Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 51, Number 4, 2019; 333-339
http://dx.doi.org/10.19106/JMedSci005104201907

Vascular endothelial growth factor (VEGF) serum levelsof patients with *Helicobacter pylori* gastritis with cytotoxin-associated gene A positive (CagA$^+$)status

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Submited: 2018-07-25
Accepted : 2019-07-02

ABSTRACT

Helicobacter pylori (*H.pylori*) is an agent that causes gastritis and the ulcer of gaster, which are ultimately caused gastric tumors. The prevalence is about 80% average in developing country and 20-50 % in developed countries. One of the virulence factor is cytotoxin-associated gene A (CagA) that plays a role in the inflammation process, cell proliferation and metaplasia in gastric mucosa. Vascular endothelial growth factor (VEGF) is one of angiogenic factors that plays a role in the process of making new mucosal tissue after the inflammation by *H.pylori*. The escalation of VEGF expression levels contribute to the beginning of gastric carcinogenesis. The study was aimed to analyze of VEGF serum levels between CagA(+) and CagA(-) in patients with *H.pylori* gastritis. Cross sectional study was conducted on 30 patients with *H.pylori* gastritis after conducted gastroscopy, biopsy and CLO test, which were continued with VEGF serum examination using ELISA test and perfomed PCR test to determine the CagA status. The data was analyzed with SPSS 22 version and p value <0.05 was considered significant. The results of this study were18 men (60 %) and 12 women (46%) from 30 total subjects, with a median age average was 53.5 years old. Majority ethnic was Batak with 16 subjects (53.3%). *Helicobacterpylori* gastritis with CagA(+) was about 21 subjects (70%) and *H.pylori* gastritis with CagA(-) was about 9 subjects (30%). We found that median VEGF serum levels of patients with *H.pylori* gastritis with CagA(+)[480.3 pg/dL (115.5-2185.2)] significantly higher compared to that with CagA(-) [291.1 pg/dL (158.4-556.7)] (p<0.05). In conclusion, the VEGF serum levels of patients with *H.pylori* gastritis with CagA(+) is higher compared to that with CagA(-).

ABSTRAK

Helicobacter pylori (*H.pylori*) merupakan agen penyebab gastritis dan ulkus gaster, yang pada akhirnya menyebabkan terjadinya tumor gaster.Prevalensinya sekitat 80% di negara berkembang dan 20-50% di negara maju. Salah satu faktor virulensinya yaitu *cytotoxin-associated gene A* (CagA) yang berperan dalam peningkatan inflamasi, proliferasi sel dan metaplasia mukosa gaster. *Vascular endothelial growth factor* (VEGF) merupakan faktor angiogenik yang berperan dalam proses pembentukan jaringan mukosa baru setelah proses inflamasi oleh *H.pylori*. Peningkatan ekspresi VEGF berkontribusi terhadap proses awal terjadinya karsinogenesis gaster. Penelitian ini bertujuan untuk mengetahui perbandingan kadar serum VEGF penderita gastritis *H.pylori* denganstatus CagA(+) dan CagA(-). Penelitian dilakukan dengan rancangan potong lintang terhadap 30 pasien gastritis *H.pylori* setelah menjalani tindakan gastroskopi, biopsi dan pemeriksaan CLO, dilanjutkan dengan pemeriksaan kadar serum VEGF dengan metoda ELISA serta CagA dengan metode PCR. Data dianalisis dengan SPSS versi 22 dan perbedaan signifikan jika p<0,05. Dari 30 subyek penelitian yang sudah dianalisis secara statistik, 18 orang (60%) pria, 12 orang perempuan (46%), median umur 53.5 tahun (20-68), mayoritas bersuku batak 16 orang (53.3%). Penderita gastritis *H. pylori* dengan CagA (+) sebanyak 21 orang (70%) dan penderita gastritis *H. pylori* dengan CagA(-) sebanyak 9 orang (30%). Didapatkan median kadar serum VEGF pasien gastritis *H.pylori* dengan CagA(+) [480,3 pg/dL (115,5-2185,2)] lebih tinggi secara nyata dibandingkan dengan kadarnya pada pasien dengan pasien dengan Cag(-) [291,1 pg/dL (158,4-556,7)] (p<0,05). Dapat disimpulkan, kadar serum VEGF pasien gastritis *H.pylori* dengan CagA(+) dibandingkan dengan kadarnya pada pasien dengan CagA(-).

