Correlation Between TNF- α and Degree of Gastritis

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ABSTRACT

Background: *TNF- α is a cytokine that plays an active role in the pathogenesis of gastritis. The correlation of TNF-α levels in the gastric mucosa with the severity of gastritis has long been known. However, few studies have assessed TNF-α levels in serum of gastritis patients. This study aims to evaluate correlation between serum TNF-α level with the degree of gastritis based on histopathology.*

Method: *A cross sectional study on eighty gastritis patients that fulfilled the inclusion criteria underwent serum TNF-α examination, endoscopy, and biopsy. Rapid urease test was used for diagnosis of H. pylori infection. The severity of gastritis based on lymphocyte infiltration, neutrophil infiltration, atrophy, and intestinal metaplasia according to Updated Sydney System. Univariate and bivariate analysis (Chi-square, fisher's exact, spearman correlation, and independent t-test) were done using SPSS version 22.*

Results: *There were 41.25% patients infected with Helicobacter pylori. Serum TNF-α levels in the infected group were significantly higher compared to negative H. pylori ($p < 0.05$). There was significant positive correlation between serum TNF-α levels and degree of gastritis based on lymphocyte infiltration ($r = 0.333$; $p = 0.003$).*

Conclusion: *There was a significant positive correlation between serum TNF-α level with the severity of gastritis based on lymphocyte infiltration.*

Keywords: *cytokine, TNF-α, Helicobacter pylori, gastritis, Updated Sydney System*

ABSTRAK

Latar belakang: *TNF- α merupakan sitokin yang berperan aktif pada patogenesis gastritis. Korelasi kadar TNF- α pada mukosa lambung dengan derajat keparahan gastritis telah lama diketahui. Meskipun demikian, hanya sedikit penelitian yang menilai kadar TNF- α dari serum pasien gastritis. Penelitian ini bertujuan menilai kadar serum TNF-α dan korelasinya dengan derajat keparahan gastritis secara histopatologi.*

Metode: *Penelitian potong lintang terhadap 80 pasien gastritis yang memenuhi kriteria inklusi. Dilakukan pemeriksaan kadar serum TNF-α, endoskopi, dan biopsi. Rapid urease test digunakan untuk diagnosis infeksi H. pylori. Derajat keparahan dinilai dari infiltrasi limfosit, infiltrasi neutrofil, atrofi, dan metaplasia intestinal yang mengacu pada Updated Sydney System. Analisis univariat dan bivariat (Chi-square, fisher's exact, korelasi spearman dan independent t-test) dengan SPSS versi 22.*

Hasil: *Pasien yang terinfeksi H. pylori sebesar 41,25%. Kadar TNF-α serum secara signifikan lebih tinggi pada kelompok yang terinfeksi H. pylori dibandingkan kelompok yang tidak terinfeksi H. pylori ($p < 0,05$). Terdapat korelasi positif antara kadar serum TNF-α dengan derajat gastritis berdasarkan infiltrasi limfosit [$r = 0,333$ dengan $p = 0,003$].*

Simpulan: *Terdapat korelasi positif yang signifikan antara kadar TNF- α serum dengan derajat keparahan gastritis berdasarkan infiltrasi limfosit.*

Kata kunci: *sitokin, TNF-α, Helicobacter pylori, gastritis, Updated Sydney System*

INTRODUCTION

Gastritis is a process of inflammation in the mucosal and submucosa layer of gaster in response to injury (injury). Gastritis differs from dyspepsia, which is a clinical syndrome. Gastritis is a diagnosis that can be established histologically, but not clinically.[1] In gastritis, there is an acute or chronic inflammatory response in which proinflammatory factors, called cytokines, are activated and cause mucosal inflammation. Infection of *H. pylori* is the most common cause of chronic active gastritis worldwide. It is estimated that 50% of the world's population had *H. pylori* infection where 70-90% of these infections occur in developing countries and only 40-50% in developed countries.[2-7]

Prolonged chronic gastritis is at risk of developing gastric mucosal atrophy and metaplasia leading to gastric cancer. Endoscopy and stomach tissue biopsy are the most accurate screening method in assessing the severity of gastritis and evaluating the risk of metaplasia. Cytokines such as TNF-α, IL-6, and IL-8 in gastric mucosa play a role in the immune response associated with an inflammatory disease. Several studies have suggested an increase in the secretion of proinflammatory cytokines such as IL-6, IL-8, and TNF-α in gastritis due to *H. pylori* infection.[8-13] The aim of study to determine the association of proinflammatory cytokines levels in serum, especially TNF-α, with the severity of gastritis based on histopathology.

