Are Mucosa CD4+/CD8+ T-Cells Expressions Correlated with the Endoscopic Appearance of Chronic Gastritis Related with *Helicobacter pylori* Infection?

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Abstract- Local inflammatory processes in the gastric mucosa are followed by extensive immune cell infiltration, resulting in chronic active gastritis characterized by a marked infiltration of T(h)1 cytokine-producing CD4+ and CD8+T-cells Objective. To investigate the correlation between CD4+/CD8+ T-cells in gastric mucosa with endoscopic appearance in chronic gastritis with or without *H.pylori* infection. Prospective, cross sectional study is performed in a chronic dyspepsia population in July-November 2009 at Dr. Sardjito General Hospital Yogyakarta, Indonesia. The update Sydney system was used to analyze the gastroscopy appearance. Biopsy specimens were stained with HE-stain and IHC-stain. Data were analyzed by t-test, Mann-Whitney and Spearman correlation test. Number of 88 consecutive subjects are enrolled the study (50% male; 50% female), age 46±15 years; 25% *H.pylori* positive. The expression of CD4+ and CD8+ were higher in *H.pylori* negative subjects, but only the CD4+ was significant (P=0.011). A significant correlation was found between CD4+ and CD8+ in both subjects (r(Hp+)=0.62 and r(Hp-)=0.68; P<0.05). The expression of CD4+ and CD8+ in *H.pylori* positive showed a significant correlation with gastric lesions (r(CD4+)=-0.60; r(CD8+)=-0.42 ; P<0.05), only erosion showed a significant difference in both subjects. A positive correlation was found between CD4+ and CD8+ infiltration in both subjects with or without *H.pylori* infection, and a negative correlation was only found between gastric lesion with CD4+ and CD8+ infiltration in *H.pylori* subject.

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Keywords: CD4+/CD8+ T-cells; *H. pylori*; Gastric lesion; Chronic gastritis

Introduction

Dyspepsia is the most common problem encountered in gastroenterology practice. Multiple and diverse causes influence structural disorders, treatment, degree of inflammation and metabolic disorders (1). Helicobacter pylori (*H.pylori*) is the most important cause of chronic active gastritis, gastro-duodenal ulcers and plays an important role in the development of gastric cancer and mucosa-associated lymphatic tissue (MALT) lymphomas. Helicobacter pylori colonizes in approximately 50% of the world's population, resulting in persistent stomach inflammation in infected individuals (1,2). In Asia, the prevalence of *H.pylori* infection varies markedly in different Asian countries. Higher prevalence rates are found in developing Asian countries while lower rates have been reported in more industrialized and developed countries (3). Prevalence of *H.pylori* infection at Yogyakarta, Indonesia, based on positive IgG *H.pylori*, is lower than Japanese people (5% vs. 62% in men and 4% vs. 57% women) (4).

Persistent gastro-duodenal infection causes the *H.pylori* to produce humoral and cellular immune response (5-7). Mucosal inflammation in acute *H.pylori* infection is a response of humoral immune (involves IgM) caused by invasive *H.pylori* or water-soluble proteins passing the mucosal barrier. Acute infection of *H.pylori* may induce mucosal infiltration dominated by neutrophils, and within a few weeks develops into chronic active inflammations dominated by neutrophils, macrophages, lymphocytes, and plasma cells. High expressions of CD4+ T-cells gastric mucosa are usually present in *H.pylori* acute infections as a dominant role of regulating or suppressing local inflammation. CD8+ T-

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cells take a role in initiating and maintaining gastric inflammation. Both CD4+ and CD8+ T-cells are present in chronic active *H.pylori* infection (8-10).

There are few studies that are concerned in investigating the correlation between objective parameters such as endoscopic features and pathological examinations. To further explore the correlation between CD4+and CD8+ T-cell infiltration in gastric mucosa with endoscopic features in patients with chronic dyspepsia *H.pylori* infection, a clinical investigation was designed with an analytical method as proposed in this study.