Keywords:
gastritis
H.pylori
CagA
VEGF
gastric tumor

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INTRODUCTION

Helicobacter pylori (*H.pylori*) is the causative agent of gastritis (GIs) and gastric ulcers (GU), which are eventually led to gastric tumors (GT). *Helicobacter pylori* is therefore classified into class I carcinogenic substances by WHO. Developing countries have an average *H.pylori* prevalence of about 80% compared with developed countries of about 20-50%. NSAID-related gastritis is also a common medical problem in clinical practice and the second most important risk factor of peptic ulceration after *H.pylori* gastritis. About 11% of the US population are experiencing this problem.[1-5]

Associated with virulence, this bacteria is able to produce a kind of protein that has long been regarded as a marker. This protein known as CagA which is also associated with increased inflammation, cell proliferation, and gastric mucosal metaplasia.[5] Vascular endothelial growth factor (VEGF), a well-known angiogenic factor, plays a role in many processes in the formation of new mucosal tissue after *H. pylori* inflammation by stimulated the formation of angiogenesis, which are aimed to supply the nutrients and oxygen to newly formed tissues. Many studies have shown that levels of VEGF increased in cases of malignancy, including gastric tumors. Research by Maciorkowska *et al*.[6] against *H. pylori*-infected children found that VEGF was highest in moderate and severe gastritis conditions. Some studies were made to evaluate the expression of VEGF in response to chronic inflammation due to *H.pylori* infection in the gastric mucosa.[5,7-9] Based on the information above, in this study we investigated the VEGF serum levels in patients with *H.pylori* gastritis with CagA(+) and CagA(-) status.

MATERIALS AND METHODS

Subjects

This was a cross-sectional study to compare the VEGF serum levels in patients with *H.pylori* gastritis with CagA(+) status and that with CagA(-) status. The independent variable wasthe status of CagA of patients with *H.pylori*gastritis, whereas the dependent variable was serum VEGF level. Patients with discomfort abdominal complaints who meet the inclusion criteria would fill the PADYQ score questionnaire. Patients with PADYQ score ≥ 6 (dyspepsia) would undergo gastroscopy to confirm gastritis.

Laboratory analysis

The patients with gastritis positive then uderwent Compylobacter-like organism (CLO) test to detect *H. pylory* infection. Patients with *H. pylori* positive, CagA status was ditermined using PCR method. The serum VEGF levels was then examined using ELISA method. Protocol of the study has been approved by the Research Committee of Medical Field, Faculty of Medicine, University of North Sumatra, Medan, Indonesia.

Statistical analysis

Univariate analysis was used to analyze the frequency distribution of the independent and the dependent variables, while bivariate analysis was used to analyze the independent variables which are suspected to have relation with the dependent variable. Thedata normality was analyzed by using the Shapiro-Wilk (sample < 50) obtained that data were not normally distributed. Furthermore, unpaired two-groups numerical comparative analytical test was done by Mann-Whitney test. A p value< 0.05 was considered significant.

RESULTS

The study involved 30 patients with *H. pylori* gastritiswho met the inclusion and exclusion criteria. The characteristics of patients are presented in TABLE 1. A total of 18 patients (60%) were male and 12 patients (46%) were female. The median age was mostly 53.5 years (20-68). The majority of respondents were Bataknese, which were 16 people (53.3%). The majority of respondents' religion were Moslem as many as 23 people (76.7%). Based on the level of education, respondents of senior high school level were 20 people (66.7%). Based on the occupation, the majority of respondents were working as entrepreneurs which were 14 people (46.7%). In this study, PCR result of *H.pylori* with CagA(+) was obtained as many as 21 respondents (70%) and CagA(-) 9 respondents (30%). While on serum VEGF examination of all respondents by using ELISA test, the median VEGF serum was 424.7 pg/dL (155.5 - 2185.2).