METHOD

This is a cross sectional study with a sample size of 80 people. The study was conducted in Endoscopy Unit at Adam Malik General Hospital and Permata Bunda Hospital, Medan, Indonesia from September 2015 until December 2015. This study was conducted after the approval of Local Health Research Ethics Committee and each subject has signed informed consent before the research procedure is performed. Inclusion criteria include patients aged >18 years with dyspepsia according to Rome III criteria and are willing to participate. Exclusion criteria are patients who have received *H. pylori* eradication therapy, consumed proton pump inhibitors, NSAIDs, steroids, or alcohol for the last 48 hours, and patients with systemic and inflammatory diseases.

The subjects of the study underwent gastroscopy and biopsy examinations at 5 sites. Biopsy specimens were stained with H & E staining for histopathological examination to evaluate the severity of gastritis based on The Updated Sydney System. This system classifies gastritis severity based on lymphocyte infiltration, neutrophil infiltration, atrophy, and intestinal metaplasia with score 0 for absence, 1 for mild, 2 for moderate, 3 for severe.[14] Rapid urease test was used for diagnosis infection of *H. pylori.*

Serum TNF-α were examined using enzyme-linked immunosorbent assay (ELISA) method that reacts TNF-α with antibody. This study used Human TNF-α Elisa KIT reagents 2.10. The samples were incubated and coated with antibodies specific for TNF-α. A substrate was added to give colour to the samples. TNF-α concentrations were measured by optical density of the colour of each sample. Under normal circumstances TNF-α levels are almost undetectable in the blood. TNF-α value is considered low if TNF-α ≤ mean and high if its value > mean.

All data is statistical analysed using SPSS program. Univariate and bivariate tests were performed using Mann Whitney U test, Spearman correlation. Significant was considered if $p < 0.05$, with 95% confidence interval.

RESULTS

In this study, a total of 80 patients have met the inclusion criteria. It consists of 41 males (51.3%) and 39 were female (38.7%). The mean age of the subjects was 49.3 years old. Most patients who became respondents in this study were housewives (36.3%), followed by 26 entrepreneurs (32.5%), 12 employees (15%), 6 farmers (7.5%). The mean of respondent's BMI was 23.59 kg/m^2. There were 58.75% patients infected with *H. pylori*. The mean value of TNF-α in serum was 4.34 with a minimum value of 1.13 and a maximum value of 15.88.

Table 1. Characteristic of study population

Characteristic	n = 80
Sex	
Male	41 (51.3)
Female	39 (48.7)
Age (year)	49.33 (13.38)*
Ethnic group	
Bataknese	48 (60)
Javanese	21 (26.3)
Acehnese	7 (8.8)
Malay	3 (3.8)
Minang	1 (1.3)
Occupation	
Self employed	26 (32.5)
Housewife	29 (36.3)
Employee	12 (15)
Farmer	6 (7.5)
Others	7 (8.8)
Body mass index (kg/m^2)	23. 59 (3.76)*
< 18.5	7 (8.75)
18.5 – 24.9	42 (52.5)
≥ 25	31 (38.75)
Campylobacter like organism (CLO)	
Negative	47 (58.75)
Positive	33 (41.25)
TNF-α mean value	4.34*
minimum	1.13
maximum	15.88

*mean

Table 2. TNF-α in *H. pylori* positive and negative

	H. pylori (+)	Non *H. pylori*	p
TNF-α, mean (SD)	4.34 (3.72)	2.76 (1.51)	0.029

TNF-α level was significantly higher in patients with *H. pylori* positive (4.34) than negative (2.76), p = 0.029.

