Serum TNF-α, IL-8, VEGF Levels in *Helicobacter pylori* Infection and Their Association with Degree of Gastritis

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ABSTRAK

Tujuan: *untuk mengetahui kadar serum TNF-α, IL-8, VEGF pada infeksi Helicobacter pylori dan hubungannya dengan derajat histopatologi gastritis.* ***Metode:*** *studi potong lintang terhadap 80 pasien gastritis secara konsekutif yang datang ke unit endoskopi Rumah Sakit Umum Adam Malik dan Rumah Sakit Permata Bunda, Medan, Indonesia dari Juli-Desember 2014. Rapid urease test digunakan untuk diagnosis infeksi H. pylori. Dinilai derajat keparahan inflamasi kronik, infiltrasi neutrofil, atrofi, dan metaplasia intestinal. Dilakukan pemeriksaan kadar serum TNF-α, IL-8, dan VEGF. Analisis univariat dan bivariat (chi square, fisher's exact, dan mann-whitney test) dengan SPSS versi-22.* ***Hasil:*** *sebanyak 41,25% dari 80 pasien terinfeksi H. pylori. Kadar serum TNF-α dan VEGF secara signifikan lebih tinggi pada kelompok yang terinfeksi H. pylori dibandingkan kelompok H. pylori negatif, tetapi tidak ada perbedaan signifikan antara kadar serum IL-8 pada H. pylori positif dan negatif. Terdapat hubungan yang signifikan antara kadar serum TNF-α dan IL-8 dengan derajat inflamasi kronik, serta antara kadar serum IL-8 dengan derajat infiltrasi neutrofil. Terdapat hubungan yang signifikan antara kadar serum VEGF dengan derajat atrofi dan derajat metaplasia intestinal.* ***Kesimpulan:*** *kadar serum TNF-α yang tinggi berhubungan dengan derajat inflamasi kronik yang berat, kadar serum IL-8 yang tinggi berhubungan dengan derajat inflamasi kronik dan infiltrasi neutrofil yang berat, serta kadar serum VEGF yang tinggi berhubungan dengan derajat lesi gaster prakeganasan yang berat.*

Kata kunci: *sitokin, neoangiogenesis, Helicobacter pylori, gastritis atrofi, metaplasia intestinal.*

ABSTRACT

Aim: *to investigate the serum levels of TNF-α, IL-8, VEGF in Helicobacter pylori infection, and their association with the degrees of gastritis histopathology.* ***Methods:*** *a cross-sectional study was done on 80 consecutive gastritis patients admitted to endoscopy units at Adam Malik General Hospital and Permata Bunda Hospital, Medan, Indonesia from July-December 2014. The Rapid Urease test was used for the diagnosis of H. pylori infection. The severity of chronic inflammation, neutrophil infiltration, atrophy, and intestinal metaplasia were assessed. Serum samples were obtained to determine circulating TNF-α, IL-8, and VEGF. Univariate and bivariate analysis (chi square, fisher's exact, and mann-whitney test) were done using SPSS version-22.* ***Results:*** *there were 41.25% of 80 patients infected with Helicobacter pylori. Serum TNF-α and VEGF levels in the infected group were significantly higher compared to H. pylori negative, but there were no significant differences between serum levels of IL-8 in H. pylori positive and negative. There were significant associations between serum level of TNF-α and IL-8 with degree of chronic inflammation, and also between serum level of IL-8 and degree of neutrophil infiltration. There were significant associations between serum level of VEGF and degree of atrophy, and also between serum level of VEGF and degree of intestinal metaplasia.*

Conclusion: *High levels of TNF-α were associated with severe degree of chronic inflammation, high levels of IL-8 associated with severe degree of chronic inflammation and neutrophil infiltration, and high levels of VEGF associated with severe degree of premalignant gastric lesion.*

Key words: *cytokine, neoangiogenesis, Helicobacter pylori, atrophic gastritis, intestinal metaplasia.*

INTRODUCTION

Helicobacter pylori is the most common bacterial infection in humans that is specific for gastric epithelial cells. It is a Gram negative, microaerophilic bacterium associated with chronic gastritis and peptic ulcer disease as well as gastric cancer and mucosa related tissue lymphoma (MALT).[1] The prevalence of *H. pylori* seems to be dependent on geographical location and the socioeconomical status of the population and it was found that approximately 50% of the world adult population was infected by *H. pylori*. The bacterium colonizes the human stomach and triggers gastric inflammation, promoting neutrophils and monocyte recruitment, and increases the release of cytokines which causes gastric mucosa damage.[2] Local production of inflammatory cytokines are thought to play a central role in the recruitment of inflammatory cells to the gastric mucosa in the presence of *H. pylori*.[3]

