacter pylori became the most cause of gastritis and changes the gastric mucosa of gastritis patients. This change influenced by the inflammatory response of the host and also the virulence of *H. pylori* bacteria.[1] One of the host factor which influences inflammatory response is angiogenesis by vascular endothelial growth factor (VEGF).[2]

Nowadays prevalence of *H. pylori* has been increasing, especially in developing countries.[3] In Indonesia, the incidence of gastritis is high, according to the World Health Organization the incidence of gastritis in Indonesia reaches 40.8%. Acute inflammation occurs briefly characterized by neutrophil infiltration, while chronic inflammation occurs in the long term characterized by mononuclear cell infiltration especially lymphocytes.[4] Based on histopathology examination of all dyspepsia patients in India, there were 33.3% with neutrophil infiltration, 12.3% with atrophy, and 7% with intestinal metaplasia.[5] Histopathology examination in gastritis patients do not directly correlate with clinical symptoms.[6]

Mechanism of *H. pylori* can cause histopathology changes and gastric mucosal damage was influenced by the inflammatory response of the host and also the virulence of *H. pylori* bacteria. Activation of angiogenesis which stimulated by VEGF in host cells has been shown to play a role in the response to damaged gastric mucosal.[2] Expression and release of gastrin from G cells in the mucosal infected with *H. pylori* will increase the expression of VEGF.[7] VEGF also

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plays a role in the process of tumor angiogenesis and shown to be overexpression in gastric carcinoma.[2]

Vascular endothelial growth factor (VEGF) gene located on chromosome 6p21.3 and consists of 8 exons separated by 7 introns. This gene is very polymorphic. Single nucleotide polymorphisms (SNP) is a genetic variation that is inherited and found in human genes. Changes in the deoxyribonucleic acid (DNA) sequence in some individuals will cause genetic polymorphisms, one of which can occur in the sequence of SNPs. Change in a single nucleotide sequence, namely adenine (A), thymine (T), cytosine (C) or guanine (G) is the cause of variations. If it occurs in less than 1% of the population will cause lethal abnormalities called mutations but if it occurs in more than 1% of the population will cause phenotypic variations.[8] Several studies have shown that the presence of polymorphisms in the VEGF gene is associated with an increased risk of several disorders including preeclampsia, age-related macular degeneration (AMD), colorectal malignancy, and precancerous lesions in gastric.[9]

There are three most common variations of SNP VEGF which associated with the angiogenesis process namely -460T/C and + 405G/C in the 5′-untranslated region and + 936C>T chains in the 3′-untranslated region chain.[10] Zhen Xia et al in China showed that there was a relationship between VEGF + 936C > T gene polymorphism and increasing gastric carcinoma in smokers patients.[11] Bae et al. in 2008 also found that VEGF 936 T allele was associated with an increased risk of gastric cancer in Korea.[12] But Tahara et al. in 2009 found VEGF + 936 +C> T gene polymorphism was not significantly associated with gastric premalignant lesions.[13] The newest study about association VEGF +936 C> T gene polymorphism in gastritis with *H. pylori* by Siregar et al found that no association between VEGF +936 C>T gene polymorphism with gastric premalignant lesions.[14]

Acute gastritis caused by *h. pylori* characterized by infiltration of neutrophils in the gastric mucosa while chronic inflammation characterized by lymphocyte infiltration. This condition will complicate to glandular atrophy and intestinal metaplasia in the gastric mucosal epithelium which subsequently causes gastric malignancy.[4] Some study have shown that gastric cancer has association with VEGF +936 C>T gene polymorphism, but there was no study about association between VEGF +936 C> T gene polymorphism with degree of neutrophils and lymphocyte infiltration in gastritis patients. Hypothesis of the study is an association between both of VEGF +936 C>T gene polymorphism and degree of neutrophils and lymphocyte infiltration in gastritis patients. The aim of this study to analyzed association between VEGF +936C>T polymorphism with the degree of neutrophil and lymphocyte infiltration in gastritis patients with *H. pylori*.

Methods

There were 60 samples obtained through consecutive sampling in patients who present with dyspepsia complaints in April-August 2019. All patients treated using eradication therapy and proton pump inhibitors were exclude in this study. Gastritis was ensured by endoscopy (Olympus, Tokyo, Japan). Mucosa undergoes edema, erythema (spotted, patchy, linear), exudate, bleeding, erosion and histopathology that marked by inflammatory cells in the gastric mucosa were diagnosed with gastritis. Histologic features evaluated on each slide and score scaled and defined by Sydney system. H.pylori is established through changes in color from yellow to red, magenta, pink, and orange in the examination of Campylobacter Like Organism test (CLO). VEGF + 936 C> T gene polymorphism was ensured from the serum in the laboratory using VEGF (R&D system), TagMan SNP Genotyping rs 3025039.

