Correlation Between TNF-α and Degree of Gastritis

Ricky Rivalino Sitepu, Darmadi, Gontar Alamsyah Siregar
Division of Gastroentero-Hepatology, Department of Internal Medicine,
Faculty of Medicine, Universitas Sumatera Utara/Adam Malik General Hospital, Medan

Corresponding author:
Gontar Alamsyah Siregar. Division of Gastroentero-hepatology, Department of Internal Medicine, Universitas Sumatera Utara. Jl. Bunga Lau No. 17 Medan Indonesia. Phone/facsimile: +62-61-8365742. E-mail: gontarsiregar@gmail.com

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 dini 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. 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.

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. 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. 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/m². 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  |       |
| Batakansese   | 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/m²) |       |
| < 18.5        | 23.59 (3.76)* |
| 18.5 – 24.9   | 7 (8.75) |
| ≥ 25          | 42 (52.5) |
| Campylobacter like organism (CLO) |       |
| Negative      | 47 (58.75) |
| Positive      | 33 (41.25) |
| TNF-α mean value |       |
| minimum       | 1.13 |
| maximum       | 15.88 |

*mean
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 | \( p \)  | \( r \)  |
|--------------------|---------|---------|
| Lymphocyte infiltration | 0.003  | 0.333  |
| Neutrophil infiltration | 0.088  | 0.186  |
| Atrophy            | 0.239  | 0.133  |
| Intestinal metaplasia | 0.121  | 0.175  |

\( *p < 0.05 \)

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.\(^\text{15-17}\)

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.\(^\text{10,18,19}\)

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.\(^\text{20-24}\) 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.\(^\text{12,13}\) However, studies from Bayraktaroğlu et al and Abdollahi et al which assessed serum TNF-α showed no correlation with the severity of gastritis.\(^\text{25,26}\) Klausz et al also stated that TNF-α serum did not affect the severity of duodenal ulcers.\(^\text{27}\) 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.

