The Comparison of TNF α (Tumor Necrosis Factor α) Serum Levels Between Cytotoxin - Associated Gene A (Cag A) Positive and Negative in Patients with Gastritis *Helicobacter pylori*

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ABSTRACT

Background: *Helicobacter pylori (H. pylori) infection is the common cause of chronic gastritis in the world that is around 80% in addition to other causes such as autoimmune diseases, drugs, idiopathic and others. The pathogenesis of H. pylori associated with virulence factors consisting of cytotoxin - associated gene A (Cag A) and vacuolating cytotoxin A (Vac A). In the case of gastritis occurred acute and chronic inflammatory responses and activation cytokines that cause inflammation of mucous which TNF-α levels increased in patients gatritis H. pylori. Levels of serum TNF-α was found higher in patients infected with H. pylori with Cag A positive. The purpose of this study is to investigate the comparison between TNF-α serum level in H. pylori gastritis patients with Cag A (+) and Cag A (-).*

Method: *The study was conducted with a cross-sectional design in 30 patients with dyspepsia, using PADYQ score. We performed gastroscopy, biopsy, and CLO test to prove the existence of H. pylori. Furthermore, we used PCR to assess Cag A (+) and Cag A (-), and ELISA method to measure TNF-α serum level.*

Results: *From 30 subjects, 18 men (60%), 12 women (40%), and the mean age was 53.5 years, the majority of the ethnic was Bataknese (53.3%), patients with H. pylori gastritis with Cag A (+) were 21 (70%) and patients with H. pylori gastritis with Cag A (-) were 9 (30%). We found the mean serum levels of TNF-α was higher (3.48) in H. pylori gastritis with Cag A (+) than the Cag A (-) (1.29) with p was 0.001.*

Conclusion: *We found increased serum levels of TNF-α in patients with Cag A (+) compared to Cag A (-) H. pylori gastritis.*

Keywords: *Gastritis, H. pylori, Cag A, TNF-α*

ABSTRAK

Latar belakang: *Infeksi Helicobacter pylori (H.pylori) merupakan penyebab tersering gastritis kronik di seluruh dunia yaitu sekitar 80% disamping penyebab lain berupa penyakit autoimun, obat-obatan, idiopatik dan lain-lain. Patogenesis H.pylori berhubungan dengan faktor virulensi yang terdiri dari cytotoxin - associated gene A (Cag A) dan vacuolating cytotoxin A (Vac A). Pada gastritis terjadi respon inflamasi akut maupun kronik dan terjadi aktivasi sitokin-sitokin yang menyebabkan terjadinya inflamasi mukosa dimana kadar TNF-α meningkat pada pasien gatritis H.pylori. Kadar serum TNF-α dijumpai lebih tinggi pada pasien yang terinfeksi H. pylori dengan Cag A positif. Tujuan penelitian ini adalah untuk mengetahui perbandingan kadar serum TNF α antara*

pasien gastritis H. pylori dengan Cag A (+) dengan Cag A (-).

Metode: *Penelitian dilakukan dengan desain cross sectional terhadap 30 penderita dyspepsia, menggunakan PADYQ skor. Selanjutnya dilakukan gastroskopi dan biopsi untuk melihat adanya H.pylori menggunakan CLO dan pemeriksaan PCR untuk menilai Cag A (+) dan Cag A (-), serta serum TNF-α dengan metoda ELISA.*

Hasil: *Dari 30 subyek, 18 orang (60%) pria, 12 orang perempuan (40%), rerata umur 53,5 tahun, mayoritas bersuku batak 16 orang (53,3%), penderita gastritis H. Pylori dengan CagA positif 21 orang (70%) dan penderita gastritis H. Pylori dengan Cag A negatif 9 orang (30%). Ditemukan rerata kadar serum TNF-α lebih tinggi (3,48) pada gastritis H.pylori dengan Cag A (+) dibandingkan gastritis H.Pylori dengan Cag A (-) (1,29) dengan (p = 0,001)*