Table 3. Correlation of TNF-α with degrees of lymphocyte & neutrophil infiltration, atrophy, and metaplasia

Degree of gastritis	p	r
TNF		
Lymphocythe infiltration	0.003	0.333
Neutrophil infiltration	0.098	0.186
Atrophy	0.239	0.133
Intestinal metaplasia	0.121	0.175

*p < 0.05

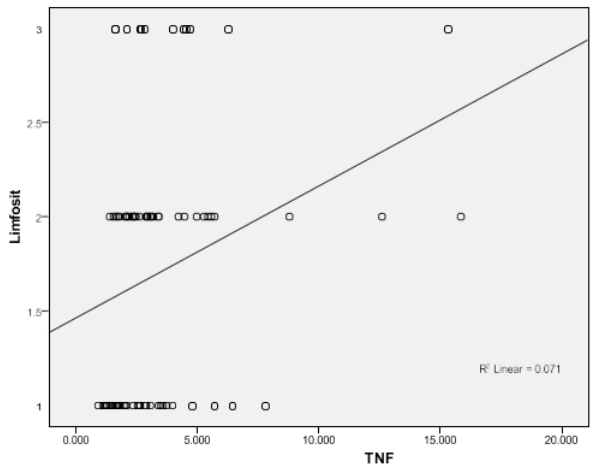

Figure 1. Scatterplot Chart of correlation between TNF-α and degree of lymphocyte infiltration

The correlation of TNF-α and the severity of gastritis based on histopathology was determined using Spearman correlation test. Table 3 shows that there was a significant positive correlation between TNF-α and severity of gastritis based on degree of lymphocyte infiltration (r = 0.333; p = 0.003). A positive r value indicates that the higher the TNF-α value will be followed by increasing lymphocyte values. Significant correlations were not shown between TNF-α with degree of neutrophil, atrophy and metaplasia (p > 0.05).

DISCUSSION

The baseline characteristic shows that the number of male suffering from gastritis was higher than female. All subjects had chronic inflammation in which there was lymphocyte infiltration in gastric histopathology, while neutrophil infiltration was 41.25%, gastric atrophy 38.75%, and gastric metaplasia 27.5%. Previous studies have shown mixed results.[15-17]

Serum TNF-α levels were significantly increased in subjects with *H. pylori* infection compared with those without *H. pylori* infection (p < 0.05). This suggests that *H. pylori* infection not only stimulates immune reactions in the gastric mucosa but also can stimulate systemic immunity. Equivalent results were obtained in Perri et al, Collodel et al, and Russo et al. Patients with *H. pylori* infection also tend to have more severe gastric mucosal damage that may increase cytokines level in serum.[10,18,19]

Theoretically, levels of TNF-α in the blood could increase as a result of immune stimulation in the gastric mucosa. This is because chronic inflammation, like gastritis, could trigger macrophages to release TNF-α both locally and systematically.[20-24] This study found significant positive correlation between level of serum TNF-α and degree of chronic inflammation. Meanwhile, there was no correlation between level of serum TNF-α with the degree of neutrophil infiltration, atrophy, and intestinal metaplasia. These results suggest that high levels of inflammation in the gastric mucosa may increase levels of cytokines in the blood. This is in line with the results of studies assessing TNF-α in the gastric mucosa, suggesting a correlation between severity of gastritis with cytokines.[12,13] However, studies from Bayraktaroğlu et al and Abdollahi et al which assessed serum TNF-α showed no correlation with the severity of gastritis.[25,26] Klausz et al also stated that TNF-α serum did not affect the severity of duodenal ulcers.[27] Thus it can be concluded from this study that the greater of lymphocyte infiltration degree in gastritis, the higher serum TNF-α level.

This study has many limitations, especially on histopathological severity assessments that are subjective by pathologists. But to reduce this bias, the entire study sample was examined by the same pathologist. Furthermore, there was no comparison of mean TNF-α values in gastric and serum mucosa. TNF-α levels in the gastric mucosa are important because it is a better representation compared with serum TNF-α.

CONCLUSION

There was a significant positive correlation between serum TNF-α level with the severity of gastritis based on lymphocyte infiltration.

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