TABLE 1. The characteristics of patients with *H. pylori*

Variables	n (%)
Gender [n (%)]	
• Men	18 (60) [a]
• Women	12 (40)
Age [median(min-max years)]	53.5 (20 – 68) [b]
Ethnic [n (%)]	
• Bataknese	16 (53.3) [a]
• Javanese	6 (20)
• Acehnese	5 (16.7)
• Malay	2 (6.7)
• Indian	1 (3.3)
Religion[n (%)]	
• Moslem	23 (76.7) [a]
• Christian	6 (20)
• Hindi	1 (3.3)
Education [n (%)]	
• Primary	3 (10) [a]
• Junior High School	4 (13.3)
• Senior High School	20 (66.7)
• Bachelor	3 (10)
Occupation [n (%)]	
• Entrepreneur	28 (56) [a]
• Housewife	18 (36)
• Employee	3 (6)
• College student	1 (2)
CagA [n (%)]	
• Positive	21 (70) [a]
• Negative	9 (30)
Serum VEGF level [median(min-max pg/dL]	424.7 (155.5 – 2185.2) [b]

Note: a. Categorical data: n (%); b. Numerical data, not normal distribution: median (min-max)

TABLE 2 presents the characteristics patients with *H. pylori* based on CagA status. The male respondents were more in the group with CagA(+), which was 12 (66.7%) of the total male sample, while in women with CagA(+) was 9 (75%) of the total female samples studied. The mean of age in both groups was not different which was 50.5 ± 12.3 years in the CagA(+) *H.pylori* and 52.3 ± 12.84 years in the CagA(-) *H.pylori* group. Most of the ethnics in both groups were Bataknese with the most occupation in the group of CagA(+) were housewives, 9 people (75%), and the employees in the group of CagA(-), 6 people (42.9%).

TABLE 2. The characteristics of patients with *H. pylori* gastritis based on CagA

Variables	CagA(+)(n=21)	CagA(-) (n=9)	Total	p
Gender [n (%)]				
• Men	12 (66.7)	6 (33.3)	18 (100)	0.626
• Women	9 (75)	3 (25)	12 (100)	
Age (mean ± SD years)	50.5±12.3	52.3±12.8	51.1±12.3	0.718
Ethnic[n (%)]				
• Bataknese	10 (62.5) [a]	6 (37.5)	16 (100)	
• Javanese	6 (100)	0 (0)	6 (100)	
• Acehnese	2 (40)	3 (60)	5 (100)	
• Malay	2 (100)	0 (0)	2 (100)	
• Indian	1 (100)	0 (0)	1 (100)	
Religion [n (%)]				
• Moslem	15 (65.2)	8 (34.8)	23 (100)	
• Christian	5 (83.3)	1 (16.7)	6 (100)	
• Hindi	1 (100)	0 (0)	1 (100)	
Education [n (%)]				
• Primary	2 (66.7)	1 (33.3)	3 (100)	
• Junior High School	3 (75)	1 (25)	4 (100)	
• Senior High School	15 (75)	5 (25)	20 (100)	
• Bachelor	1 (33.3)	2 (66.7)	3 (100)	
Occupation [n (%)]				
• Entrepreneur	3 (100)	0 (0)	3 (100)	
• Housewife	9 (75)	3 (25)	12 (100)	
• Employee	8 (57.1)	6 (42.9)	14 (100)	
• College student	1 (100)	0 (0)	1 (100)	

Note: a. Categorical data: n (%);
b. Numerical data, not normal distribution: median (min - max)

There was significant difference of serum VEGF level between *H.pylori* CagA(+) and CagA(-) as presented in TABLE 3. The mean VEGF in the group with *H.pylori* CagA(+) was significantly higher with median 480.3pg/dL compared with the group with *H.pylori*CagA(-) with median 291.1 pg/dL (p=0.005).

