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# ASSOCIATION BETWEEN VEGF +936 C>T GENE POLYMORPHISM WITH THE DEGREE OF ATROPHY IN GASTRITIS PATIENTS

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## Abstract

In the process of gastritis become its deadly sequel, gastric cancer, some genetic factors are thought to play a role. VEGF is a neoangionesis factor that involved in this process. Some polymorphisms are suspected play a role in determining of its plasma levels. The aim of this study is to analyze the association between VEGF936C>T polymorphism and degree of atrophy in gastritis patient. Atrophy is preliminary lesion before the occurrence of gastric cancer.

**Methods:** This crossectional study included gastritis patient in Permata Bunda Hospital. Endoscopy was performed to assess the gastric mucosae and the tissue biopsy was taken. VEGF936C>T was examined by Polymerase Chain Reaction (PCR).

**Results:** Among sixty gastritis patients that included in this study, 23.4% shows atrophy. There is a significant relationship between VEGF 936C>T polymorphism and the degree of atrophy. A significant association is also found between CC genotype and allele C and degree of atrophy ($p<0.05$).

**Conclusion:** There is a significant relationship between VEGF 936C>T polymorphism with the degree of atrophy.

## Introduction

Chronic gastritis is one of the most common diseases. It is estimated that almost half of the world's population has suffered from this disease. Although the prevalence has decreased significantly, gastritis is still a serious disease because of its deadly sequel.[1] *Helicobacter pylori*(*H. pylori*)is one etiology of chronic gastritis besides unhealthy diet, drugs and autoimmune.[2] *H. pylori* infection has been established as an important event in the development of gastro-duodenal diseases such as peptic ulcer, chronic atrophic gastritis and gastric cancer. However, there are many variations in the rate of gastric inflammation. Only a small percentage of chronic gastritis with *H. pylori* infection actually develop into peptic ulcer or gastric cancer. This suggests that *H. pylori* infection is not the only one cause gastro-duodenal disease. Some genetic factors are thought to play an important role in the long-term outcome of H. pylori infection.[3]

Neoangiogenesis is a common pathophysiological mechanism involved in the pathogenesis of inflammatory epithelial lesions of gastritis into malignant and metastatic cancer. Among the known pro-angiogenesis factors so far, vascular endothelial growth factor (VEGF) is one of the most powerful stimuli of neoangiogenesis. Siregar GA et al found that VEGF levels are associated with the degree of intestinal atrophy and metaplasia in gastritis patients.[4] Intestinal atrophy and metaplasia are preliminary lesion of gastric cancer.[2] Siregar GA et al, also found that VEGF levels were significantly higher in the gastritis group with positive *H. pylori* ($p <0.05$).[4] So it was concluded that *H. pylori* can activate angiogenesis. Another study by Feng et al,found that VEGF expression increased in chronic atrophic gastritis and metaplasia before the onset of gastric cancer occurred, giving the impression that VEGF plays a role in the initial process of gastric carcinogenesis.[5]A number of VEGF polymorphisms are thought to play a role in determining the levels of circulating VEGF in the plasma. One of the most important VEGF polymorphisms is VEGF +936 C>T which is located in the 3'untranlated region (3'UTR) VEGF gen.[6]

Several previous studies have examined the involvement of VEGF + 936C>T and risk of gastric cancer in gastritis patients. However, there seems a contradictory in the results. Xia et al found that TT genotype in VEGF + 936C>T gene is associated with the increasing risk of gastric cancer, through increased plasma VEGF levels.[7] In contrast, another study reported that VEGF levels decreased in +936C>T gene polymorphisms include CC,CT and TT genotypes.[8]A meta-analysis study in 2018 found that + 936C >T gene polymorphism was not associated with risk of gastric cancer, but this study reported that T allele and TT genotypes in VEGF + 936C >T gene, were related to the size of gastric tumors. [9]

So far there have been no studies examining the relationship between VEGF +936C>T with premalignant lesions, especially the degree of atrophy in patient with gastritis. That's why the author wants to examine the relationship between VEGF + 936C>T gene polymorphism and the degree of atrophy in gastritis patients.

## Material and Methods

This study was a cross-sectional study on 60 consecutive gastritis patients that were admitted to the Endoscopy Unit at Permata Bunda General Hospital, Medan, Indonesia. The data was collected between April 2019 until August 2019. All patients with diagnosis gastritis based on their histopathological examination, at least 18 years old and willing to take part were included. The exclusion criteria were have history of *H. pylori* eradication treatment in the last 6 month or currently get antibiotic that was one of regimen for eradication; history of using proton pump inhibitor, H2 receptor antagonist, NSAID, alcohol, steroid, within last 48 hours; patients with systemic disease or malignancy. This study was approved by the Institutional Review Board of Universitas Sumatera Utara.