Data analysis of association between VEGF + 936 C>T gene polymorphism with degrees of neutrophils and lymphocytes infiltration in patients with *h. pylori* gastritis were univariate and bivariate. (Figure 1) Univariate analysis was used in determining the characteristics and prevalence of h. pylori gastritis patients. Bivariate analysis was used in determining the association between VEGF +936 C>T gene polymorphism and degree of neutrophils and lymphocytes infiltration using chi-square analysis. VEGF + 936 C>T gene polymorphism became independent variable and degree

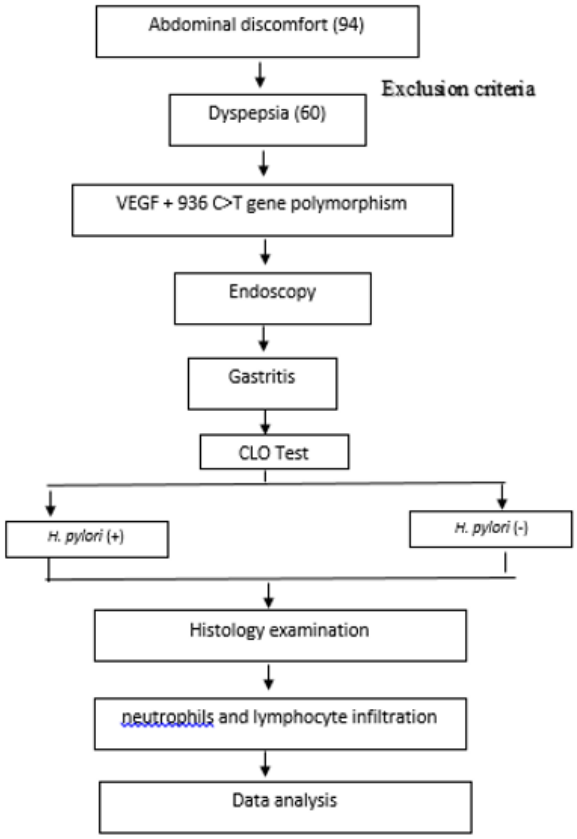

Figure 1. Flow Chart of the Study

of neutrophils and lymphocytes infiltration as the dependent variable. All data were analyzed by SPSS 23 version. A value of $p< 0.05$ with a 95% confidence interval was considered statistically significant.

Results

The proportion of gastritis was more in males (60%) than in females (24%). Based on h. pylori status, patient with *h. pylori* positive was 33.3% and *H. pylori* negative was 66.7%. Based on ethnicity, the highest proportion of gastritis sufferers was Batak (58.3%), and Acehnese (8.3%) as the lowest. Based on the occupation, the highest was a private employee (46.7%) and civil servants (6.7%) as the lowest. Based on education, the highest proportion of patients is at high school level (45.0%) and the lowest proportion at the elementary school (5%). Mean of body mass index (BMI) was 23.65+3.84 kg / m^2 (Table I).

In this study, VEGF Polymorphism +936 C>T genotypes CC, CT, and TT were examined. Genotype frequency distribution CC, CT, and TT of VEGF++936 C>T polymorphism in gastritis patients can be seen in Table II, which most gastritis patients have CT genotype (37.5%), followed by CC genotypes (36.7%), and TT genotypes (35%).

There were 23 patients gastritis without neutrophil infiltration and 37 patients experienced neutrophil infiltration (40% mild, 18.3% moderate, and 3.3% severe). (Table III)

There was a relationship between VEGF +936 C>T polymorphism and the degree of neutrophil infiltration. Patients with CC genotype increased the risk of 18 times moderate and severe neutrophil infiltration compared to CT+TT genotype (p=0.001). Allele C increased the risk of 11.29 times having moderate and severe neutrophil infiltration compared to the T allele (p=0.001) (Table IV).

