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**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 gastric mucosa as one of the inflammatory cells due to its
high titer antibodies production circulated in blood system. We aimed to identify the
correlation between total plasma cells and *H. pylori* density in gastric mucosa.*

Method: Endoscopic gastric biopsy tissues were stained by two different stains, Haematoxylin-Eosin and Modified Giemsa. The examination was performed by experienced pathologist.

Results: The average age of chronic gastritis patients in this study was 48.80 years with standard deviation of 14.356. Out of 30 samples, 17 (56.7%) were female chronic gastritis patients. Female patients were dominating in most of 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. The average of total plasma cells in this study was 17.30 cells with standard deviation of 5.838. Importantly, there was no statistically significant correlation between total plasma cells and *H. pylori* density in gastric mucosa (P value = 0.536). Although the distribution of total plasma cells was normal, there was no statistically significant difference of total plasma cells between positive and negative *H. pylori* density (P value = 0.944).

Conclusion: Plasma cells in gastric mucosa is unspecific for diagnosing *Helicobacter pylori* infection. Other causes associated with plasma cells are needed to be assessed for further study.

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

INTRODUCTION

Helicobacter pylori infection is a worldwide problem to be solved because almost of each population is infected by the bacterium, whether they realize it or not. The infection is not recognizable easily because most of infection is not exhibit the visible symptoms in the stomach. Estimatedly 90% of the 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 CD4⁺ T cell is one of the T lymphocytes which is usually initiated and known as T helper cell. T helper cell contributes in the activation of B lymphocyte to differentiate as plasma cell, the antibody-producing cell.⁶

As the effector of humoral adaptive immune response, plasma cell is produced by the body in peripheral lymphoid organs and went 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 titer of anti-*H. pylori* IgM in the blood serum indicated the colonization of *H. pylori* has just happened in the stomach.⁷ While anti-*H. pylori* IgG is found in a high titer following the escalation of *H. pylori* density in gastric mucosa.^{8,9} Titer of anti-*H. pylori* IgA in blood serum was high when mild inflammation is presented in stomach lining.¹⁰

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

MATERIALS AND METHODS

Population and sample

This observational analytic study was performed with cross-sectional approach. The population of this study included every paraffin block which consisted of endoscopic gastric biopsy tissues of chronic gastritis patient in Anatomical Pathology Installation Dr. Soetomo General Hospital Surabaya. The samples were the paraffin blocks of 2017 period and a total of 30 samples were purposively collected by Taro Yamane equation. Every paraffin block was cut twice into 5 micrometers thick by microtome. Then it was placed on microscopic slide after the paraffin wax got dissolved by alcohol. This study has obtained approval of ethical clearance from ethics commission of Faculty of Medicine Universitas Airlangga/Dr. Soetomo General Hospital Surabaya (No. 0715/KEPK/X/2018).

Counting of *H. pylori* density

As the independent variable of this study, *H. pylori* density was counted by a semiquantitative visual analogue scale from Updated Sydney System. Previously, endoscopic gastric biopsy tissues of chronic gastritis patient 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 it went homogenized, we poured it over the slides for 25 minutes. Then washed it twice with pH 7.2 buffer solution for 1 minute each. After it dried, the examination of *H. pylori* was done through light microscope using x40 objective lens. All high-power fields were examined.

Counting of total plasma cells

Endoscopic gastric biopsy tissues of chronic gastritis patient were stained with Haematoxylin-Eosin in order to count total plasma cells as the dependent variable of this study. The slides ²⁷ stained in Harris haematoxylin solution for 8 minutes. Then it went ⁸ differentiated in 1% acid alcohol for 30 seconds after it got washed with tap water in 5 minutes. The slides went washed again with tap water for 1 minute then ⁴ blued in 0.2% ammonia water for 30 seconds. It got washed again for 5 minutes before getting rinse in ⁴ 95% alcohol. We applied counterstain in eosin-phloxine solution to the slides for 1 minute then it went dehydrated through 95% alcohol for 5 minutes. 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 counted.

²² Statistical analysis

The data of this study was analyzed with SPSS Statistics 17.0. ¹ Kendall's tau-b test was used to determine the correlation between total plasma cells and *H. pylori* density in 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	Frequency	Percentage
Normal	9	30.0
Mild	14	46.7
Moderate	5	16.7
Marked	2	6.7
Total	30	100.0

Female patients were dominating in most of 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 5 patients (60.0%) in moderate *H. pylori* density. An equal number of patients between male and female patients was happened in marked *H. pylori* density. Although there was no statistically significant correlation between gender and *H. pylori* density in gastric mucosa (P value = 0.996). *H. pylori* will be appeared in spiral form and blue-greyish colored. 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. Red circle and red arrow=*H. pylori*.

