IJAM

RESEARCH PAPER

CORRELATION OF SERUM VEGF LEVELS WITH IL-10 IN GASTRITIS PATIENTS H. PYLORI AND NON H. PYLORI

Otto Harris Edison Lumbantoruan Gastroentero-hepatology Division of Internal Medicine Department Medical Faculty of University of Sumatera Utara/Haji Adam Malik General Hospital

Gontar Alamsyah Siregar Gastroentero-hepatology Division of Internal Medicine Department Medical Faculty of University of Sumatera Utara/Haji Adam Malik General Hospital

Masrul Lubis Gastroentero-hepatology Division of Internal Medicine Department Medical Faculty of University of Sumatera Utara/Haji Adam Malik General Hospital

ABSTRACT The objective of this study was to determine the correlation of serum VEGF levels with IL-10 in gastritis patients H. pylori and Non H. pylori. The study was conducted with a cross sectional design of 60 H. pylori and Non H. pylori gastritis patients after undergoing gastroscopy, biopsy and CLO test, followed by examination of serum VEGF and IL-10 levels by ELISA method. Data were analyzed by SPSS version 22. The difference was significant if $p < 0.05$. The mean VEGF in the group with H. pylori was much higher with a mean of 580.65 while in the group with Non H. pylori only with a mean of 335.65 ($p = 0,0001$). The mean IL-10 level in the H. pylori group was 0.94 and in patients with Non H. pylori it was 0.58 ($p = 0.04$). There was a significantly higher increase in serum VEGF and IL-10 levels in H. pylori gastritis patients compared to Non H. pylori.

KEY WORDS : Gastritis, H. pylori, IL-10, VEGF

INTRODUCTION

Dyspepsia is a clinical condition that is often found in daily practice. In Indonesia it is estimated that there are 30% of cases in general practice and 60% in specialist practice. The term dyspepsia is related to food, and describes complaints or a collection of symptoms consisting of pain or discomfort in the epigastrium, nausea, vomiting, bloating, full satiety, full stomach feeling, belching, regurgitation and a burning sensation that spreads in the chest. Gastritis is a health problem that is most often found in clinical practice. Gastritis is an inflammatory process in the gastric mucosa and submucosa in response to injury (injury) which can be acute or chronic.[1]

H. pylori plays an important role in the occurrence of peptic ulcer gastritis and ulcers. H. pylori infection is estimated to occur in 50% of the population in the world where most of these infections occur in developing countries, namely 70-90% and only 40-50% in industrialized countries. NSAID-related gastritis is also a medical problem that is often found in clinical practice. About 11% of the US population experiences this problem.[2,3]

In gastritis there is an acute and chronic inflammatory response. Based on the results of the study it was reported that Th-1 cytokines cause gastritis while Th-2 cytokines including Interleukin-10 (IL-10) have protection against the stomach. IL-10 is also thought to play a role in determining the type of gastric mucosal lesions with H. pylori infection. On the one hand IL-10 is a potent anti-inflammatory cytokine, which can inhibit the synthesis of proinflammatory cytokines such as IL-6, IL-8, and TNF-α. So that it is estimated that IL-10 can cause failure of the immune response to eradicate H. pylori infection.[4] IL-10 responses are associated with increased bacterial density and decreased pathology.[5,6] It was concluded from Robinson et al's study that IL-10 helps maintain bacterial colonization. But most of these studies are still limited to animals.[7-9]

The common mechanism involved in the pathogenesis of inflammation and ulcerative epithelial lesions is neoangiogenesis which is the development of new blood vessels from existing endothelial precursors. Vascular endothelial growth factor (VEGF) is one of the important markers for neoangiogenesis. There is an increase in VEGF gene expression in the process of healing peptic lesions. Many studies have shown that there is an increase in VEGF levels in malignant cases including gastric ca. But from the study it was found that VEGF increased in pre-gastric malignant lesions such as chronic gastritis atrophy and intestinal metaplasia, which showed an increase in VEGF expression contributing to the initial process of gastric carcinogenesis. Research by Maciorkowska E, et al., On children infected with H. pylori, found that VEGF was highest in moderate and severe gastritis conditions.[10,11]

Based on the information above, this study was prepared to determine serum VEGF levels as angiogenesis markers and IL-10 as anti-inflammatory cytokines in H. pylori and non H. pylori gastritis patients and the correlation of these markers in H. pylori and non H. gastritis patients. pylori.