It remains unclear whether this inflammation is limited to gastric mucosa or causes systemic inflammation, since the stomach has a large surface area. It has been suggested that the chronic gastric mucosal inflammation induced by *H. pylori* potentially may have systemic effects based on the increase in serum proinflammatory cytokines.[4]

H. pylori plays a critical role in the pathogenesis of benign and malignant gastric diseases and is associated with activation of the host's angiogenesis.[5] Among the pro-angiogenic factors known so far, vascular endothelial growth factor (VEGF) represents one of the most potent stimuli of neoangiogenesis.[6] VEGF promotes vascular permeability. It is thus thought to contribute to growth of tumor and tumor metastasis.[7]

Although cytokine-based gastric mucosal immune response and expression VEGF to *H. pylori* infection have been documented very well, only few data on circulating levels of particular inflammatory cytokines and VEGF are available. In the present study we aimed to investigate whether we can show increased circulating TNF-α, IL-8, and VEGF levels in *H. pylori*-infected patients with gastritis without systemic diseases and their association with with the degree of histopathology.

METHODS

Patient Selection

The present study was a cross sectional study on eighty consecutive gastritis patients admitted to endoscopy units at the Adam Malik General Hospital and Permata Bunda Hospital, Medan, Indonesia from July-December 2014. Inclusion criteria included all adult patients with gastritis. All patients gave informed consent and the study was approved by the local ethics committee. None of the patients had received antibiotics, bismuth compounds, H2 antagonists, proton pump inhibitors or immune modulating drugs within the last four weeks before endoscopy. Patients with evidence of malignancy, immunosuppression, metabolic disorders, or gastrointestinal hemorrhage and patients who had a history of gastric surgery were excluded.

Histological Assessment of Gastritis

The histopathological degree of gastritis was evaluated from biopsies of the mucosa of gastric antrum and body. 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 University of Sumatra Utara referring to visual analogue scale of the updated Sydney System. The higher degree was used if differences of degree were found between the body and antrum. The degree of chronic inflammation, neutrophil

infiltration, atrophy, and intestinal metaplasia were scored 0 to 3, i.e., normal (0), mild (1), moderate (2), and severe (3).[8]

Helicobacter pylori Detection

The Rapid urease test (CLO test, Kimberly-Clark, Utah, USA) was used to establish diagnosis of H. pylori infection. The results were read within 24 hours. Yellow is considered a negative result. A positive result was reported if the color changed from yellow to red, magenta, pink or deep orange within 24 h of incubation at room temperature.[9]

Serum Levels of TNF-α, IL-8, VEGF

Venous blood was drawn using a serum separator tube and allowed to clot for 30-45 minutes at room temperature before centrifugation for 15 minutes at approximately 1,000 g. Serum was immediately stored frozen in aliquots at -20oC until assays for TNF-α, IL-8, VEGF were performed. Circulating TNF-α and IL-8 levels were measured by means of a high sensitivity ELISA that uses an additional amplification step (HS Quantikine, R&D Systems, Inc., Minneapolis).[10,11] Circulating VEGF levels were examined in serum using the Quantikine Human VEGF-ELISA (Quantikine, R&D System, Inc., Minneapolis). The levels above the mean were categorized as high level and the levels below the mean were categorized as low levels.[12]

Statistical Methods

SPSS version 22 (SPSS Inc., Chicago) was used for the analysis. The data were analyzed using univariate and bivariate analysis with 95% confidence interval. Bivariate analysis was carried out using fisher's exact test, chi-square test, and Mann Whitney tests with a significance levels set at $p<0.05$.

RESULTS

Demographics of Respondents

There were 80 subjects, consisted of 41 males (51.25%) and 39 females (48.75%). Mean age was 49.3±13.4 (SD) years old. The highest number of age group was from the age group of 46-60. The majority of subject`s employment status was housewife (36.25%) and self-employed (32.5%). The majority of the subjects had a normal nutrition status (43 subjects, 53.75%). There were 33 patients (41.25%) infected with *Helicobacter pylori*.

Table 1. Characteristics of the subjects

Variables	*H. pylori* positive	*H. pylori* negative
Sex		
- Males	21 (26.25%)	20 (25%)
- Females	12 (15%)	27 (33.75%)
Age (years)		
- <30	5 (6.25%)	4 (5%)
- 30-45	10 (12.5%)	13 (16.25%)
- 46-60	10 (12.5%)	20 (25%)
- >60	8 (10%)	10 (12.5%)
Job		
- Self employed	11 (13.75%)	15 (18.75%)
- Employee	5 (6.25%)	7 (8.75%)
- Farmer	3 (3.75%)	3 (3.75%)
- Housewife	9 (11.25%)	20 (25%)
- Other	5 (6.25%)	2 (2.5%)
Nutritional status		
- Normal	16 (20%)	27 (33.75%)
- Underweight and Overweight	17 (21.25%)	20 (25%)
Total	33 (41.25%)	47 (58.75%)