Endoscopy was performed to assess the gastric mucosae (presence of oedema, erythema, bleeding, erosion). The tissue biopsy was performed on the greater and lesser curvature of the distal antrum, the lesser curvature at incisura angular, the anterior and posterior wall of the proximal corpus. The specimen were then sent to laboratory for microscopic evaluation. Histopathologic examination was done by two different pathologist blindly. Degree of atrophy was determined based on *Updated Sydney System.*

Venous blood sample was drawn from all participant and sent to laboratorium. Genomic DNA then extracted and purified using the High Pure PCR Template Preparation Kit. Analysis of the VEGF +936C>T was performed using real- time polymerase chain reaction (RT-PCR).

*Statistical Methods*

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

## Result

**Subject Characteristic**

Among sixty subjects who participated in the study, thirty six of them was male and twenty four of them was female. The average age was 46.9 years old (Table 1.)

Majority respondent have Batak ethnic (58,35%), followed by Java (33,3%), and Aceh by 8.3%. Based on occupation, majority of patients in this study were employee (46.7%), followed by housewives (21.7%), entrepreneurs (25%), and civil servants (6.7%).

Histopathological results of gastric mucosal biopsy shows atrophy in 14 speciment (23.4 %), with 6.7% have moderate and16.7% have mild severity. (Table 2.)

Polymorphism of VEGF 936 C>T gene analysis identified 22 subjects (18,3%) have genotype CC, 17 subjects (14,2) have genotype TT and 21 subjects (17,5%) have genotype CT (Table 2)

***Table 1. Subject Characteristics***

| Subject Characteristics | n (%) |
|---|---|
| Age | 46.9 (6,6) |
| Gender. n (%) | |
| Male | 36 (60) |
| Female | 24 (40) |
| Etnics | |
| Batak | 35 (58,3) |
| Jawa | 20 (33,3) |
| Aceh | 5 (8,3) |
| Occupation | |
| Employee | 28 (46,7) |
| Housewives | 13 (21,7) |
| Entrepreneurs | 15 (25) |
| Civil servants | 4 (6,7) |

***Table 2. Histopathological and PCR Analysis***

| | n (%) |
|---|---|
| Atrophy Grading | |
| Normal | 46 (76,7) |
| Mild | 10 (16,7) |
| Moderate | 4 (6.7) |
| Severe | 0 (0) |
| Polimorfisme VEGF 936C>T | |
| CC | 22 (18,3) |
| TT | 17 (14,2) |
| CT | 21 (17,5) |

*Polymorphism VEGF 936C>T and the Degree of Atrophy*

Based on Chi square analysis, there are significant association between VEGF polymorphism 936 C> T with the degree of atrophy in patients with gastritis (p = 0.025). Chi square analysis found a significant association between genotype CC with the degree of atrophy (p = 0.015) and allele C with the degree of atrophy (p = 0.007), the value of P <0.05. Chi square analysis on the TT genotype showed no significant results (p = 0.287).

***Table 3. Association Between VEGF +936 C>T Gene Polymorphism***

| | **Normal-Mild** | **Moderate-Severe** | **p** |
|---|---|---|---|
| Genotip | | | |
| CC | 18 | 4 | *0.025 |
| CT | 17 | 0 | |
| TT | 21 | 0 | |
| Genotip CT | | | |
| CT | 17 | 0 | 0.570 |
| CC + TT | 39 | 4 | (1.002-1.213) |
| Genotip CC | | | |
| CC | 18 | 4 | *0.015 |
| CT + TT | 38 | 0 | (0.672-0.996) |
| Genotipe TT | | | |
| TT | 21 | 0 | 0.287 |

| | | | |
|---|---|---|---|
| CT+CC | 35 | 4 | (0.802-0.998) |
| Alelle | | | |
| C | 55 | 6 | *0.007 |
| T | 59 | 0 | (0.809-0.969) |

## Discussion

Some single nucleotide polymorphism (SNP) form of VEGF gene have an impact on the expression of VEGF genes. One of them is the 936C>T polymorphism in 3'UTR of the VEGF gene that has an impact on plasma VEGF levels. Xia *et al*, found that the polymorphism VEGF 936C>T genotype TT increased plasma VEGF levels and was associated with an increased risk of gastric cancer.[7] In contrast, another study reported that VEGF levels decreased in VEGF 936C>T polymorphism in all CC, CT, and TT genotype.[8] Many studies have examined the relationship of VEGF 936C>T to cancer risk, but there are still no studies examining the relationship between 936C>T polymorphisms to premalignant lesion.

