

Gastric Mucosa Plasma Cells is Unspecific for Diagnosing *Helicobacter pylori* Infection

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ABSTRACT

Background: A high density of *Helicobacter pylori* is believed to trigger an accumulation of plasma cells in the gastric mucosa. This accumulation stimulated the production of antibodies, causing high antibodies titer being circulated in the blood. The aims of this study is to identify the correlation between the number of plasma cells and *H. pylori* density in gastric mucosa.

Method: This observational analytic study was performed with cross-sectional approach. The samples were the paraffin blocks which consisted of endoscopic gastric biopsy tissues of chronic gastritis patient in Anatomic Pathology Laboratory Dr. Soetomo General Hospital Surabaya in 2017 period. A total of 30 samples were purposively collected. Endoscopic gastric biopsy tissues were stained by two stains, Haematoxylin-Eosin and Modified Giemsa. The examination was performed by experienced pathologist. The correlation between total plasma cells and *H. pylori* density in gastric mucosa and the difference of total plasma cells between gastric mucosae with different density of *H. pylori* were determined.

Results: There was no significant correlation between total plasma cells and *H. pylori* density in gastric mucosa. And there was no significant difference of the number of plasma cells found with different density of *H. pylori*.

Conclusion: The number of plasma cells in the gastric mucosa is unspecific for diagnosing *H. pylori* infection. Other causes associated with plasma cells need to be assessed in further studies.

Keywords: *Helicobacter pylori*, plasma cell, antibody, gastric mucosa, chronic gastritis

ABSTRAK

Latar belakang: Peningkatan densitas *Helicobacter pylori* dipercaya memicu peningkatan jumlah sel plasma yang menetap di jaringan mukosa gaster sebagai salah satu sel inflamasi. Akumulasi sel plasma menghasilkan titer antibodi tinggi yang bersirkulasi di dalam darah. Peneliti bertujuan untuk menemukan korelasi antara jumlah sel plasma dengan densitas *H. pylori* pada mukosa gaster.

Metode: Penelitian analitik observasional ini dilakukan dengan pendekatan cross-sectional. Penelitian ini menggunakan sampel blok parafin berisi jaringan biopsi endoskopi gaster dengan gastritis kronis pada tahun

2017 di Instalasi Patologi Anatomi Rumah Sakit Umum Daerah (RSUD) Dr. Soetomo Surabaya, dengan besar sampel sebesar 30 buah yang dihitung dengan rumus Taro Yamane. Jaringan biopsi endoskopi gaster dengan gastritis kronis dipulas dengan teknik pewarnaan Hematoksin-Eosin dan Modified Giemsa. Penghitungan dilakukan oleh ahli patologi yang berpengalaman. Uji Kendall's tau-b digunakan untuk menentukan hubungan antara jumlah sel plasma dengan densitas *H. pylori* pada mukosa gaster, dengan nilai signifikansi sebesar 0,05. Untuk menentukan perbedaan jumlah sel plasma antara mukosa gaster yang memiliki perbedaan tingkat densitas kolonisasi *H. pylori*, One-Way ANOVA digunakan dengan nilai signifikansi yang sama seperti sebelumnya.

Hasil: Tidak ada hubungan yang signifikan secara statistik antara jumlah sel plasma dengan densitas *H. pylori* pada mukosa gaster ($p = 0,536$). Dan tidak ditemukan perbedaan jumlah sel plasma yang signifikan secara statistik antara mukosa gaster yang memiliki perbedaan tingkat densitas kolonisasi *H. pylori* ($p = 0,944$).

Simpulan: Sel plasma yang menetap di jaringan mukosa gaster tidak spesifik untuk mendiagnosis infeksi *H. pylori*. Penyebab lain yang berhubungan dengan peningkatan jumlah sel plasma perlu diteliti lebih lanjut.

Kata kunci: *Helicobacter pylori*, sel plasma, antibodi, mukosa gaster, gastritis kronis

INTRODUCTION

Estimatedly 90% of *Helicobacter pylori* infection doesn't give its visible symptoms, while 10% shows its symptoms which are needed to be handled immediately.¹ *H. pylori* is able to live in unfriendly environment of stomach for a long time.² The entrance of *H. pylori* into stomach triggers the immune system to respond by activating the inflammatory reaction. This reaction will not stop until *H. pylori* is fully eradicated.³ Various kinds of cell and mediator are involved in this inflammatory reaction. Mediators, such as interleukin-8 (IL-8), interleukin-1 β (IL-1 β), tumor necrosis factor α (TNF α), interleukin-6 (IL-6), and interleukin-12 (IL-12), are released when stomach lining contacted with *H. pylori* through pattern recognition receptor on the gastric epithelium. These mediators attract neutrophils, macrophages, dendritic cells, natural killer cells, and lymphocytes to come abundantly to the location of *H. pylori* infection.⁴ As antigen presenting cell, dendritic cell is able to initiate the role of T lymphocyte as the effector of cellular adaptive immune response.⁵ One of the T lymphocytes which is usually initiated is CD4+ T cell, which is known as T helper cell. This T helper cell contributes in the activation of B lymphocyte to differentiate as plasma cells, the antibody-producing cells.⁶