Serum TNF-α, IL-8, VEGF Levels in *Helicobacter pylori* Infection

TNF-α and VEGF levels were significantly higher in infected group compared to *H. pylori* negative ($p<0.05$), but there were no significant differences between serum levels of IL-8 in *H. pylori* positive and negative

Table 2. Serum levels of TNFα, IL-8, and VEGF in HP (+) and HP (-) (mean±SD)

Serum Level (pg/ml)	*H. pylori*		p
	Positive	Negative	
TNF-α	4.34±3.72	2.76±1.51	0.029*
IL-8	38.96±25.92	36.01±24.69	0.635
VEGF	683.18±428.37	485.26±306.75	0.048*

Association Serum TNF-α, IL-8, VEGF Levels and Degree of Histopathology of Gastritis

There was significant association between serum levels of TNF-α and degree of chronic

inflammation [Unadjusted OR (95% CI): 2.750 (1.11-6.83), p=0.027]. There were significant associations between serum levels of IL-8 and degree of chronic inflammation [Unadjusted OR (95% CI): 2.496 (1.01-6.16), p=0.045], and also between serum levels of IL-8 and degree of neutrophil infiltration [Unadjusted OR (95% CI): 2.926 (1.04-8.20), p=0.037]. There were no associations between VEGF levels and degree of chronic inflammation and also between VEGF levels and degree of neutrophil infiltration.

There were significant associations between serum levels of VEGF and degree of atrophy [Unadjusted OR (95% CI): 4.333 (1.27-14.78), p= 0.027], and also between serum levels of VEGF and degree of intestinal metaplasia [Unadjusted OR (95% CI): 4.678 (1.19-18.34), p= 0.037]. There were no associations between serum levels of TNF-α and degree of atrophy and also between serum levels of TNF-α and degree of intestinal metaplasia. There were no associations between serum levels of IL-8 and degree of atrophy, and also between serum levels of IL-8 and degree of intestinal metaplasia.

DISCUSSION

Mean age of gastritis subjects in this study was 49.3±13.4 (SD) years, which is considered a productive age group. In addition, the age groups with most frequent gastritis were those in the age group of 46-60 and 30-45. This result is in accordance with the results from the previous studies such as that by Garg B, et al.[10], which reported the mean age of gastritis patients of 47 years and the study by Mustapha SK, et al.[14], which reported a mean age of 47.2 years.[13,14]

All subjects experienced chronic inflammation, while neutrophil infiltration, atrophy, and intestinal metaplasia were found in 41.25%, 38.75%, and 27.5%, respectively. There were different results among studies. Garg B, et al.[10], for instance, reported chronic inflammation 100%, neutrophil infiltration in 33.33%, atrophy in 12.33%, and intestinal metaplasia in 7%,[13] while Zhang et al.[15], reported chronic inflammation in 90.3%, neutrophil infiltration in 56.2%, atrophy in 36.8%, and intestinal metaplasia in 37%.[15] Another study by Hashemi et al.[16] found active chronic gastritis in

Table 3. Association serum levels of TNF-α, IL-8, VEGF and degree of chronic inflammation and neutrophil infiltration

Cytokines VEGF		Chronic Inflammation			Neutrophil Infiltration		
		Normal + Mild	Moderate + Severe	OR (95% CI)	Normal + Mild	Moderate + Severe	OR (95% CI)
TNF-α	High	14 (17.5%)	28 (35%)	2.75 (1.11-6.83)	29 (36.25%)	13 (16.25%)	1.255 (0.47-3.33)
	Low	22 (27.5%)	16 (20%)		28 (35%)	10 (12.5%)	
IL-8	High	14 (17.5%)	27 (33.75%)	2.496 (1.01-6.16)	25 (31.25%)	16 (20%)	2.926 (1.04-8.20)
	Low	22 (27.5%)	17 (21.25%)		32 (40%)	7 (8.75%)	
VEGF	High	17 (21.25%)	23 (28.75%)	1.224 (0.51-2.96)	27 (33.75%)	13 (16.25%)	1.444 (0.55-3.83)
	Low	19 (23.75%)	21 (26.25%)		30 (37.5%)	10 (12.5%)	

Table 4. Association serum levels of TNF-α, IL-8, VEGF and degree of atrophy and intestinal metaplasia

Cytokines VEGF		Atrophy			Intestinal Metaplasia		
		Normal + Mild	Moderate + Severe	OR (95% CI)	Normal + Mild	Moderate + Severe	OR (95% CI)
TNF-α	High	31 (38.75%)	11 (13.75%)	1.892 (0.62-5.75)	33 (41.25%)	9 (11.25%)	1.80 (0.55-5.95)
	Low	32 (40%)	6 (7.5%)		33 (41.25%)	5 (6.25%)	
IL-8	High	30 (37.5%)	11 (13.75%)	2.017 (0.66-6.12)	33 (41.25%)	8 (10%)	1.333 (0.42-4.27)
	Low	33 (41.25%)	6 (7.5%)		33 (41.25%)	6 (7.5%)	
VEGF	High	27 (33.75%)	13 (16.25%)	4.333 (1.27-14.78)	29 (36.25%)	11 (13.75%)	4.678 (1.19-18.34)
	Low	36 (45%)	4 (5%)		37 (46.25%)	3 (3.75%)	