METHODS

Patient Selection

The design used was cross sectional with the independent variables were H. pylori and non H. pylori gastritis and the dependent variable was serum IL-10 with VEGF. The research will be conducted at the Endoscopic Unit of the Adam Malik General Hospital Medan and the Medical Faculty USU Hospital after receiving approval from the Health Research Ethics Commission and related agencies. Research begins with library searches, title consultations, preparation of proposals, proposal seminars, research and data analysis and preparation of reports that require time from May 2015 to August 2015.

The inclusion criteria of this study were men and women aged ≥ 18 years, gastritis patients from endoscopic examination, receiving voluntary and written information and participation approval to undergo physical, laboratory, radiological examinations that were known and approved by the Health Research Ethics Committee. The exclusion criteria of this study were patients who had received H.

Article History	Received	Accepted	Published
	05/11/2017	21/12/2017	20/01/2018

***Corresponding Author** Otto Harris Edison Lumbantoruan

Gastroentero-hepatology Division of Internal Medicine Department Medical Faculty of University of Sumatera Utara/Haji Adam Malik General Hospital ,ottolumbantoruan@gmail.com

pylori eradication therapy in the last 6 months or were on antibiotic therapy commonly used in eradication therapy, taking Proton Pump Inhibitors, H2 receptor antagonists, NSAIDs, steroids, alcohol for the last 48 hours, and endemic systemic diseases.

Dyspepsia Scoring and Detection of H. pylori infection

In this study respondents were interviewed based on a questionnaire. Patients were interviewed about the characteristics of respondents (including age, sex, duration of illness, weight, height), interviews were conducted using The Porto Alegre Dyspeptic Symptoms Questionnaire (PADYQ) which is a quantitative analysis instrument of dyspeptic symptoms. There were 11 questions to assess frequency (score 0-4), duration (score 0-3), and intensity (score 0-5) of 5 symptoms of dyspepsia (upper abdominal pain, nausea, vomiting, bloating of the upper abdomen, fast stomach full) for the past 30 days. Scores range from 0 (asymptomatic) to 44 (severe symptoms). Patients with a total score of 6 or more are diagnosed as dyspepsia (Sander GB, et al, 2004).

To detect H. pylori serology (CLO) is performed. If there is a change in color from yellow to red, magenta, pink, dark orange indicates the presence of H. pylori expressed by positive H. pylori infection.

Statistical analysis

Analysis of the data used is univariate analysis, namely by analyzing the frequency distribution of independent and dependent variables, while bivariate analysis is an analysis of the variables studied (independent) that are thought to have a relationship with the dependent variable. In this analysis to determine the correlation of serum VEGF levels with IL-10: Spearman correlation test was used to analyze the correlation between VEGF and IL-10 and Mann Whitney U test to analyze differences in VEGF levels with IL-10 between H. pylori and non gastritis H. pylori. The desired deviation (α) is 0.05.

RESULTS

The study was attended by 60 patients who had met the inclusion criteria. A total of 32 patients (53.3%) were men with an average age of 49.15 years. The majority of respondents with Batak as many as 34 people (56.7%). Most patients who were the subjects in this study were entrepreneurs and housewives totaling 20 people (33.3%). The mean BMI of the subject was 23.56 kg / m2.