Materials and Methods

Patients

A total of 88 consecutive adult patients with chronic gastritis, who were diagnosed through gastroduodenocopy and gastric mucosal biopsy, were included in the study. The study was conducted at the Dr. Sardjito General Hospital Yogyakarta Indonesia on July-November 2009. Among them, 44 were males and 44 were females, with a mean of age 46±15 years. The exclusion criteria were subjects who suffered from severe sepsis, diabetes mellitus, liver failure, renal failure, and malignancy. All patients were given informed consent regarding the procedure and their participation in the study before the gastroduodenoscopy and biopsy examinations were conducted.

Detection of gastric mucosa lesion

Gastroduodenoscopy (Video endoscope Fujinon® EG-530WR) examination was used to evaluate gastric mucosa lesions and performed by expert gastroenterologists. The severity of the gastric lesions was analyzed using the Update Sydney System classification, such as: normal, flare, exudation, even erosion, bulging erosion, hemorrhagic flux, atrophic, hyperplasia of mucosal fold and ulcer. The frequency of the endoscopic features from each lesion was calculated for further statistical analysis (11,12).

Histological diagnosis of H. pylori infection and grading (staging) of chronic gastritis

Three specimens (2 from the antrum and 1 from the corpus) of gastric mucosal biopsy were obtained from each subject via endoscopy. The presence of *H.pylori* was determined by pathological staining with hematoxylin and eosin (HE) followed by Giemsa. Under a microscope, *H.pylori* could be observed as a typical curve like "S". The results of the histology examination were confirmed by expert pathologists. The semi quantitative scoring system advocated in the Update Sydney Systems for grading and staging of spectrum gastric lesions was used (13,14).

Detection of CD4 and CD8 cells expression in gastric mucosa

Samples of gastric mucosa tissues were taken by gastric mucosa biopsy from the antrum (2 specimens) and the corpus (1 specimen) of each patient. Immunohistochemical (IHC) assay was used to detect the infiltration of CD4+ and CD8+ T-cells in gastric mucosa sections. Positive granules could be observed and the average of positive granules of three samples from Q-win DC100image analysis (400x) was used for further statistical analysis. The IHC staining of CD4+ and CD8+ in gastric biopsy are shown in (Figure 1) and (Figure 2).

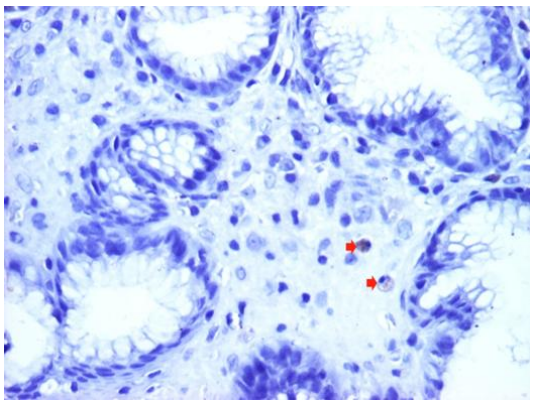

Figure 1. $CD4^+$ expression by IHC staining in the lymphocyte (red arrows) from gastric biopsy (400x)

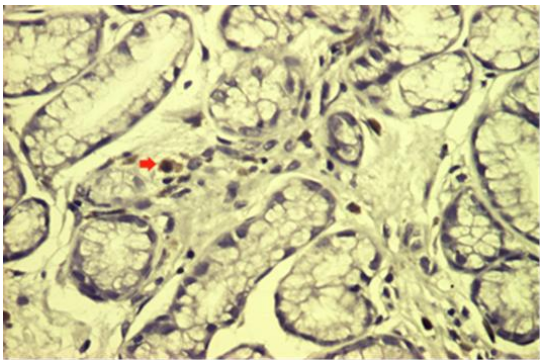

Figure 2. CD8+ expression by IHC staining in the lymphocyte (red arrow) from gastric biopsy (400x)

Statistical analysis

The data were analyzed using the computer system (IBM SPSS statistic version 20.0). Based on the distribution sample test by Kolmogorov-Smirnov test, the data were analyzed by the t-test (normal distribution) and the Mann-WhitneyU-test (not normal distribution). The Spearman's Rho correlation test was used for analyzing the correlation between CD4+ and CD8+ gastric mucosal expression with the endoscopic features. The $P<0.05$ were considered to denote statistical significance.

