Association between VEGF-634G>C Gene Polymorphism with Degree of Neutrophil and Lymphocyte Infiltration in *Helicobacter pylori* Gastritis Patients

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Keywords: *Helicobacter pylori*, VEGF, Polymorphism, Neutrophil, Lymphocyte.

Abstract: Previous study showed that VEGF-634G>C polymorphism was associated with VEGF expression. VEGF can enhance inflammatory processes, resulting in more severe inflammation. This study held to analyze association between VEGF -634G>C polymorphism and the degree of gastric neutrophil and lymphocyte infiltration in *H. pylori* gastritis patients. The aim of this study is to investigate association between VEGF -634G>C polymorphism and the degree of gastric neutrophil and lymphocyte infiltration in *H. pylori* gastritis patients.This cross-sectional study included patients with *H. pylori* gastritis at Haji Adam Malik General Hospital, Permata Bunda General Hospital, and Universitas Sumatera Utara Hospital, Medan, Indonesia. Detection of *H. pylori* infection was made using positive results of 14C-UBT, and/ or rapid urease test. gaster. The degree of neutrophil and lymphocyte infiltrations were evaluated from biopsies of the mucosa gaster, referring to visual analogue scale of the updated Sydney System. Real time polymerase chain reaction (RT-PCR) was used to examine VEGF -634G>C gene polymorphism. Data were analyzed using SPSS version 22. There was significant association between VEGF-634 G>C polymorphism and degree of neutrophil infiltration. Patients with the G allele were at risk of 2.07 times for moderate + severe degree of neutrophil infiltration compared to C allele ($p = 0.008$). There was no significant association between VEGF -634 G>C polymorphism and degree of lymphocyte infiltration ($p>0.05$). G allele of VEGF-634 G>C polymorphism was associated with moderate + severe neutrophil infiltration.

1 INTRODUCTION

Helicobacter pylori (*H. pylori*) is the main etiology of chronic gastritis that plays a role in activation of host angiogenesis. In all angiogenic factors, vascular endothelial growth factor (VEGF) is the most potent neoangiogenesis stimulus (Siregar, 2017). *H. pylori* upregulates VEGF expression in gastric epithelial cells through several mechanisms such as NF-κβ, cyclooxygenase-2 (COX-2), and epidermal growth factor receptor (EGFR) signaling (Kang, 2014).

VEGF levels can be influenced by inflammation, hypoxia, oncogenes, tumor suppressor gene, and genetic factors, one of which variations of VEGF gene (Lee, 2015; Logsdon, 2014). VEGF gene located in 6p21.3 chromosome which is consist of 8 exons separated by 7 introns as known very polymorphic (has 140 variants) (Eng, 2012; Eng, 2013). Several single nucleotide polymorphisms (SNPs) on the VEGF gene are thought to affect their expressions. A previous study by Oh et al found that the GG genotype of VEGF-634G>C polymorphism was associated with higher VEGF serum levels (Oh, 2013). VEGF can enhance inflammatory processes, resulting in more severe inflammation, so that VEGF is not the only mediator of angiogenesis but also acts as inflammatory mediator (Shaik-Dasthagirisaheb, 2013). This study held to analyze association between VEGF -634G>C polymorphism and the degree of gastric neutrophil and lymphocyte infiltration in *H. pylori* gastritis patients.

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2 METHODS

2.1 Patients Selection

This cross-sectional study was perfomed on 80 consecutive *H. pylori* gastritis patients who were admitted to the Endoscopy Unit at Haji Adam Malik General Hospital, Permata Bunda General Hospital, and Universitas Sumatera Utara Hospital, Medan, Indonesia between April and June 2018. Inclusion criteria include gastritis patients diagnosed based on histopathological examination, positive results of ^{14}C-UBT and/ or rapid urease test, at least 18 years old, and willing to take part in the study. Exclusion criteria are as follows: history of *H. pylori* eradication treatment in the last 6 months or currently on antibiotics therapy commonly used in *H. pylori* eradication; history of proton pump inhibitor, H2 receptor antagonist, NSAID or steroid 1 month prior to the study; patients with systemic disease, and malignancy. This study was approved by the local ethics committee of Universitas Sumatera Utara.

A routine endoscopy was conducted in order to evaluate gastric mucosa for the presence of edema, erythema (spotted, patchy, linear), exudate, bleeding, and erosion; as well as to take a tissue sample for the rapid urease test and histopathology. Additionally, tissue biopsy was performed within the greater and lesser curvature of the distal antrum, the lesser curvature at incisura angularis, the anterior and posterior wall of the proximal corpus. An additional biopsy was also carried out in suspicious regions that were not included in the preliminary biopsy.

