Comparison of *Helicobacter pylori* Detection Using Immunohistochemistry and Giemsa and Its Association with Morphological Changes in Active Chronic Gastritis

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ABSTRACT

Background: Gastritis is an inflammation of the gastric mucosa as a response to infection or irritation of the gaster. The most common aetiology of chronic gastritis is Helicobacter pylori (H. pylori) infection. Presence of H. pylori is associated with the occurrence of inflammation, atrophy, and intestinal metaplasia. In terms of morphology, H. pylori is known in 2 forms, which are rod-shaped and coccoid-shaped. Coccoid-shaped bacteria are difficult to detect using Giemsa staining. Therefore, immunohistochemistry staining of H. pylori and evaluation of the sensitivity of coccoid-shaped of H. pylori are needed.

Method: Cross-sectional study on 90 biopsy tissues of chronic gastritis patients in year 2015 and 2014, which included 30 Giemsa cases with positive H. pylori, 30 cases of active chronic gastritis with negative H. pylori but coccoid-shaped was found, and 30 non-active chronic gastritis, were subsequently stained with immunohistochemistry staining of H. pylori.

Results: Expression of coccoid-shaped H. pylori in active chronic gastritis was significantly different (p < 0.05) in immunohistochemistry staining. There was a significant difference between active chronic gastritis with positive H. pylori and negative H. pylori in immunohistochemistry staining with degree of inflammation. Sensitivity and specificity test between Giemsa and immunohistochemistry staining showed sensitivity of 65% and specificity of 100%.

Conclusion: Immunohistochemistry staining in active chronic gastritis was more sensitive compared to Giemsa staining in detecting H. pylori, particularly the coccoid-shaped bacteria.

Keywords: active chronic gastritis, H. pylori immunohistochemistry

ABSTRACT

Background: Gastritis merupakan suatu peradangan pada mukosa lambung sebagai respon terhadap infeksi atau iritasi lambung. Penyebab gastritis kronik yang paling sering adalah infeksi Helicobacter pylori (H. pylori). Adanya H. pylori berkaitan dengan terjadinya inflamasi, atropi, serta metaplasia intestinal. Bakteri H. pylori secara morfologi dikenal dengan 2 bentuk yaitu berupa batang dan coccoid. Bakteri yang berbentuk coccoid sulit terdeteksi dengan pewarnaan Giemsa. Untuk itu diperlukan pewarnaan imunohistokimia H. pylori dan mengukur sensitivitas H. pylori berbentuk coccoid.

Method: Studi potong lintang terhadap 90 jaringan biopsi pasien gastritis kronik pada tahun 2015 dan 2014 yang meliputi 30 kasus Giemsa dengan H. pylori positif, 30 kasus gastritis kronik aktif dengan H. pylori negatif tapi ditemukan bentuk coccoid, dan 30 kasus gastritis kronik non-aktif, kemudian dilakukan pewarnaan imunohistokimia H. pylori.

Results: Ekspresi H. pylori bentuk coccoid pada kronik aktif memiliki perbedaan yang bermakna (p < 0,05) pada pulasan imunohistokimia. Terdapat perbedaan yang bermakna antara gastritis kronik aktif H. pylori positif dan H. pylori negatif pada pulasan imunohistokimia dengan derajat inflamasi. Uji sensitivitas dan spesifisitas antara pemeriksaan Giemsa dan pulasan imunohistokimia, hasil sensitivitas 65% dan spesifisitasnya 100%.

Conclusion: Pewarnaan imunohistokimia pada gastritis kronik aktif lebih sensitif dibandingkan dengan pewarnaan giemsa untuk mendeteksi H. pylori terutama jenis coccoid .