The Medical and Health Research Ethic Commission of the Medical Faculty of Gadjah Mada University Indonesia approved the study protocol.

Results

Among 88 subjects, the endoscopy features of 75 subjects were pan-gastritis (85.2%), 10 subjects (11.4%) antrum gastritis, 2 subjects (2.3%) normal endoscopic features, and 1 subject (1.1%) corpus gastritis. Based on The Update Sydney system criteria, the mucosal lesion severity of subjects were 69.3% erosion, 18.2% hyperemia, 10.2% atrophic and 21.6% ulcer, respectively. The histological examination (HE and Giemsa staining) showed 22 specimens (25%) to be *H.pylori* positive. There was no statistical difference between the endoscopic features (topography and gastric lesion) of the *H.pylori* positive and negative subjects with $P>0.05$. The frequency of peptic ulcer in *H.pylori* positive subjects was lower than *H.pylori* the negative subjects (13.6% vs. 24.2% with $P>0.05$) (Table 1).

Table 1. The endoscopic feature in *H.pylori* (+) and *H.pylori* (-) subjects

Endoscopic features	Total (n=88)	*H.pylori* (+) (n=22)	*H.pylori* (-) (n=66)	p^*
Topography				
- **Antral gastritis**	10 (11.4%)	3 (13.6%)	7 (10.6%)	0.231
- **Corpus gastritis**	1 (1.1%)	1 (4.5%)	0 (0%)	
- **Pan gastritis**	75 (85.2%)	18 (81.8%)	57 (86.4%)	
- **Normal**	2 (2.3%)	0 (0%)	2 (3.0%)	
Gastric Lesion				
- **Normal**	2 (2.3%)	0 (0%)	2 (3.0%)	0.474
- **Hyperemia**	16 (18.2%)	6 (27.3%)	10 (15.2%)	
- **Erosion**	61 (69.3%)	15 (68.2%)	46 (69.7%)	
- **Hemorrhagic flux**	0 (0%)	0 (0%)	0 (0%)	
- **Atrophic**	9 (10.2%)	1 (4.5%)	8 (12.1%)	
Ulcer	19 (21.6%)	3 (13.6%)	16 (24.2%)[#]	0.298

*Wilcoxon-test with significant $P<0.05$; [#]ulcer occurred together with other lesions.

The expression of CD4+ and CD8+ in the gastric mucosa was assessed by IHC staining, and the positive granules were calculated from the average of three samples from Q-win DC100 image analysis (400x). The mean of CD4+ and CD8+ mucosa in *H.pylori* positive subjects was lower than *H.pylori* negative subjects

(CD4+: 11.68±6.90 vs. 14.61±6.61 with *P*=0.079; CD8+: 4.14±2.00 vs. 5.76±2.68 with *P*=0.011). The ratio of CD4+ to CD8+ was calculated and the mean ratio of CD4+/CD8+ in positive *H.pylori* was higher than the negative *H.pylori* subjects (3.49±1.54 vs. 2.95±1.50; *P*=0.139). However it was statistically not significant (Table 2).

Table 2.The Mean of $CD4^+$, $CD8^+$ cells and ratio CD4/CD8 in *H pylori* positive and negative subjects

IHC stain¶	*H.pylori*(+)(22)	*H.pylori*(-) (66)	95% CI	*P**
	Mean±SD	Mean±SD		
$CD4^+$	11.68 ± 6.90	14.61 ± 6.61	-0.341 ; 6.196	0.079
$CD8^+$	4.14 ± 2.00	5.76 ± 2.68	0.387 ; 2.864	*0.011*
CD4/CD8	3.49 ± 1.45	2.95 ± 1.50	-1.277 ; 0.181	0.139

*t-test ; significant *P*<0,05
¶Expression $CD4^+$ and $CD8^+$ mucosa: observed of immunohistochemistry (IHC) staining/100 lymphocytes (400X).