Simpulan: *Dijumpai peningkatan kadar serum TNF-α pada penderita gastritis H.Pylori Cag A (+) dibandingkan dengan gastritis H.Pylori dengan Cag A (-)*

Kata kunci: *Gastritis, H.pylori, Cag A, TNF-α*

INTRODUCTION

H. pylori infection is a very important cause of gastritis. Prevalence of *H. pylori* infection in adults in developing countries ± 90%.[1] Indonesia research report of *H. pylori* prevalence among the lowest 10.2-64% from Jakarta and highest from Bandung. The study of seropidemology in Indonesia shows the prevalence of 36-46.1% with the youngest age of 5 months. In the younger age group under 5 years, 5.3-15.4% had been infected.[7] The pathogenicity of *H. pylori* is determined by its virulence factor known as cytotoxin-associated gene A (Cag A), which plays an important role in invasion, colonization, and cell proliferation.[2,3,4] Not all *H. pylori* contains Cag A (+), and it has been attributed that peptic ulcers are also significantly associated with Cag A (+). Treatment effects were also rated higher in patients infected with *H. pylori* with Cag A (+). Even from other studies, it was mentioned that Cag A (+) was detected in 85% of patients were examined, and most of the *H. pylori* strains with Cag A (+) tend to be esophagitis compared to Cag A (-).[3.5]

Gastritis *H. pylori* is associated with the infiltration of neutrophils and severe mononuclear cells in the gastric mucosa characterized by elevated proinflammatory cytokines such as tumor necrosis factor (TNF-α). TNF α is a proinflammatory cytokine produced primarily by macrophage activation, causing tissue damage and host leukocyte activation. Many studies have reported that TNF α is produced by *H. pylori* infection in the gastric mucosa and is involved in gastric inflammation as well as in apoptosis. In addition, *H. pylori* may also induce the production of TNF α, which is closely related to epithelial cell damage. 5.6. High serum TNF-α levels are also associated with degrees of gastritis based on lymphocyte infiltration. In addition, serum TNF-α levels were also significantly higher in patients infected with *H. pylori*.[10]

TNF α plays an important role in host defense against infection. But from the research that has been done, there is a possibility of increasing the concentration of TNF α-genotype 308, altering the immune response, resulting in susceptibility to Caga (+) gastritis *H. pylori* in patients with gastritis in Korea.[8] In addition, existing studies also revealed that TNF - α expression level of the mucosa was significantly higher in individuals with positive *H. pylori* compared with *H. pylori* (-).[2] The association between *H. pylori* virulence factor, CagA and TNF-α expression level on gastric mucosa is very little studied around the world, and has not even been done in Indonesia. Based on this information, a study was conducted to determine differences in serum TNF α levels with cytotoxin - associated gene a (Cag A) (+) and (-) in patients with *H. pylori* gastritis.

METHOD

This study was cross sectional and analytic descriptive, conducted from September 2016 till March 2017. Inclusion Criteria: Man and Non-pregnant women > 18 years old, patients with a diagnosis of gastritis H pylori, receive informed and voluntary and written participation approval to undergo a physical, laboratory, gastroscopy and biopsy examination known and approved by the Research Ethics Committee Health. Exclusion Criteria Patients who have received H.pylori eradication therapy in the past 6 months or are currently on antibiotic therapy commonly used in eradication therapy, patients taking proton pump inhibitors, H2 receptor antagonists, NSAIDs, steroids, alcohols for the last 48 hours, patients are uncooperative

The population were the patients with gastritis H pylori who came to the Endoscopic Unit of Adam Malik Hospital Medan & FK USU Hospital on September 2016-November 2016, and the samples in this study were Patients with Gastritis *H pylori* who met the inclusion and exclusion criteria consecutively sampled.