As the effector of humoral adaptive immune response, plasma cell is produced by the body in peripheral lymphoid organs and migrates to the gastric mucosa through circulatory system.⁶ Plasma cell is able to produce three different isotypes of antibody: immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA). Each of these antibodies has its own correlation with *H. pylori* density in gastric mucosa. A high anti-*H. pylori* IgM titer in the blood

serum indicate the colonization of *H. pylori* has just occurred in the stomach,⁷ while a high anti-*H. pylori* IgG titer is observed following the escalation of *H. pylori* density in the gastric mucosa.^{8,9} Anti-*H. pylori* IgA titre in blood serum is high in the presence of mild inflammation in the stomach lining.¹⁰

Continuous inflammatory reaction caused by *H. pylori* infection may lead to chronic gastritis.² A high density of *H. pylori* is believed to trigger an accumulation of plasma cells in the gastric mucosa as one of the inflammatory cells. This accumulation produces a high titer of antibodies to circulate in the blood system. Therefore, we determined the correlation between total plasma cells and *H. pylori* density in the gastric mucosa.

METHOD

This observational analytic study was performed with a cross-sectional approach. The population of this study consist of all paraffin block of the endoscopic gastric biopsy tissues from chronic gastritis patients in Anatomical Pathology Department, Dr Soetomo General Hospital Surabaya. The samples were the paraffin blocks in the year 2017 and a total of 30 samples were purposively collected based on Taro Yamane equation. Every paraffin block was cut twice into 5 micrometres thick using a microtome. Then, it was placed on microscopic slide after the paraffin wax was dissolved using xylene. This study has been approved by ethics commission of Faculty of Medicine Universitas Airlangga/Dr. Soetomo General Hospital Surabaya (No. 0715/KEPK/X/2018).

As the independent variable of this study, *H. pylori* density was counted by a semi-quantitative visual analogue scale from Updated Sydney System.

Previously, endoscopic gastric biopsy tissues of chronic gastritis patients were stained with Modified Giemsa. We took 5 mL of Azur-Eosin-Methylene Blue according to Giemsa, modified solution and diluted in 50 ml of a pH 7.2 buffer solution. After homogenization, we poured the solution over the slides for 25 minutes. Then, we washed the slide twice with pH 7.2 buffer solution for 1 minute. After being dried, the examination of *H. pylori* was performed through light microscope using x40 objective lens. All high-power fields were examined.

Endoscopic gastric biopsy tissues of chronic gastritis patients were stained with Haematoxylin-Eosin in order to count the total plasma cells as the dependent variable of this study. The slides stained in Harris haematoxylin solution for 8 minutes and eosin-phloxine solution for 1 minute. The examination was performed through light microscope using x40 objective lens. Only one high-power field with the most cells were examined and the exact number of cells was counted.

The data of this study was analysed using SPSS Statistics 17.0. Kendall's tau-b test was used to determine the correlation between total plasma cells and *H. pylori* density in the gastric mucosa, with 0.05 as the value of significance level. To determine the difference of total plasma cells between positive and negative *H. pylori* density, Shapiro-Wilk was used as the normality test followed by One-Way ANOVA test, using the same previous value of significance level.

RESULTS

The average age of chronic gastritis patients in this study was 48.80 years with standard deviation of 14.356. The minimum age in this study was 18 years and the maximum age reached 74 years. Out of 30 samples, 17 (56.7%) were female chronic gastritis patients.

Table 1. Frequency of *H. pylori* density

Density	n (%)
Normal	9 (30.0)
Mild	14 (46.7)
Moderate	5 (16.7)
Marked	2 (6.7)
Total	30 (100.0)

Female patients were predominant in most of the categories of *H. pylori* density including 5 out of 9 patients (55.6%) in normal *H. pylori* density, 8 out of 14 patients (57.1%) in mild *H. pylori* density, and 3 out of 5 patients (60.0%) in moderate *H. pylori* density.

An equal number of patients between male and female patients was observed in marked *H. pylori* density, although there was no statistical significant correlation between gender and *H. pylori* density in gastric mucosa ($p = 0.996$). *H. pylori* was observed in spiral form and in blue-greyish colour. The histological appearances are shown in Figure 1.



Figure 1. *H. pylori* were examined through light microscope using x40 objective lens with Modified Giemsa (Diff Quik) staining. Circle and red arrow = *H. pylori*

The average of total plasma cells in this study was 17.30 cells with a standard deviation of 5.838. The minimum number of total plasma cells was 9 cells and the maximum number reached 31 cells.