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

Table 2. Frequency of total plasma cells

Total Plasma Cells	Frequency	Percentage
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

¹ There was no statistically significant correlation between total plasma cells and *H. pylori* density in gastric mucosa (P value = 0.536) using Kendall's tau-b test as one of non-parametric correlation tests. The distribution of total plasma cells was found normal ¹⁷ in this study, although there was no statistically significant difference of total plasma cells between positive and negative *H. pylori* density (P value = 0.944) using One-Way ANOVA test. Plasma cell will be appeared as a cell with eccentric nucleus and perinuclear halo. The histological appearances are shown in Figure 2.

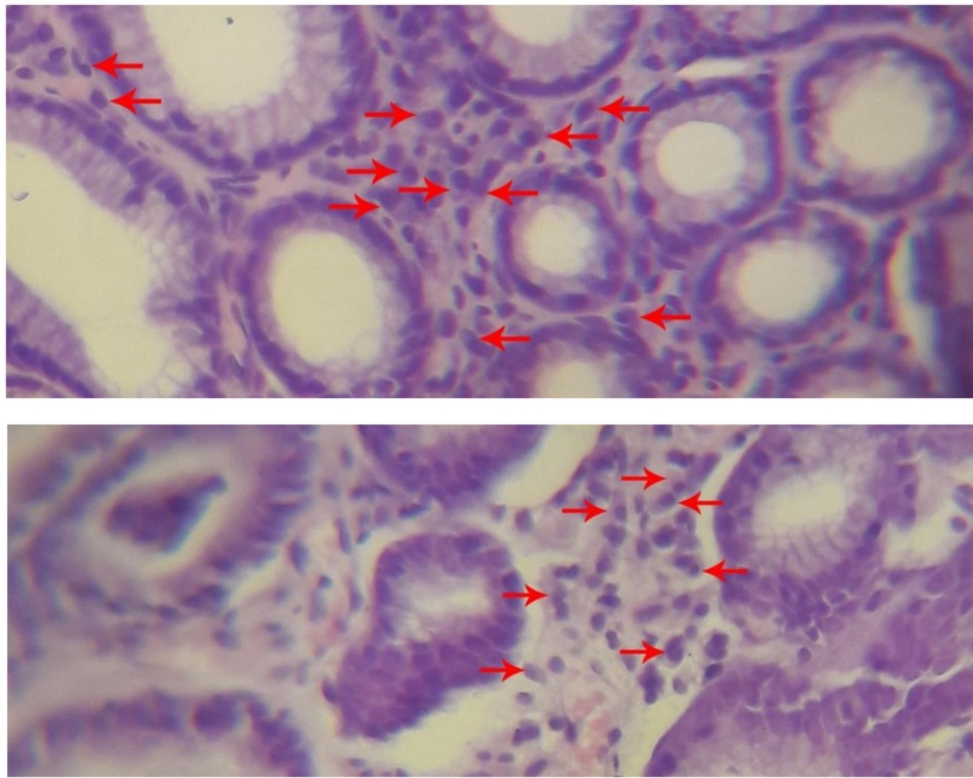


Figure 2. Plasma cells were examined through light microscope using x40 objective lens with Haematoxylin-Eosin (HE) staining. Red arrow=plasma cell.

DISCUSSION

In this study, we revealed that there was no statistically significant correlation between total plasma cells and *H. pylori* density, thus plasma cells in 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 tend to make the students had a greater risk of having gastritis.¹¹ However, no further analyzing can be performed to determine the correlation between total plasma cells and other factors related. Total mononuclear cells in gastric mucosa is defined normal

if there are only ¹³ 2-5 lymphocytes, plasma cells, and macrophages in one high-power field.¹² In this study, plasma cells were found exceeding the normal margin in every endoscopic gastric biopsy tissue. We concluded the escalation of plasma cells was happening in this study.

²⁰ In this study, the average age of chronic gastritis patients was almost similar with previous study conducted in Dr. Sardjito General Hospital, Yogyakarta.¹³ Female chronic gastritis patients were dominating in this study with 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% and still ¹⁹ no statistically significant correlation was found between gender and *H. pylori* density in gastric mucosa.¹² The research used two different stains, 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 sensitivity and specificity reached almost 100%, but Modified Giemsa and Haematoxylin-Eosin are the most suitable staining for this research because of its affordable cost and simple technique.^{15,16}

It's been found 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 ⁹ 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 ¹² high 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 is the counting methods used for total plasma cells is less specific for *H. pylori* infection. Therefore, a further study needed to be performed with better counting methods.

CONCLUSION

Plasma cells in gastric mucosa is unspecific for diagnosing *Helicobacter pylori* infection and it's the reason why ¹ there was no statistically significant correlation between total plasma cells and *H. pylori* density. Other causes associated with plasma cells are needed to be assessed for further study.

ACKNOWLEDGMENT

There is no acknowledgment.

¹⁵

CONFLICT OF INTEREST

There is no conflict of interest.

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