47.1%, atrophic changes in 25%, and intestinal metaplasia in 8.9%.[16]

The purpose of the present study was to assess the serum levels of TNF-α, IL-8 as proinflammatory cytokines, and the VEGF as a marker of angiogenesis in *H. pylori* infection, and their association with degree of histopathology of gastritis. At present there are few data on circulating levels of inflammatory cytokines in *H. pylori* and the results have been contradictory. In the present study, we studied the levels of circulatory cytokines, which may theoretically increase as a consequence of intense gastric mucosal cytokine activation.

This study found that serum TNF-α levels in the infected group were significantly higher compared to *H. pylori* negative group ($p<0.05$). This result is in accordance with the results from the previous studies such as that by Russo et al.[17] and Perri et al.[18] which reported that *H. pylori* infection was associated with increased serum levels of TNF-α. Our findings suggest that a strong immune response to *H. pylori* enhanced the systemic inflammation, which was reflected in an increased serum levels of TNF-α.

Several investigators have detected IL-8 as an important mediator in *H. pylori*-associated gastritis as the most likely substance responsible for inducing further steps of the signal transducing pathway because it is up-regulated in epithelial cells infected with *H. pylori*.[19,20] Lindholm et al.[21] found increased levels of mucosal IL-8 in the *H. pylori*-infected subjects whereas Cichoz-Lach et al.[22] found that mean levels of IL-8 in patients with biliary gastritis was higher than the control group, and was found to be more increased in *H. pylori* infected patients than in uninfected ones.[22] This study found that serum levels of IL-8 were higher in *H. pylori* associated gastritis, but not significant compared to *H.pylori* negative. Kim et al.[23] showed that *H. pylori* strains that express CagA were found to upregulate epithelial IL-8 secretion and gene expression, a direct CagA effect on IL-8 induction by gastric epithelial cells.[23,24] Probably our limitations in this study was that we did not determine the CagA status of our patients so we possibly had patients infected with less pathogenic strains of this bacterium such as with Cag A-negative *H. pylori*. This might be a reason that there were no significant differences between serum levels of IL-8 in *H. pylori* positive and negative.

High serum levels of TNF-α were associated with severe degree of chronic inflammation, but not with the degree of neutrophil infiltration, atrophy, and intestinal metaplasia. High serum levels of IL-8 were associated with severe degree of chronic inflammation and neutrophil infiltration, because of its potent chemotactic and stimulatory activity on neutrophils and lymphocytes.

This study also found that serum VEGF levels in the infected group were significantly higher compared to *H. pylori* negative ($p<0.05$). Previous investigations suggested that *H. pylori* can activate host angiogenesis.[3]

Mangia et al.[25] and Caputo et al.[26] reported that *H. pylori* was able to upregulate VEGF expression in gastric mucosa cells. Tucillo et al.[27] reported that *H. pylori* up-regulates the expression of VEGF in human gastric epithelial cells in vitro and that this is apparently mediated through an EGFR-, COX-2-related pathway. Through the increased production of prostaglandins, this leads to further activation of EGFR, and to the increased expression of angiogenetic VEGF. All these events may ultimately be pivotal in the progression from chronic gastritis to adenocarcinoma in the multi-step model of gastric carcinogenesis.[27]

Atrophic gastritis and intestinal metaplasia are known to be premalignant gastric lesions, and patients with premalignant gastric lesions are at considerable risk of gastric cancer.[28] Feng et al.[29] found that VEGF expression was elevated in chronic atrophic gastritis as well as in metaplastic areas before the onset of gastric cancer and the detection of high levels of serum VEGF in gastric premalignant lesions such as atrophic gastritis and intestinal metaplasia, suggesting that VEGF may also contribute early in the process of gastric carcinogenesis. We did not find association between VEGF levels and the degree of chronic inflammation or neutrophil infiltration. In this study, we found that high serum levels of VEGF were associated with severe degree of atrophy and intestinal metaplasia.

CONCLUSION

Serum TNF-α and VEGF levels were significantly increased in the infected H. pylori group, but there were no significant differences between serum levels of IL-8 in H. pylori positive and negative group. High levels of TNF-α were associated with severe degree of chronic inflammation, high levels of IL-8 were associated with severe degree of chronic inflammation and neutrophil infiltration, and high levels of VEGF were associated with severe degree of premalignant gastric lesion.

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