REFERENCES

1. El-Zimaity H. Recent advances in the histopathology of gastritis. Curr Diag Pathol 2007;13:340-8.
2. Rugge M, Genta RM. Staging and grading of chronic gastritis. Human Pathol 2005;36:228-33.
3. Crabtree J. Role of cytokines in pathogenesis of Helicobacter pylori-induced mucosal damage. Dig Dis Sci 1998;43:46-55.
4. Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. J Clin Invest 2007;117:60.
5. Cesar ACG, Cury PM, Payao SLM. Comparison of histological and molecular diagnosis of Helicobacter pylori in benign lesions and gastric adenocarcinoma. Brazilian J Microbiol 2005;36:12-6.
6. Rehnberg-Laiho L, Rautelin H, Koskela P, Sarna S, Pukkala E, Aromaa A, et al. Decreasing prevalence of helicobacter antibodies in Finland, with reference to the decreasing incidence of gastric cancer. Epidemiol Infect 2001;126:37-42.
7. Matsukura N, Yamada S, Kato S, Tomtitchong P, Tajiri T, Miki M, et al. Genetic differences in interleukin-1 betapolymermorphisms among four Asian populations: an analysis of the Asian paradox between H. pylori infection and gastric cancer incidence. J Exp Clin Cancer Res 2003;22:47-55.
8. 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;30:83-8.
9. Andersen LP, Holck S, Janulaityte-Gu’tner d, Kucpienskas L, Kiudelis G, Jonaitis L, et al. Gastric inflammatory markers and interleukins in patients with functional dyspepsia, with and without Helicobacter pylori infection. FEMS Immunol and Medical Microbiol 2005;44:233-8.
10. Perri F, Clemente R, Festa V, De Ambroisio CC, Quitadamo M, Fusillo M, et al. Serum tumour necrosis factor-alpha is increased in patients with Helicobacter pylori infection and CagA antibodies. Italian journal of gastroenterology and hepatology 1999;31:290-4.
11. Noach LA, Bosma NB, Jansen J, Hook FJ, Van Deventer SJH, Tytgat GNJ, et al. Mucosal Tumor Necrosis Factor-α, Interleukin-1/3, and Interleukin-8 Production in Patients with Helicobacter pylori Infection. Scan J Gastroenterol 1994;29:425-9.
12. Yamaoka Y, Kita M, Kodama T, Sawai N, Kashima K, Imanishi J. Induction of various cytokines and development of severe mucosal inflammation by cagA gene positive Helicobacter pylori strains. Gut 1997;41:442-51.
13. Bauditz J, Ortnr M, Bierbaum M, Niedobitek G, Lochs H, Schreiber S. Production of IL-12 in gastritis relates to infection with Helicobacter pylori. Clin Exp Immunol 1999;117:316-23.
14. Dixon MF, Genta RM, Yardley H, Correa P. Classification and grading of gastritis: the updated Sydney system. Am J Surg Pathol 1996;20:1161–81.
15. Garg B, Sandhu V, Sood N, Sood A, Malhotra V. Histopathological analysis of chronic gastritis and correlation of pathological features with each other and with endoscopic findings. Pol J Pathol 2012;3:172-8.
16. Zhang C, Yamada N, Wu YL, Wen M, Matsuhita T, Matsukura N. Helicobacter pylori infection, glandular atrophy and intestinal metaplasia in superficial gastritis, gastric erosion, erosive gastritis, gastric ulcer and early gastric cancer. World J Gastroenterol 2005;11:791-6.
17. Hashemi MR, Rahnavardi M, Bikdeli B. H. pylori infection among 1000 southern Iranian dyspeptic patients. World J Gastroenterol 2006;12:5479-82.
18. Russo F, Jirillo E, Clemente C, Messa C, Chiloiro M, Ricizzo G, et al. Circulating cytokines and gastrin levels in asymptomatic subjects infected by Helicobacter pylori. Immunopharmacoimmunotoxicol 2001;23:13-24.
19. Collodel G, Moretti E, Campagna MS, Capitani S, Lenzi C, Figura N. Infection by CagA-positive Helicobacter pylori strains may contribute to alter the sperm quality of men with fertility disorders and increase the systemic levels of TNF-α. Dig Dis Sci 2010;55:94-100.
20. Parham P. Activation of resident macrophages induces inflammation at sites of infection. The Immune System. Garland Science 2014.p.49-57.
21. Deltrick B, Naginenci C, Hooks J. Cytokines: Regulators of Immune Responses and Key Therapeutic Targets. In: MRGOGaAD Donnenberg. Human Immunology. CRC Press, Taylor & Francis Group, 2008.p.495-516.
22. Baratawidjaja KG. Sitokin. Imunologi Dasar. Jakarta: FKUI, 2006.p.119-35.
23. Bodger K, Wyatt J, Heatley R. Gastric mucosal secretion of interleukin-10: relations to histopathology, Helicobacter pylori status, and tumour necrosis factor-alpha secretion. Gut 1997;40:739-44.
24. 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. Current Topics in Gastritis, 2012.p.114.
25. Abdollahi H, Shams S, Zahedi MJ, Darvish MS, Hayatbakhsh MM, Jafarzadeh A. IL-10, TNF-α and IFN-γ levels in serum and stomach mucosa of Helicobacter pylori-infected patients. Iran J Allergy Asthma Immunol 2011;10:267-71.
26. Bayraktaroğlu T, Aras AS, Aydemir S, Dayutoglu C, Ustundag Y, Atmaca H, et al. Serum levels of tumor necrosis factor-α, interleukin-6 and interleukin-8 are not increased in dyspeptic patients with Helicobacter pylori-associated gastritis. Mediators Inflamm 2004;13:25-8.
27. Klausz G, Tiszlavicz L, Gyulai Z, Lonovics F, Ikonomoudis N, MrGOGA AD Donnenberg. Human Immunology. CRC Press, Taylor & Francis Group, 2014.p.49-57.
28. Hall PowerPoint presentation.