2.2 *Helicobacter pylori* Detection

A positive result of ^{14}C-UBT and / or rapid urease test will confirm the diagnosis of *H. pylori* infection. Participants were asked to fast for at least 6 h, prior to ^{14}C-UBT examination, usually overnight. Patients swallowed 37 kBq (1 μCi) of encapsulated ^{14}C urea/citric acid composition in 25 ml water. Heliprobe Breath Cards (Noster system) 10 min after administration of ^{14}C urea were used. The next step is to exhale into the breath cards until its color indicator changed from orange to yellow. The breath samples were measured using the Heliprobe analyzer (Noster system), and the activity was counted for 250s. Results were articulated as counts per minute (cpm) and counts < 25 cpm were defined as Heliprobe 0 = not infected, counts between 25 cpm and 50 cpm as Heliprobe 1 = equivocal and counts > 25 cpm as Heliprobe 2 = infected (Ghanaei, 2011).

The rapid urease test (Pronto Dry®, France) was also another primary tool for diagnosis of *H. pylori* infection. When the color from amber to pink-red at room temperature within 24 hours, it would confirm a positive result. A negative result would divulge a yellow colour on the indicator (Rojborwonwitaya, 2005).

2.3 Degree of Neutrophil and Lymphocyte Infiltration

The degree of neutrophil and lymphocyte infiltrations were evaluated from biopsies of the mucosa gaster. Biopsy specimens were fixed in 10% formalin and embedded in paraffin. The samples were stained using Hematoxylin-Eosin and were evaluated by the Pathologist of anatomic pathology of the medical faculty of the Universitas Sumatra Utara referring to visual analogue scale of the updated Sydney System. The degree of neutrophil and lymphocyte infiltration were scored 0 to 3, i.e., normal (0), mild (1), moderate (2), and severe (3) (Rugge, 2011).

2.4 VEGF -634 G>C Polymorphism

Genomic DNA was extracted and purified from peripheral blood smear using High Pure PCR Template Preparation Kit (Roche Applied Science), and stored until processed for genotyping. Analysis of the VEGF SNP -634G>C was performed using real time polymerase chain reaction (RT-PCR). The PCR primers used for the –634G>C polymorphisms were 5'-CGACGGCTTGGGGAGATTGC-3' (forward) and 5'-GGGCGGTGTCTGTCTGTCTG-3' (reverse). The PCR cycle conditions consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 62 °C, 30 s at 72 °C, and a final elongation at 72 °C for 10 min.

2.5 Statistical Methods

Data analysis was performed through univariate, bivariate (Chi-Square test) analyses using SPSS 22nd version (SPSS Inc., Chicago). A value of $p < 0.05$ with 95% confidence interval was considered statistically significant.

3 RESULTS

3.1 Baseline and Clinical Characteristics of Subjects

The characterized by male (61.3%) and mean age 50.8, and most of the subject were Bataknese (53.8%). There were 43 polymorphism VEGF-634G>C GC genotype patient (53.8%), followed 22 patient (27.5%) GG genotype and 15 patient (18.8) CC genotype (Table 1).

Table 1. Baseline and clinical characteristics of subjects

Characteristics	n=80
Gender Male Female	49 (61.3%) 31 (38.8%)
Age, mean ± SD (years)	50.8 ± 12.2
Ethnicity Batak Javanese Aceh Malay	43 (53.8%) 17 (21.3%) 11 (13.8%) 9 (11.3%)
Occupation Entrepreneur Housewife Employee Civil servants University students	14 (17.5%) 31 (38.8%) 29 (36.3%) 4 (5%) 2 (2.5%)
Education Elementary school Middle school High school University	 6 (7.5%) 10 (12.5%) 53 (66.3%) 11 (13.8%)
VEGF-634 G>C polymorphism GG genotype GC genotype CC genotype	 22 (27.5%) 43 (53.8%) 15 (18.8%)

3.2 Degree of Neutrophil and Lymphocyte Infiltration

Lymphocyte infiltration degree showed normal + mild degrees (55%) and moderate + severe degrees (45%). Neutrophil infiltration degree showed normal + mild degrees (71.2%) and moderate-severe degrees (28.8%) (Table 2).

Table 2. Degree of neutrophil and lymphocyte

Degree of Neutrophil		Degree of lymphocyte		Total
Moderate + Severe	Normal + Mild	Moderate + Severe	Normal + Mild	
23 (28.8%)	57 (71.2%)	36 (45%)	44 (55%)	80 (100%)

3.3 Association between VEGF -634 G>C Polymorphism and Degree of Neutrophil and Lymphocyte Infiltration

There was a significant association between VEGF-634 G>C polymorphism and degree of neutrophil. Patients with the G allele were at risk of 2.07 times for moderate + severe degree of neutrophil infiltration compared to C allele (p = 0.008) (Table 3). There was no significant association between VEGF -634 G>C polymorphism and degree of lymphocyte infiltration (p>0.05) (Table 4).