Keywords: gastritis kronik aktif, Giemsa, imunohistokimia H. pylori

INTRODUCTION

Gastritis is one of the most common digestive tract problems. Worldwide, the incidence of gastritis is 1.8 - 2.1 million, while in South East Asia, 583,635 per year. The incidence of gastritis in Indonesia is quite high, which is 247,396 cases from 238.452.952 population.[1] Gastritis is an inflammatory condition of the gastric mucosa as an inflammatory response towards infection or irritation of the gaster.[2] Generally, the cause of chronic gastritis is the infection of *Helicobacter pylori (H. pylori),* a gram-negative bacteria, presence of autoimmune disease and reaction towards chemical and drugs.[3]

Globally, the prevalence of *H. pylori* varies. Infection is more common to be found in the developing countries compared to developed countries. Infection may affect all ages, starting from childhood to adulthood.[4] *H. pylori* infection in developing countries can reach up to 25-30%, where 5-27% are found in early childhood and 50-60% are found in adults aged more than 60 years old.[5] Based on the report from several studies, it was known that the prevalence of *H. pylori* infection in Indonesia varied. *H. pylori* infection in Dr. Mohammad Husein Palembang Hospital reached 46.7% in 2009, 24.3% in Tugurejo Semarang Hospital in year 2004-2010, 20.1% in Surakarta in year 1997.[6,7,8] Meanwhile, in Jakarta based on serologic examination in 150 primary school children, the obtained prevalence was 27%.[9]

Along with the increased prevalence of *H. pylori* infection, various methods have been developed to detect it, either using invasive or non-invasive methods. Some known non-invasive methods include urea breath test, nitrogen excretion test, blood immunoglobulin (Ig) G and IgA serologic examination, and faecal *H. pylori* antigen test. On the other hand, invasive methods include microbial culture test, urease examination in biopsy tissue, histopathology, and polymerase chain reaction (PCR) of the biopsy tissue. Appropriate *H. pylori* diagnostic test is chosen based on the sensitivity and specificity of the methods being used, cost, and equipment availability.[10] Invasive detection of *H. pylori* is performed through gastric biopsy. Gastric biopsy that fulfilled Sydney system criteria is biopsy from the antrum and corpus.[11] *H. pylori* detection in gastric biopsy can be performed through Giemsa staining, immunohistochemistry staining, McMullen modification method, and silver staining method. Rotimi et al studied 63 gastric samples to detect *H. pylori*. Sensitivity of all the four methods being used were immunohistochemistry using *H. pylori* antibody (98.3%), McMullen modification (90%), Giemsa (86.7%), and silver staining (85%), respectively.[12]

Based on its morphology, *H. pylori* bacteria are known in two forms, rod-shaped and coccoid-shaped. Rod-shaped *H. pylori* has tendency to become coccoid-shaped in several environmental conditions, such as: oxygen exposure, base pH, starvation, long-term treatment, and inadequate proton pump inhibitor (PPI) or antibiotic administration.[13,14] Coccoid-shaped bacteria is hard to detect using Giemsa staining due to the difficulty in differentiating coccoid-shaped bacteria from artefact or other bacteria. Therefore, immunohistochemistry staining of *H. pylori* is needed. A different opinion from several researchers stated that the coccoid-shaped is a transformation form leading to degenerative state or death, while some others considered it as active and viable form.[13,14]

Coccoid-shaped *H. pylori* may also be caused by increased oxygen pressure and antibiotic administration. Coccoid-shaped *H. pylori* is form that cannot be cultured but is still alive and can be induced back to the virulent form (spiral). Coccoid-shaped *H. pylori* is thought to play role in bacterial transmission and

some are responsible in the recurrence of infection after antimicrobial treatment; however, the pathogenesis of coccoid-shaped *H. pylori* is still unclear and has not been much studied.[13,14] In the study performed by She et al, there were 3 strains of coccoid-shaped *H. pylori* which changed from spiral-shaped due to exposure to metronidazole.[14] In this study, we would like to know the correspondence between immunohistochemistry and Giemsa staining to detect *H. pylori* in chronic gastritis and to observe the morphological or histological difference of chronic gastritis with rod-shaped and coccoid-shaped *H. pylori*. Immunohistochemistry staining method becomes a consideration in increasing the sensitivity in the detection of *H. pylori* as it relies on more specific antigen and antibody binding.