This study also analyzed the difference of gastric mucosa CD4+ and CD8+ in each type of gastric lesions. The different expressions of CD4+ and CD8+ in *H.pylori* positive subjects were compared with the *H.pylori* negative. Significant results were only foind in erosions and ulcers (erosion: CD4+9.53±5.44 vs.14.13±5.43, *P*=0.035; CD8+3.99±2.08 vs. 5.89±2.55 *P*=0.011; ulcer: CD4+5.13±3.44 vs. 15.77±7.50 *P*=0.030; CD8+3.07±1.90 vs. 6.30±2.81 *P*=0.078). There were two subjects in the *H.pylori* negative group with normal gastric mucosa. The CD4+ and CD+ expressions in atrophic mucosa were lower in the *H.pylori* positive subjects than the negative (Table 3).

Table 3. The difference of $CD4^+$ and $CD8^+$ mucosa based on mucosa lesion in *H.pylori* (+) and *H.pylori* (-).

Lesions based on CD	CD mucosa *H.pylori* (+) Mean± SD (n)	CD mucosa *H.pylori* (-) Mean± SD (n)	*P**
$CD4^+$- normal	-	11.40±7.35 (2)	-
- Hyperemia	18.27±6.37 (6)	19.34±10.62 (10)	0.827
- Erosion	9.53±5.44 (16)	14.13±5.43 (46)	*0.035*
- Atrophy	4.40 (1)	12.27±4.54 (8)	-
- Ulcer¶	5.13±3.44 (3)	15.77±7.50 (16)	*0.030*
$CD8^+$- normal	-	3.10±2.69 (2)	-
- Hyperemia	5.04±1.26 (6)	6.76±3.18 (10)	0.098
- Erosion	3.99±2.08 (16)	5.89±2.55 (46)	*0.011*
- Atrophy	1.00 (1)	4.40±2.20 (8)	-
- Ulcer¶	3.07±1.90 (3)	6.30±2.81 (16)	0.076
$CD4^+$/$CD8^+$- normal	-	4.24±1.31 (2)	-
- Hyperemia	4.68±1.48 (6)	3.81±3.17 (10)	0.541
- Erosion	3.33±1.52 (16)	2.66±1.04 (46)	0.062
- Atrophy	4.40 (1)	3.05±0.95 (8)	-
- Ulcer¶	2.93±0.41 (3)	2.91±1.52 (16)	0.982

*t-test, significant *P*<0.05; ¶ulcer occurred together with other lesions

The mean of CD4+ in erosions and ulcers in the *H.pylori* positive subjects were lower than the *H.pylori* negative subjects, statistically significant with *P*<0.05. However, the mean of CD8+ mucosa in *H.pylori* positive subjects were lower than the *H.pylori* negative but were only significant in erosions (*P*<0.05). There were no significant difference in the ratio of CD4+/CD8+ in gastric lesions between *H.pylori* positive and *H.pylori* negative subjects (*P*>0.05).

A strong significant positive correlation between

mucosal expression of CD4+ and CD8+ was found in both of *H.pylori* positive (r=0.62; *P*=0.002) and *H.pylori* negative subjects (r= 0.68; *P*<0.001). The correlation of CD4+ and CD8+ mucosa expressions with gastric lesion was only significant in the *H.pylori* positive subjects (CD4+ r= -0.60; *P*=0.003 and CD8+ r= -0.43; *P*=0.044). There was no significant correlation between the ratio of CD+/CD8+ mucosa with gastric lesion in both subjects (Table 4).

Table 4. Correlation between $CD4^+$ and $CD8^+$ mucosa with gastric lesions in *H.pylori* (+) and *H.pylori* (-)

Correlation	*H.pylori* (+)		*H.pylori* (-)	
	r	*p**	r	*p**
$CD4^+$ with $CD8^+$	+0.62	*0.002*	+0.68	*<0.001*
$CD4^+$ with gastric lesion	-0.60	*0.003*	+0.02	0.872
$CD8^+$ with gastric lesion	-0.43	*0.044*	-0.05	0.694
Ratio CD4/CD8 with gastric lesion	-0.07	0.757	+0.18	0.148

*Spearmen-rho correlation test, significant *P*<0.05
Category of gastric lesion: normal, hyperemia, erosion, atrophy