Table 1. Baseline characteristics

Characteristics	Non *H. Pylori* n = 30	*H. pylori* n = 30	p
Sex			
Male	13 (43,3)	19 (63,3)	0,121[a]
Female	17 (56,7)	11 (36,7)	
Age, Mean (yeas)	49,07 (13,06)	49,23 (15,64)	0,964[b]
Ethnics			
Bataknese	17 (56,7)	17 (56,7)	0,786[a]
Javanese	9 (30)	8 (26,7)	
Acehnese	2 (6,7)	4 (13,3)	
Melayunese	1 (3,3)	1 (3,3)	
Minangnese	1 (3,3)	0	
Job, n (%)			
Entrepreneur	10 (33,3)	10 (33,3)	0,437[a]
Housewife	12 (40)	8 (26,7)	
Employee	5 (16,7)	4 (13,3)	
Farmer	2 (6,7)	3 (10)	
Etc	1 (3,3)	5 (16,7)	
MBI, Mean (SB), kg/m^2	22,27 (3,56)	24,85 (3,63)	0,007[b]

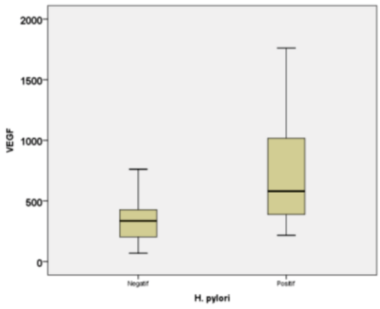
Using the Mann Whitney test it was found that there were significant mean differences (p = 0.0001) of VEGF levels in subjects with H. pylori and non H. pylori. The mean VEGF in the group with H. pylori was much higher with an average of 580.65 while in the group with non H. pylori only with a mean of 335.65. (Table 2, Figure 1)

Table 2. Difference between VEGF and IL-10 levels between H. pylori and non H. pylori Gastritis

	H. pylori	*Non H. pylori*	p
VEGF	580,65 (216,5-1761,1)	335,65 (67,9-761,6)	0,0001*
IL-10	0,94 (0,2-6,68)	0,58 (0,09-12,6)	0,04*

*Mann Whitney

Figure 1. VEGF Difference Boxplot Diagram between Groups of Patients with H. pylori and non H. Pylori

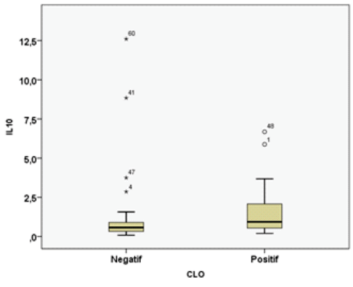


Using the Mann Whitney test found significant mean differences (p = 0.04) for IL-10 levels between patients with H. pylori and non H. pylori. The mean IL-10 level in the H. pylori group was 0.94 and in patients with non H. pylori it was 0.58. The results of the analysis using the Spearman correlation test showed that there was no significant correlation between VEGF and IL-10 (p = 0.580). (Table 3, Figure 2)

Table 3. Correlation of VEGF to IL-10 in the Patient Group with H. pylori

	IL-10	
	P	r
VEGF	0,580	0,105

Figure 2. Box-plot diagram of IL-10 differences between groups of patients with H. pylori and non H. pylori

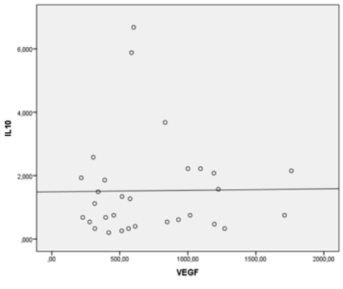


The results of the analysis using the Spearman correlation test showed that there was no significant correlation between VEGF and IL-10 (p = 0.863) in patients with non H. pylori. (Table 4, Figure 3)

Table 4. Correlation of VEGF to IL-10 in groups of patients with non-H. pylori

	IL-10	
	P	r
VEGF	0,863	0,033

Figure 3. Scatter Dot Diagram of VEGF Correlation with IL-10 in the Patient Group with H. Pylori