Table 2. Frequency of total plasma cells

Total Plasma Cells	n (%)
6 – 10	4 (13.3)
11 – 15	10 (33.3)
16 – 20	6 (20.0)
21 – 25	7 (23.3)
26 – 30	2 (6.7)
31 – 35	1 (3.3)
Total	30 (100.0)

No statistical significant correlation between total plasma cells and *H. pylori* density in gastric mucosa ($p = 0.536$) was observed using Kendall's tau-b test as one of the non-parametric correlation tests. The distribution of total plasma cells was normal in this study, although there was no statistical significant difference of total plasma cells between positive and negative *H. pylori* density ($p = 0.944$) using One-Way ANOVA test. Plasma cell appear as a cell with eccentric nucleus and perinuclear halo. The histological appearances are shown in Figure 2.

In this study, we revealed that there was no statistically significant correlation between total plasma cells and *H. pylori* density, thus plasma cells in the gastric mucosa is unspecific for diagnosing *H.*

pylori infection. Additional information of patients, such as eating habits, lifestyle, and drug consumption, including non-steroidal anti-inflammatory drug (NSAID), were not assessed in this study. Several other factors such as sex, smoking habits, consuming irritant foods, high-risk occupation, experiencing stress in academics and personal matters also can interfere this study result.¹¹ However, no further analysis could be performed to determine the correlation between total plasma cells and other related factors related. Total mononuclear cells in the gastric mucosa is defined normal if there are only 2-5 lymphocytes, plasma cells, and macrophages in one high-power field.¹² In this study, plasma cells exceeded the normal margin in all endoscopic gastric biopsy tissue. We concluded the escalation of plasma cells was observed in this study.

In this study, the average age of chronic gastritis patients was almost similar with the previous study conducted in Dr. Sardjito General Hospital, Yogyakarta.¹³ Female chronic gastritis patients dominated in this study with a total of 17 out of 30 patients (56.7%). The similar results were found in the study conducted in Iran with female domination reached 51.84%, but no statistical significant correlation between gender and *H. pylori* density in the gastric mucosa was found.¹² This study used two different staining methods, Modified Giemsa and Haematoxylin-Eosin, as recommended by Lee and Kim.¹⁴ Even though a study confirmed that Warthin-Starry is the best staining because of its high sensitivity and specificity which reached almost 100%, Modified Giemsa and Haematoxylin-Eosin are the most suitable staining for this study for its affordability and ease use of technique.^{15,16}

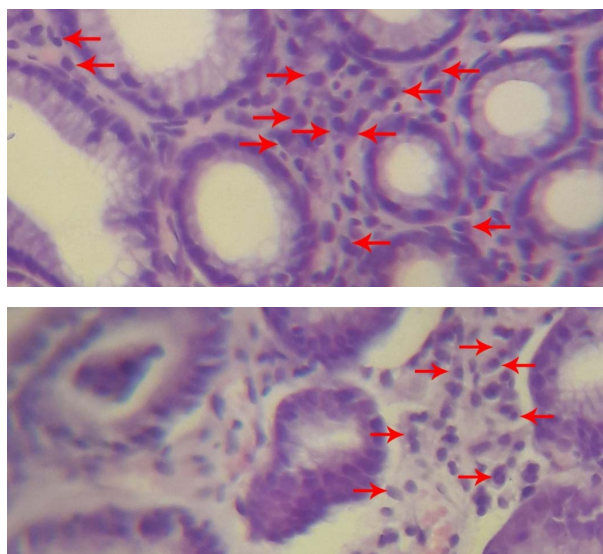


Figure 2. Plasma cells were examined through light microscope using x40 objective lens with Haematoxylin-Eosin (HE) staining. Arrow = plasma cell.

It was found that 9 out of 30 samples were negative *H. pylori* density in endoscopic gastric biopsy tissues of chronic gastritis patient in this study. This happened because there are still uncertain reasons why Indonesia, as one of the developing countries, has a low prevalence of *H. pylori* infection.¹⁷ In Surabaya itself, the prevalence of *H. pylori* infection was 11.5% after going through five different methods of diagnosing.¹⁸ However, a higher prevalence of *H. pylori* infection was found in several Indonesia's ethnic groups, such as Papuan, Bugis, and Batak.¹⁷

The limitation of this study was that the counting methods used for total plasma cells was less specific for *H. pylori* infection. Therefore, further studies with better counting methods are needed.

CONCLUSION

Plasma cells in the gastric mucosa was unspecific for diagnosing *Helicobacter pylori* infection and it was the reason why there was no statistical significant correlation between total plasma cells and *H. pylori* density. Other causes associated with plasma cells are needed to be assessed in further studies.

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