Table 3. Association between VEGF -634 G>C polymorphism and degree of neutrophil

VEGF-634G>C Polymorphism	Degree of neutrophil		Total	p	PR (95% CI)
	Moderate + Severe	Normal + Mild			
Genotype					
GG	10 (45.5%)	12 (54.5%)	22 (100%)	0.051	6.82 (0.97-47.83)
GC	12 (27.9%)	31 (72.1%)	43 (100%)	0.150	4.19 (0.59-29.5)
CC	1 (6.7%)	14 (93.3%)	15 (100%)		1 (ref.)
GG+GC	22 (33.8%)	43 (66.2%)	65 (100%)	0.058	5.08 (0.74-34.76)
CC	1 (6.7%)	14 (93.3%)	15 (100%)		1 (ref.)
Allele					
G	32 (36.8%)	55 (63.2%)	87 (100%)	0.008*	2.07 (1.17-3.63)
C	14 (19.2%)	59 (80.8%)	73 (100%)		1 (ref.)

*p<0.05

Table 4. Association between VEGF -634 G>C polymorphism and degree of lymphocyte

VEGF-634G>C Polymorphism	Degree of lymphocyte		Total	p	PR (95% CI)
	Moderate + Severe	Normal + Mild			
Genotype					
GG	11 (50%)	11 (50%)	22 (100%)	0.156	1.88 (0.73-4.79)
GC	21 (48.8%)	22 (51.2%)	43 (100%)	0.135	1.83 (0.75-4.47)
CC	4 (26.7%)	11 (73.3%)	15 (100%)		1 (ref.)
Genotype					
GG+GC	32 (49.2%)	33 (50.8%)	65 (100%)	0.113	1.85 (0.77-4.43)
CC	4 (26.7%)	11 (73.3%)	15 (100%)		1 (ref.)
Allele					
G	43 (49.4%)	44 (50.6%)	87 (100%)	0.219	1.24 (0.87-1.77)
C	29 (39.7%)	44 (60.3%)	73 (100%)		1 (ref.)

4 DISCUSSION

Inflammatory process are characterized by neutrophil and lymphocyte activity. Neutrophil activity in gastritis indicates an active course of the disease, whereas lymphocyte activity indicates a chronic course of disease. When mucosal damage occurs due to both infection and other stimulating factors, IL-8 inflammatory mediator expenditure acts as a chemotactic agent for neutrophils. IL-8 will activate neutrophils to release the lysosomal enzyme and induce neutrophil adherence in endothelial cells. This adherence is followed by neutrophil migration from the capillaries to the lamina propria and arise between the epithelial cells. These neutrophils can be found in gastric mucosa during acute phase gastritis and *H. pylori*-associated gastritis. This theory is in accordance with the results of the research presented by Atayan et al that there is a significant correlation between *H. pylori* infection with chronic gastritis severity with neutrophil and lymphocyte infiltration (r= 0.309, 0.226, respectively) (Atayan, 2017). The higher density of neutrophils, the higher density of bacterial infections (Dhakwa, 2012; Tuccillo, 2005). *H. pylori* infection in the gastric mucosa induces the production of IL-1β, IL-6, IL-8, and TNF-α cytokines. IL-1 or TNF-α alone, as well as TNF-α synergize with IFN-γ induce IL-8 production in gastric cells (Caputo, 2003; Tuccillo, 2005).

Induction of pathological angiogenesis is that inflammation precedes and is accompanied by the formation of neovessels as evidenced by increased vascular permeability and the recruitment of inflammatory cells. However, VEGF itself will not only promote angiogenesis, but also has the potential to induce inflammatory response. VEGF can influence the inflammatory process in several ways. VEGF increases vascularization at the site of inflammation causing the reaction to be more severe. Furthermore, VEGF can promote the recruitment of inflammatory cells (Sinnathamby, 2015; Angelo, 2007).

Certain SNP on the VEGF gene is thought to affect its expression. Allele variation may lead to overexpression of the transcription factor that will bind to the promoter site, which serves as the initial RNA polymerase binding site that will initiate transcription (Corvalan, 2012). Results of this study showed that there was a significant association between VEGF-634 G>C polymorphism and degree of neutrophil. Patients with the G allele were at risk of 2.07 times for moderate + severe degree of neutrophil infiltration compared to C allele. Patients with GG genotype were more likely to have moderate+severe degree of lymphocyte infiltration than patients with CC genotypes, however not statistically significant ($p>0.05$). The association between VEGF-634 G>C polymorphism with degree of neutrophil infiltration may be due to elevated levels of VEGF as a result of the G allele that plays a role in increasing neutrophil cell recruitment in gastric mucosa. Oh et al showed that patients with GG genotype of VEGF-634G>C polymorphism had significantly higher VEGF serum level than patients with CC genotype (Oh, 2013).

It was concluded that the G allele of VEGF-634 G>C polymorphism was associated with moderate + severe neutrophil infiltration. The limitations of our study include the small sampel size which might make our study underpowered. Further studies are necessary to examine other SNPs that may affect the degree of neutrophil and lymphocyte infiltration.

5 CONCLUSION

G allele of VEGF-634 G>C polymorphism was associated with moderate + severe neutrophil infiltration

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