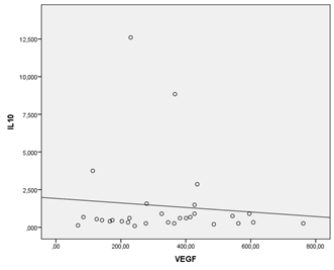


The results of the analysis using the Spearman correlation test showed that there was no significant correlation between VEGF and IL-10 (p = 0.863) in patients with non H. pylori. (Table 5, Figure 4)

Table 5. Correlation of VEGF to IL-10 in All Research Subjects

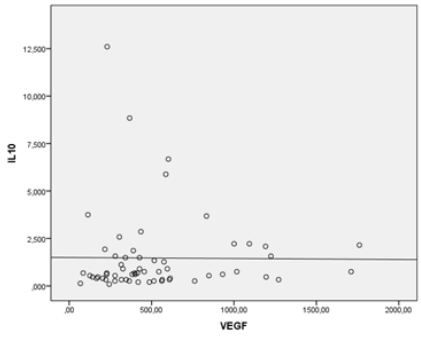
	IL-10	
	P	r
VEGF	0,184	0,174

Figure 4. Scatter Dot diagram of VEGF correlation with IL-10 in a group of patients with non-H. Pylori



The results of the analysis using the Spearman correlation test showed that there was no significant correlation between VEGF and IL-10 (p = 0.184) in all research subjects. (Figure 5)

Figure 5. Diagram of Scatter Dot VEGF Correlation with IL-10 for All Research Subjects



DISCUSSION

Gastritis is a health problem most often found in clinical practice.[12] Gastritis is an inflammatory process in the gastric mucosa and submucosa in response to injury (injury) which can be acute or chronic.[1] Infection with Helicobacter pylori (H. pylori) is the most common cause of active chronic gastritis worldwide, which is around 80% of all causes of chronic gastritis.[13]

H. pylori infection is a multi-factorial pathology and each host (genetic and nutritional status) and bacteria (virulent strains and several strains) depend on factors that have their influence on the immune system.[14] In gastritis there is an acute and chronic inflammatory response.[15] Based on the results of the study it was reported that Th-1 cytokines cause gastritis while Th-2 cytokines including Interleukin-10 (IL-10) have protective properties on the stomach. IL-10 is also thought to play a role in determining the type of gastric mucosal lesions with H. pylori infection.[4]

Vascular endothelial growth factor (VEGF) is also one of the important markers for neoangiogenesis.[16] There is an increase in VEGF gene expression in the process of healing peptic lesions. Many studies have shown that there is an increase in VEGF levels in cases of malignancy including gastric ca. But from the study it was found that VEGF increased in pre-gastric malignant lesions such as chronic gastritis atrophy and intestinal metaplasia, which showed an increase in VEGF expression contributing to the initial process of gastric carcinogenesis.[17]

A total of 60 patients who met the inclusion criteria were included in this study. A total of 32 patients (53.3%) were male and 28 patients (46.7%) were women with an average age of 49.15 years. Then divided into two groups with 30 people each based on the presence or absence of H. pylori infection.

In this study we found significant mean differences (p = 0.04) for IL-10 levels between patients with H. pylori and Non H. pylori. The mean IL-10 level in the H. pylori group was 1.52 and in patients with Non H. pylori it was 1.42. Lundin et al (2007) and Goll et al (2007) reported high levels of IL-10 in the gastric mucosa of H. pylori patients.[5,6] Activation of cytokines which causes mucosal inflammation occurs. Levels of IL-6 and IL-8 mucosa have been reported to increase in dyspepsia patients infected with H. pylori.[18]

Another study also stated that IL-10 was associated with the incidence of H. pylori infection. IL-10 as a potent anti-inflammatory cytokine, which can inhibit the synthesis of proinflammatory cytokines such as IL-6, IL-8, and TNF-α. So that it is estimated that IL-10 can cause failure of the immune response to eradicate H. pylori infection.[4] From research conducted by Robinson et al. Also concluded that IL-10 helps maintain bacterial colonization.[19] Research conducted by Dlugovitzky et al. (2005) also found that in H. pylori IL-2 and IFN levels were lower and IL-4, IL-10 and TGF-β were higher compared to Non H. pylori subjects.[20] This indicates that IL-10 can be a biomarker for H. pylori infection.