In this study, we found that there is a significant relationship between VEGF 936C>T polymorphism on the degree of atrophy which is a premalignant lesion in the occurrence of gastric cancer. This association may be related to C allele in this polymorphism. The study found that there is a significant association between VEGF 936C>T polymorphism CC genotype and the degree of atrophy ($p=0.015$, $p<0.05$). We also found a significant association between allel C and degree of atrophy.

Certain allele variation allegedly sparked overexpression of the transcription factor that will bind to the promoter site. This factor then bind to promoter site and serves as the initial RNA polymerase binding site that will initiate transcription.[10] This overexpression may lead to higher levels of VEGF.

VEGF is a neoagiogenesis factor that regulates vaskular formation, endothelial formation, proliferation, migration and differentiation. High VEGF levels are associated with susceptibility or severity of many diseases including the process of becoming cancerous. Siregar GA *et al.*, in his study, found that VEGF levels were related to the degree of atrophy and intestinal metaplasia in gastritis patient, where as atrophy and metaplasia are premalignant lesion that are part of the course of chronic gastritis into gastric cancer.[4]Study by Siregar GA *et al.* in 2018 found that there is higher expression of VEGF in gastric premalignant lesion such as chronic atrophic gastritis.[10]

Unfortunately, this study didn't examine the VEGF levels so we didn't know if this association was due to increasing or decreasing VEGF plasma levels.

## Conclusion

There is a significant relationship between VEGF 936C>T polymorphism and the degree of atrophy which is a premalignant lesion in the occurrence of gastric cancer. A significant association was also found between CC genotype and allele C with the degree of atrophy ($p<0.05$).

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## References

[1] Sipponen P, Maaroos HI. Chronic gastritis. Scand J Gastroenterol. 2015;50(6):657–67.
[2] Mescoli C, Gallo Lopez A, Taxa Rojas L, Jove Oblitas W, Fassan M, Rugge M. Gastritis staging as a clinical priority. Eur J Gastroenterol Hepatol. 2018; 30(2):125–9.

[3] Tahara T, Arisawa O, Shibata T, Nakamura M, Yamashita H, Yoshioka D, et al.Effect of polymorphisms in the 3′-untranslated region (3′-UTR) of VEGF gene on gastric pre-malignant condition. Anticancer Res. 2009;29(2):485–9.
[4] Siregar GA, Halim S, Sitepu RR. Serum TNF-α, IL-8, VEGF levels in Helicobacter pylori infection and their association with degree of gastritis. Indones J Intern Med. 2015;47(2):120–6.
[5] Feng CW, Wang LD, Jiao LH, Liu B, Zheng S, Xie XJ. Expression of p53, inducible nitric oxide synthase and vascular endothelial growth factor in gastric precancerous and cancerous lesions: Correlation with clinical features. BMC Cancer. 2002;2:1–7.
[6] Savabkar S, Chaleshi V, Farahbakhsh FB, Montazer Haghighi M, Zali N, Nazemalhosseini Mojarad E, et al. VEGF gene +936C/T polymorphism decreases the risk of colorectal cancer. Eur J Oncol. 2015;20(2):88–93.
[7] Xia H-Z, Wu Q, Du W-D. Association of Genetic Variants of VEGF gene with Gastric Carcinoma Risk. J Biochips Tissue Chips. 2011;01(01):1–9.
[8] Zhang C, Yamada N, Wu YL, Wen M, Matsuhisa T, Matsukura N. Helicobacter pylori infection, glandular atrophy and intestinal metaplasia in superficial gastritis, gastric erosion, erosive gastritis, gastric ulcer and early gastric cancer. World J Gastroenterol. 2005;11(6):791–6.
[9] Liu W, Dong Z, Hu R, Wang C. Association of vascular endothelial growth factor (VEGF) gene polymorphisms with gastric cancer and its development, prognosis, and survival. Technol Cancer Res Treat. 2018;17(613):1–8
[10] Siregar GA, Parwati I, Achmad TH, Syukriani YF. Association between VEGF -634G>C Gene Polymorphism with Gastric Premalignant Lesions and Serum VEGF Levels in Helicobacter pylori Gastritis Patients.2018;6(8):1328-34.