TABLE 3. Levels of VEGF serum gastritis *H.pylori* with CagA(+) and CagA(-)

CagA	VEGF serum	p
Positive	480.3 (115.5 – 2185.2)	0.005*
Negative	291.1 (158.4 – 556.8)	

Note: numerical data, not normal distribution: median (minimum - maximum) *p<0.05

DISCUSSION

The results of this study were found that the median age of all respondents was 53.5 years (20-68). This is in accordance with the research conducted by Jamaludin *et al.*[10] with 60 samples where the mean age of 49.15±14.29 years. Another study conducted by Zhu *et al.*[11] was obtained 3445 patients with *H.pylori*(+) with urea breath test (UBT) andage range of 30-39 years (82-90%) on the total sample of 5017 people. The results of Betty *et al.*[12] conducted from January to June 2012 with 42 people as samples, obtained the prevalence ofgastritis with *H.pylori* was 47.6% with variation of age 16-40 years as much as 21.4%, 41-60 years as much as 52.3% and ≥ 61 years as much as 26.2%.

Based on gender characteristics data in the study of *H.pylori* gastritis patients, it was obtained the majority of the samples was male with 18 samples (60%), while the rest, 12 people (40%) were female. This is in accordance with the results of research conducted by Jamaludin *et al.*[10] with sample of 60 *H.pylori* gastritis and non-*H.pylori* patients, the majority of the sample was male with 32 people (53.3%), while the remaining 28 people (46.7%) were female.Other studies by Zhu *et al.*[11] obtained from 5417 samples of UBT examination were obtained 3435 (63.41%) *H.pylori*(+) which mentioned women as much as 64.47% and more as *H.pylori* sufferers compared to men as much as 35.63%.

The natural response to tissue ischemia is an increase in angiogenic growth factor. VEGF is one of the important markers for neoangiogenesis. There is an increase in VEGF expression in the healing process of peptic lesions. Many studies have shown that increased levels of VEGF in cases of malignancy including gastric cancer. A study by Maciorkowska *et al.*[6] on *H.pylori*-infected children found that VEGF was highest in moderate and severe gastritis conditions. *Helicobacter pylori* through CagA proteins would activate growth factor receptors in the effector c-Met.[13] Microvascular damage occurs early in mucosal injury cause glandular cellular necrosis and increase ischemic areas. The presence of increased aggressive factors may lead to the formation of thrombus and microvascular stasis, leading to ischemia and hypoxia, resulting in local necrosis and erosion. Injury of gastric mucosa was associated with a significant 4-6 times increase of VEGF.[13] In this study, there was significantly different in the mean of VEGF levels between CagA(+) *H.pylori*group and CagA(-) *H.pylori* group (p=0.005). The mean of VEGF level in the group with CagA(+) *H.pylori*was higher with a mean 480.3 pg/dL compared to the group with CagA(-) *H.pylori*was obtained with a mean of 291.1 pg/dL. This is in line with the study by Karayiannakis *et al.*[14] with the mean of serum levels of VEGF on 58 patients suffering from gastric carcinoma was 186 (101-266) pg/dL in which no significant differences were found between men and women. As well as other studies by Mangia *et al.*[15] the *H.pylori* antigen (41% of patients) with CagA(+) was closely related to plasma VEGF levels (p=0.026). Jamaludin *et al.*[10] obtained VEGF level in patients with *H.pylori*(+) was 723.51 pg/dL compared with *H.pylori*(-) patients which was 333.3 pg/dL (p=0.0001).

CONCLUSION

The serum level of VEGF in the group with *H.pylori* CagA(+) was higher with mean value 480.3 pg/dL compared with the group with *H.pylori* CagA(-) with mean value 291.1 pg/dL.

ACKNOWLEDGEMENTS

The authors express thanks to the Research Committee of Medical Field, Faculty of Medicine, University of North Sumatra, Medan for the permission of this study and thanks to all the parties who contribute to this study.