From the results of this study it was found that there were significant mean differences (p = 0.0001) of VEGF levels in patients with H. pylori and non H. pylori. The mean VEGF in the group with H. pylori was much higher with an average of 580.65 while in the group with Non H. pylori only with an average of 335.65.

Mangia et al and Caputo et al reported that H. pylori can increase VEGF expression in gastric mucosa. Tucillo et al. Found that VEGF expression in gastric epithelial cells was mediated by the COX and EGFR pathways. Through increasing prostaglandin production triggers EGFR activation and increased VEGF expression. This pathophysiology is important because it can affect the progression of chronic gastritis to adenocarcinoma through the Correa cascade.[21] It is hoped that VEGF can be a marker of H. pylori infection in gastritis patients.

When testing hypotheses, from the results of research that has been conducted it was found that there was no significant correlation between VEGF and IL-10 (p> 0.05), both in subjects with H. pylori and non H. pylori. Although these two biomarkers are associated with H. pylori infection, they do not have a correlation so that they can only be used in each attempt to diagnose H. pylori infection.

In another study the lack of IL-10 in the host produced a strong inflammatory production capable of eradicating bacteria from the gastric mucosa.22 This is in accordance with the study, where increasing IL-10 can increase the work of the immune system to eradicate germs. Thus IL-10 can contribute to the protective response.[23]

The limitation of this study is that IL-10 and VEGF levels were taken from the blood so that the accuracy would be lower than the examination of the gastric mucosa, not evaluating H. pylori virulence status (CagA, VacA).

CONCLUSION

There was a significantly higher increase in serum VEGF and IL-10 levels in H. pylori gastritis patients compared to Non H. pylori.