There was no patient with normal lymphocyte infiltration and 38 patients (63.3%) had mild infiltration, 19 (31.7%) had moderate infiltration, and 3 (5%) had severe infiltration. There was no association between VEGF + 936 C>T polymorphism with degree of lymphocytes infiltration (p=0.293). There was no study about association between VEGF +936 C>T gene polymorphism and degree of neutrophils and lymphocytes infiltration before. This result supported the hypothesis about association between VEGF + 936 C>T polymorphism and degree of neutrophils infiltration but did not support the hypothesis between VEGF + 936 C>T polymorphism with degree of lymphocyte infiltration. (Table V)

Discussion

Result of the study showed the association between VEGF + 936 C>T polymorphism and degree of neutrophils infiltration with p<0.001 but no association between VEGF + 936 C>T polymorphism and degree of lymphocytes infiltration with p< 0.293. Previous studies suggest that VEGF plays an important role in the carcinogenesis. Activation

Table I. Baseline and clinical characteristic of subjects

Variable	n = 60
Sex (%)	
Male	36 (60)
Female	24 (40)
Age, years	46,9 ± 10,56[b]
Ethnic, n (%)	
Batak	35 (58.3)
Javanese	20 (33.3)
Acehnese	5 (8,3)
Occupation, n (%)	
Private Employee	28 (46.7)
Housewives	13 (21.7)
Enterpreneur	15 (25)
Civil servants	4 (6.7)
Education, n (%)	
Elementary	3 (5)
Midle	15 (25.0)
High School	27 (45.0)
University	15 (25.0)
BMI, kg/m^2	23,65 ± 3,84
Gastritis	
H. pylori(+)	20 (33.3%)
H. pylori(-)	40 (66.7%)

Table II. Frequency Distribution of VEGF+936 C>T Polymorphism in Gastritis Patients

Variable	n (%)
Polymorphism VEGF+936 C>T	
CC	22 (36.7%)
CT	17 (37.5%)
TT	21 (35.0%)

Table III. Degree of neutrophil infiltration (n=60)

Degree	
Normal	23 (38.3%)
Mild	24 (40.0%)
Moderate	11 (18.3%)
Severe	2 (3.3%)

Table IV. Association between VEGF +936 C>T polymorphism and degree of neutrophil infiltration

VEGF +936 C>T polymorphism	Degree of neutrophil infiltration		Total	*P*	PR (95% CI)
	Moderate-Severe	Normal-Mild			
CC	11	11	22 (100%)	0.001	
CT	1	16	17(100%)		
TT	1	20	21(100%)		
CC+CT	12	26	38 (100%)	0.01	9.69 (1.164-80.7)
TT	1	21	22 (100%)		
CC	11	11	22 (100%)	0.001	18 (3.453-2.314)
CT+TT	2	36	38 (100%)		
Allele					
C	23	38	61 (100%)	0.001	11.29 (3.16-40.29)
T	3	56	59 (100%)		

Table V. Degree of lymphocytes infiltration (n=60)

Degree	
Normal	0
Mild	38 (63.3%)
Moderate	19 (31.7%)
Severe	3 (5.0%)

of angiogenesis which stimulated by Vascular endothelial growth factor (VEGF) in host cells had been shown in playing a role in the response to damaged gastric mucosal. Mechanisms in how VEGF polymorphisms contribute to carcinogenesis remain unclear. Potential mechanisms that occur are variations in DNA sequence that may alter the production and/or activity of VEGF, causing inter- individual differences in tumor development. Alteration of the VEGF gene function (activation and repression) synergized with co-factor molecules became a rational cause.[13] Based on Zhen Xia et al in China showed that there was a relationship between VEGF + 936C > T gene polymorphism and increasing gastric carcinoma in smokers patients.[11] Bae et al in 2008 also found that VEGF 936 T allele was associated with an increased risk of gastric cancer in 154 patients in Korea. These results was contrary in pre-malignant lesion. Tahara et al and Siregar et al found 936 +C> T gene polymorphism was not significantly associated with gastric premalignant lesions.

Association between VEGF + 936 C>T gene polymorphism and degree of neutrophils infiltration showed that VEGF + 936 C>T gene polymorphism played a major role in gastric mucosa in gastritis patient by increasing transcription of VEGF.[13] Lymphocytes infiltration can complicate to glandular atrophy and intestinal metaplasia in the gastric mucosal epithelium which subsequently causes gastric malignancy.[4] No association between VEGF + 936 C>T polymorphism and degree of lymphocytes infiltration might be influenced by virulence of *h. pylori*. Serum VEGF level is correlated with *h. pylori* infection and its virulence status. The more virulence of *h. pylori,* cagA gene, the higher serum VEGF levels were found.[15] This study does not observe the virulence of *h. pylori*. We suggest further study about association between association between VEGF + 936 C>T polymorphism and virulence of *h. pylori* in gastritis patient

Conclusion

There was a significant association between VEGF + 936 C>T polymorphism and the degree of neutrophil infiltration but there was no association between VEGF + 936 C>T polymorphism and the degree of neutrophil infiltration

Disclosure

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