REFERENCES

1. Rugge M,Genta RM. Staging and grading of chronic gastritis. Hum pathol 2005; 36(3):228-33. https://doi.org/10.1016/j.humpath.2004.12.008
2. Fox JG, Megraud F. Helicobacter. In: Murray PR, editor. Manual of clinical microbiology. 9th ed. Pensylvania: Elsevier Mosby; 2007: 947-62.
3. Cesar ACG, Cury PM, Payao SLM, Liberatore PR, Silva AE. Comparison of histological and molecular diagnosis of Helicobacter pylori in benign lesions and gastric adenocarcinoma. Braz J Microbiol 2005; 36(1):12-16. https://doi.org/10.1590/S1517-83822005000100003
4. Banerjee A, Mukhopadhyay AK, Paul S, Bhattacharyya A, Swarnakar S. Unveiling the intricacies of Helicobacter pylori-induced gastric inflammation: T helper cells and matrix metalloproteinases at a crossroad. In Mozsik G, editor. Current Topics in Gastritis. Croatia: In Tech Publishers; 2013. Chapter 7. https://doi.org/10.5772/54193
5. Maciorkowska E, Marcinkiewicz S, Kaczmarski M, Kemona A. Inflammatory changes of the gastric mucosa and serum concentration of chosen growth factors in children. Adv Med Sci 2010; 55(1):59-66. https://dx.doi.org/10.2478/v10039-010-0007-6
6. Aziz F, Chen X, Yang X, Yan Q. Prevalence and correlation with clinical diseases of helicobacter pylori caga and vaca genotype among gastric patients from northeast China. Bio Med Research International 2014; 2014:1-7. https:/doi.org/10.1155/2014/142980
7. Tahara T, Arisawa T, 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 premalignant condition. Anticancer Res 2009; 29(2):485-9.
8. Matsukura N, Yamada S, Kato S, Tomtitchong P, Tajiri T, Miki M, *et al*. Genetic differences in interleukin-1 betapolymorphisms among four Asian populations: an analysis of the Asian paradox between H. pylori infection and gastric cancer incidence. J Exp Clin Cancer Res 2003; 22(1):47-55.
9. Caputo R, Tuccillo C, Manzo BA, Zarrilli R, Tortora G, Blanco CV, *et al*. Helicobacter pylori VacA toxin up-regulates vascular endothelial growth factor expression in MKN 28 gastric cells through an epidermal growth factor receptor-, cyclooxygenase-2-dependent mechanism. Clin Cancer Res 2003; 9(6):2015-21.
10. Jamaludin, Siregar GA. Perbandingan kadar serum VEGF dan MMP 9 pada pasien gastritis H.Pylori dan Non-H. Pylori. [Thesis], Magister Program Studi Ilmu Penyakit Dalam Fakultas Kedokteran USU.2015. Site: http://www.repository.usu.ac.id.
11. Zhu Y, Zhou X, Wu J, Su J, Zhang G. Risk factors and prevalence of H.Pylori infection in persistent high incidence area of gastric carcinoma in Yangzhong city. Gastroenterol Res Pract 2014; 2014:481365.

https://doi.org/10.1155/2014/481365
12. Betty, Lubis CP, Siregar GA. Infeksi Helicobacter pylori pada lesi gastritis yang didiagnosa dengan pewarnaan histokimia giemsa dan imunohistokimia Helicobacter pyloridi Laboratorium Patologi Anatomi Fakultas Kedokteran USU Medan. [Thesis]. Fakultas Kedokteran USU, 2012. http://repository.usu.ac.id/handle/123456789/34694.
13. Churin Y, Laila AG, Oliver K, Thomas FM, Walter B, Michael N. Helicobacter pylori Cag A protein targets the c-Met receptor and enhances the motogenic response. J Cell Biol 2003; 161(2):249-55. https://doi.org/10.1083/jcb.200208039
14. Karayiannakis AJ, Syrigos KN, Polychronidis A, Zbar A, Kouraklis G, Simopoulos C, *et al*. Circulating VEGF levels in the serum of gastric cancer patients correlation with pathological variables, patient survival, and tumor surgery. Ann Surg 2002; 236(1):37-42. https://doi.org/10.1097/00000658-200207000-00007
15. Mangia A, Chiriatti A, Ranieri G, Abbate I, Coviello M, Simone G, *et al*. H.Pylori status and angiogenesis factor in human gastric carcinoma. World J Gastroenterol 2006; 12(34):5465-72. https://doi.org/10.3748/wjg.v12.i34.5465