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

REFERENCES

1. El-Zimaity HMT. Recent advances in the histopathology of gastritis. Current Diagnostic Pathology. 2007;13:340-8.

2. Fox JG, Wang TC. Inflammation, atrophy and gastric cancer. The Journal of Clinical
3. Cesar ACG, Cury PM, Payao SLM. Comparison of histological and molecular diagnosis of Helicobacter pylori in benign lesions and gastric adenocarcinoma. Braz J Microbiol. 2005;36(1):261-6.
4. Bodger, K. et al. (2001) 'Interleukin 10 in Helicobacter pylori associated gastritis: Immunohistochemical localisation and in vitro effects on cytokine secretion', Journal of Clinical Pathology, 54(4), pp. 285–292. doi: 10.1136/jcp.54.4.285.
5. Goll R, Gruber F, Olsen T, Cui G, RascHpichler G, Buset M, et al. Helicobacter pylori stimulates a mixed adaptive immune response with a strong T-regulatory component in human gastric mucosa. Helicobacter. 2007;12:185-92.
6. Lundin BS, Enarsson K, Kindlund B, Lundgren A, Johnsson E, Quiding-Jarbrink M, et al. The local and systemic T-cell response to Helicobacter pylori in gastric cancer patients is characterised by production of interleukin-10. Clin Immunol. 2007;125: 205-13.
7. Tanigawa T, Watanabe T, Higuchi K, Machida H, Okazaki H, Yamagami H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, et al: Lansoprazole, a Proton Pump Inhibitor, Suppresses Production of Tumor Necrosis Factoralpha and Interleukin-1beta Induced by Lipopolysaccharide and Helicobacter Pylori Bacterial Components in Human Monocytic Cells via Inhibition of Activation of Nuclear Factor-kappaB and Extracellular Signal-Regulated Kinase. J Clin Biochem Nutr. 2009, 45(1):86–92.
8. Lee HJ, Han YM, Kim EH, Kim YJ, Hahm KB. A possible involvement of Nrf2-mediated heme oxygenase-1 up-regulation in protective effect of the proton pump inhibitor pantoprazole against indomethacin-induced gastric damage in rats. BMC Gastroenterology. 2012;12:143.
9. Zhao X, Wang Q, Qian Y, Song JL,. Ilex kudingcha C.J. Tseng (Kudingcha) prevents HCl/ethanol induced gastric injury in Sprague Dawley rats. Molecular Medicine Reports. 2013;7:1613-6.
10. Toljamo K. Gastric erosions – clinical significance and pathology. A long-term follow-up study. University of Oulu, Finland:Acta Univ Oul; 2012.hlm 1-80.
11. Mazzoleni, Luiz EdmundoSander, G. B. et al. (2011) 'Helicobacter pylori Eradication in Functional Dyspepsia', American Medical Association, 171(21), pp. 1671–1677.
12. Hagymási, K. and Tulassay, Z. (2014) 'Helicobacter pylori infection: New pathogenetic and clinical aspects', World Journal of Gastroenterology, 20(21), pp. 6386–6399. doi: 10.3748/wjg.v20.i21.6386.
13. Rugge M, Genta RM. Staging and grading of chronic gastritis. Human pathology. 2005;36:228-33.
14. Adrián, T. B. R.- (2017) 'Helicobacter pylori tion: Participation of Cytokines in the Immune Response and in the Consequences Pathological Clinic. Mini Review', EC Gastroenterology and Digestive System, 3(2), pp. 66–69.
15. Zullo, A. et al. (2014) 'Helicobacter pylori and functional dyspepsia: An unsolved issue?', World Journal of Gastroenterology, 20(27), pp. 8957–8963. doi: 10.3748/wjg.v20.i27.8957.
16. Siregar, G., Sari, D. and Sungkar, T. (2017) 'Serum VEGF levels in helicobacter pylori infection and correlation with helicobacter pylori cagA and vacA genes', Macedonian Journal of Medical Sciences, 5(2), pp. 137–141. doi: 10.3889/oamjms.2017.031.
17. Strowski, M. Z. et al. (2004) 'Helicobacter pylori stimulates host vascular endothelial growth factor-A (vegf-A) gene expression via MEK/ERK-dependent activation of Sp1 and Sp3.', The FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 18(1), pp. 218–220. doi: 10.1096/fj.03-0055fje.
18. Xuan J, Deguchi R, Yanagi H, Ozawa H, Urano T, Ogawa Y, et al. Relationship between gastric mucosal IL-8 levels and histological gastritis in patients with Helicobacter pylori infection. Tokai J Exp Clin Med. 2005;30(2):83-8.
19. Yamaoka, Y. et al. (1996) 'Helicobacter pylori cagA gene and expression of cytokine messenger RNA in gastric mucosa', Gastroenterology, 110(6), pp. 1744–1752. doi: 10.1053/gast.1996.v110.pm8964399.
20. Dlugovitzky, D. G. et al. (2005) 'Changes in cytokine levels related to the immunopathogenesis of Helicobacter pylori disease. Immunological and histological effects of triple treatment (omeprazol, azytromycin, and amoxycillin)', Inmunologia, 24(1), pp. 11–16. doi: 10.1016/ j.neuropsy cholo gia.2017.03.013.
21. Mangia, A. et al. (2006) 'H pylori status and angiogenesis factors in human gastric carcinoma', World Journal of Gastroenterology, 12(34), pp. 5465–5472. doi: 10.3748/wjg.v12.i34.5465.
22. Matsumoto, Y. et al. (2005) 'Eradication of Helicobacter pylori and resolution of gastritis in the gastric mucosa of IL-10-deficient mice', Helicobacter, 10(5), pp. 407–415. doi: 10.1111/j.1523-5378.2005.00349.x.
23. Meyer, F., Wilson, K. T. and James, S. P. (2000) 'Modulation of innate cytokine responses by products of Helicobacter pylori', Infection and Immunity, 68(11), pp. 6265–6272. doi: 10.1128/IAI.68.11.6265-6272.2000.