For the calculation of research sample used 10 preliminary data from preliminary study. To get the value of S and X1-X2. To determine the sample size of the research can be used the formula of calculating the sample size with unpaired numericalThis research was conducted in Endoscopy Unit of Adam Malik General Hospital Medan and General hospital of FK USU after obtaining the approval of Research Commission of Health Sector and related institutions. Conducted interviews with patients using The Porto Alegre Dyspeptic Symptoms Questionnaire (PADYQ). Patients with a total score of 6 or more were diagnosed as dyspepsia, then endoscopic examination, and biopsy were performed. To detect *H. pylori*, serology test (CLO) is performed. The CagA examination was performed by PCR, and serum TNF-α using ELISA. Data were analyzed using Mann Whitney test. The magnitude of the desired deviation (α) is 0.05. Data is processed and analyzed using SPSS program.

RESULTS

This study was followed by 30 patients who have met the inclusion criteria. A total of 18 patients (60%) were male and 12 patients (40%) were female, with an average age of 53.5 years. Cag A (+) is found on 21 people (70%) and Cag A (-) found 9 people (30%). The median value of TNF-α is 2.49. The demographic and clinical characteristics of the study subjects are shown in Table 1.

Table 1. Demographic and clinical characteristics of research subjects (n = 30)

Variabel	n (%)
Gender	
Man	18 (60) [a]
Women	12 (40)
Age	53,5 (20 – 68) [b]
Ethnic group	
Bataknese	16 (53,3) [a]
Javanese	6 (20)
Acehnese	5 (16,7)
Malay	2 (6,7)
India	1 (3,3)
Religion	
Moslem	23 (76,7) [a]
Christian	6 (20)
Hindu	1 (3,3)
Level of education	
Elementary school	3 (10) [a]
Junior high school	4 (13,3)
Senior high school	20 (66,7)
Bachelor degree	3 (10)
Occupation	
entrepreneur	14 (46,7) [a]
Housewife	12 (40)
Employee	3 (10)
College student	1 (3,3)
CagA	
Positive	21 (70) [a]
Negative	9 (30)
TNF-α serum	2,49 (0,74 – 37,76) [b]

[a]Categorical data: n (%)
[b]Numeric data, abnormal distribution: median (minimum-maximum)

In this data, the mean values of TNF-α in patients with *H. pylori* Cag A (+) were 3.48 (0.74-37.76) with p 0.001, and mean TNF-α values in patients with *H. pylori* Cag A (-) is 1.29 (0.87-2.51). It is attached in Table 2 and Figure 1.

Table 2. Comparison of serum TNF-α between *H. pylori* patients with Cag A (+) and (-)

CagA	TNF-α serum	p
Positive	3,48 (0,74-37,76)	0,001*
Negative	1,29 (0,87-2,51)	

Numerical data, abnormal distribution: median (minimum-maximum), *p < 0.05

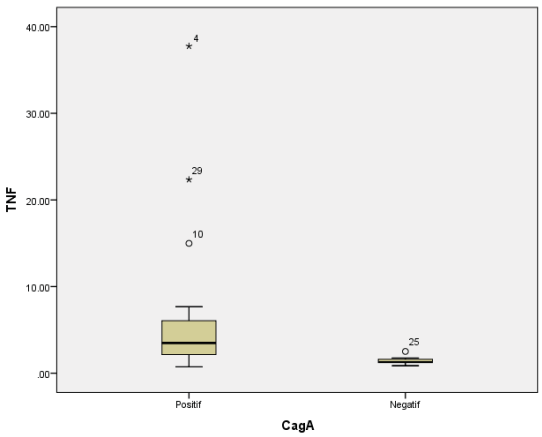

Figure 1. Diagram of TNF-α serum boxplot between patients with gastritis *H. pylori* CagA positive and negative