Discussion

Systemic and local T-cell and B-cell response in chronic *H.pylori* infection and immunologic response would be marked by the increasing number of CD4+ and CD8+T-cells in the gastric mucosa (15). T-cells from *H.pylori*-infected subjects produce larger amounts of interferon-γ than uninfected subjects (16). The co-existence of *H.pylori* with its human host has evolved elaborate adaptations that allow it to persist in the hostile environment of the stomach in the face of a vigorous innate and adaptive immune response (7). This study showed that the mean of gastric mucosa CD4+ and CD8+ in *H.pylori* positive subjects were lower than in *H.pylori* negative, but only the CD8+ was statistically significant. However, the mean of CD4+ was larger than CD8+ in both subject groups, meaning that acute on chronic inflammation was occurring in the gastric mucosa.

It was consistent with the endoscopic appearance; erosion is the most frequent in all subjects (68.2% Hp-positive; 69.7% Hp-negative), but a significant negative correlation was found between CD4+ and CD8+ with Hp-positive gastric lesions. Andersen *et al.*, 2005 (2). and Lu *et al.*, 2005 (10). revealed that CD4+ T-cell infiltrations in gastric mucosa occurred more often in patients with *H.pylori* infection, while CD8+T-cell infiltrations were similar in patients with or without *H.pylori* infection. A potentially important role for CD8+ T-cells that may mediate more severe in gastric disease resulting from *H.pylori* infection have been studied in mice with CD4 deficiency (9). This study was different from the previous study, as a negative correlation between infiltration of CD4+ and CD8+ with Hp-positive gastric lesion was found and the distribution of each lesion type was not even (there were less atrophy and ulcer types than erosion). There were many other factors that were not taken account of that may or may not influence the expression of gastric mucosa CD4+and CD8+T-cells such as other infective agents causing inflammation, environment factors (food, alcohol, lifestyle, genetics and pathogenicity of *H.pylori*), and drugs (non steroid anti inflammation).

The insignificance of cell populations increase other than the concentrations of total mononuclear cells in Hp-positive patients lead to a question, whether the mucosa in Hp-negative individuals has been infected by other infective agents causing inflammation (17). Different *H.pylori* antigens may only induce modest proliferative responses in circulating CD4+ and CD8+ T-cells, and do not increase proliferation or cytokine production, such as interferon-γ which is secreted by CD8+ T-cells. Progression of glandular atrophy and intestinal metaplasia had a key role in the distribution of *H.pylori* colonization.

H.pylori appears to be the most important risk factor for developing glandular atrophy and intestinal metaplasia, but it is not the only risk (18). In particular, environmental factors such as food, lifestyle, genetic, physiological functions and prevalence of *H.pylori* infection are widely different in Asian countries compared to the West (19). And gastric mucosal damage induced by *H.pylori* infection is also due to the bacterial virulence factors encoded by the cag pathogenicity island, such as the vacuolating cytotoxin A (VacA) and the CagA protein (CagA) (20,21,22). This study showed that Infiltration of CD4+ and CD8+ T-cells in gastric mucosa was consistent with atrophy and ulcer occurrence, meaning that atrophy and ulcer in *H.pylori* positive subjects were less frequent

compared to *H.pylori* negative subjects, and most of them were found to have erosions (69.3%) in the gastric mucosa. These conditions influenced the value of the correlation between infiltration CD4+ and CD8+ with gastric lesion.

There are limitations of this study: 1) the expressions of CD4+ and CD8+ were not enough to assess chronic active gastritis. An index of inflammatory markers can summarize gastric inflammations. 2) This study is a hospital-based study, which may not represent the true epidemiologic condition. An ideal population-based epidemiological study of functional dyspepsia would involve performing gastroduodenoscopy on symptomatic adults in the community, which is not easy to do. 3) The prevalence of *H.pylori* in our population is lower than other countries, and there are no data about the type of *H.pylori* in our country (Indonesia) especially our region (Yogyakarta).

A positive correlation was found between CD4+ and CD8+ infiltration in both subjects with or without *H.pylori* infection, and a negative correlation between gastric lesions with CD4+ and CD8+ infiltration were only in the *H.pylori* subjects.

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