DISCUSSION

Of the 30 patients with positive *H. pylori* were investigated, 18 patients (60%) were men and 12 patients (40%) were women. This is in accordance with research conducted by Siregar G et al 2014 which found that of 33 samples obtained people with positive *H. pylori* and found as many as 21 patients (26.25%) were male and 12 patients (15%) is female. In a study conducted by Calvancante et al in Brazil in 2012, which examined 134 positive *H. pylori* patients, there were different outcomes, of which women were

more likely to be 65.9%, compared with men.[8] This also Supported by Zabaglia et al in 2015 in Brazil who studied 134 patients with positive *H. pylori*, and resulting 73 patients were women, while 61 patients were male.[2] Epidemiology of *H. pylori* is about 50% of the population in the women. In western countries such as USA, the prevalence of *H. pylori* is < 30% at < 30 years and> 75% by age > 60 years. In Asia, the prevalence of *H. pylori* is very high.[7] From this study, the mean age of patients was 53.5 years. This is similar to Siregar et al research in 2015 that examined 80 patients with mean age of 46-60 years. This is contrast to a study conducted in Brazil in 2015 by Zabaglia et al, where the mean age of the 134 patients studied suffering from *H. pylori* gastritis was 40.3 ± 24.2 years.[3]

The virulence factor that has been involved in the *H. pylori* Gastritis process is Cag A secreted by *H. pylori*. Based on this study, we found CagA strains in 21 patients (70%), and Cag A strains were negative in 9 patients (30%). This is similar to a study conducted by Zalewska et al in 2009 in Poland that examined 43 gastritis patients with *H. pylori* (+), found 29 patients (67.44%) with Cag A (+) and 14 patients (32.56 %) With Cag A strain (-).[4] Zhu et al stated in the In vitro study done in 2005 that Cag A acts as a potential oncoprotein.[9] In addition, Cag A (+) strain is more commonly associated with peptic ulcers, gastritis atrophy, and gastric adenocarcinoma compared to Cag A strains (-).[9] Gzyl et al reported that the prevalence of *H. pylori* strain Cag A (+) in Poland was 72.4%.[11] Research conducted by Dzierżanowska et al reported the prevalence of Cag A(+) strains encountered 60.0%.[12] A study conducted by Zalewska et al in 2004-2005 that had piled up 43 patients with chronic gastritis with positive *H. pylori* showed that 96.6% of *H. pylori* CagA (+) strains were found and associated with Vac A genotype s1, with s1a. There is a very significant relationship between positive Cag A and Vac A genotype s1.[4]

Mucosal infection rates for TNF-α were significantly higher in *H. pylori* positive patients than in *H. pylori* negative patients. TNF-α may be the key to cytokines in *H. pylori* gastritis.[14] As Alamsyah et al in the field did in 2014, states that serum TNF-α levels were significantly increased in subjects with positive *H. pylori* compared with *H. pylori* negative , whereas high serum TNF-α levels are associated with degree of gastritis based on lymphocyte infiltration.[7] The theoretical equations and results of this study have been found, indicating that a difference in TNF-α levels in Gastritis *H. pylori* with Cag A (+) compared with Gastritis *H. pylori* with Cag A (-). Whereas TNF-α was significantly higher in Gastritis *H. pylori* with Cag A positive.

There are some limitations in this study; firstly, this study does not assess the severity of gastritis in histopathology, making it difficult to serve as a diagnostic and prognostic marker in *H. pylori* gastritis. Second, the study did not assess other *H.pylori* virulence factors such as Vac A and Bab A, so TNF-α levels could not be associated with other virulence such as Vac A and Bab A from *H. pylori* gastritis. Thirdly, no genetic polymorphisms were assessed in patients who were molecularly related to various diseases including *H. pylori*.

CONCLUSION

The results of this study can be concluded that serum TNF α levels were significantly higher in patients with H. pylori gastitis with Cag A (+) compared with Cag A (-). The need for further research, with more samples, correlates with the severity of gastritis in histopathology, assessment of other virulence factors of *H. pylori* germs such as Vac A and Bab A as well as the examination of polymorphisms more specifically to see its association with elevated levels of cytokines in the blood.

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