METHOD

This study used cross-section design, performed in Department of Anatomical Pathology Faculty of Medicine University of Indonesia/Dr. Cipto Mangunkusumo Hospital (FMUI/CMH) in November 2015 to January 2016. Accessible population of this study was active chronic gastritis cases which were diagnosed in Anatomical Pathology Department FMUI in year 2014-2015 with topographic code C15, C16, and morphologic code H544 in accordance with International Classification of Disease-10 (ICD-10) standard. Samples of active chronic gastritis with positive *H. pylori* in Giemsa staining and active chronic gastritis with negative *H. pylori* in Giemsa staining, but had coccoid-shaped were obtained through consecutive method. Samples of non-active chronic gastritis were obtained through simple random sampling. Estimation of the sample size counted with formula (paired categorical) was 51 cases.

Search and exploration of cases were performed in Anatomical Pathology Department FMUI/CMH in January 2015 to September 2015, and if results were not adequate, samples were further taken from the previous years. Anatomical pathology examination form and slides were collected; subsequently, re-evaluation towards active chronic gastritis H & E slides and Giemsa positivity were conducted. Evaluation of inflammation, atrophy, and metaplasia were performed using visual analog scale. Later, unstained slides from paraffin block which fulfilled the criteria were made and *H. pylori* (BC 7) antibody which was incubated for 1-2 hours with 1:50 dilution was examined.

Assessment of the results of *H. pylori* (BC 7) immunohistochemistry staining was performed by researcher using light microscope. Immunohistochemistry staining evaluation was based on the presence of *H. pylori* staining in gastric mucosa. Staining results evaluation was performed by researchers together. Statistical analysis was performed using Chi-square test and if criteria had not been fulfilled, Fisher's exact test would be used as an alternative. These statistical tests were performed using SPSS 20 software.

RESULTS

Active chronic gastritis samples with Giemsa positive *H. pylori* and active chronic gastritis with Giemsa negative *H. pylori* but contained coccoid-shaped were obtained through consecutive technique. Non-active chronic gastritis samples were collected through simple random sampling in one year period, which was January to December 2015, in each studied group, and if results were not adequate, samples were further taken from the previous years. This study evaluated 3 categories, which consisted of 30 cases with positive *H. pylori* with Giemsa, 30 cases of active chronic gastritis, and 30 cases of non-active chronic gastritis. Patients' age data distribution showed non-normal distribution, which was median of age 51.50 years old, the youngest age was 7 years old and the eldest was 86 years old, with the age range of 79 years old and mean age of 49.08 years old.

Table 1. Characteristic of samples

	Total (n = 90)	(%)
Age (years old)		
0-10	1	(1.1)
11-20	3	(3.3)
21-30	8	(8.9)
31-40	17	(18.9)
41-50	15	(16.7)
51-60	21	(23.3)
61-70	20	(22.2)
71-80	3	(3.3)
81-90	2	(2.2)
Mean age	49.08	-
Age range	79	-
Sex		
Male	38	(42.2)
Female	52	(57.8)

In this study, assessments on inflammation, atrophy, and metaplasia were also performed. Severe inflammation was found in 17.8% cases, severe atrophy in 5.6% cases and intestinal metaplasia was found in 6.7% cases.

Table 2. Results of Gastric Biopsy Assessment

	Total (n=90)	(%)
Inflammation		
Absence of inflammation	0	(0)
Mild inflammation	41	(45.6)
Moderate inflammation	33	(36.7)
Severe inflammation	16	(17.8)
Atrophy		
Absence of atrophy	6	(6.7)
Mild atrophy	57	(63.3)
Moderate atrophy	22	(24.4)
Severe atrophy	5	(5.6)
Intestinal metaplasia		
Absent	84	(93.3)
Present	6	(6.7)

Evaluation was performed to 60 cases of active chronic gastritis which were divided into 2 categories; first, 30 cases of active chronic gastritis with negative *H. pylori* in Giemsa staining but had coccoid-shaped, in the immunohistochemistry staining positive *H. pylori* was found in 16 cases (53.3%), but had coccoid-shaped *H. pylori* morphology (Figure 1 and 2). While, 30 cases which initially showed positive *H. pylori* with Giemsa staining, the immunohistochemistry staining also revealed positive results (Figure 3 and 4).

Table 3. Evaluation Results of Active Chronic Gastritis Based on Inflammation, Atrophy, and Metaplasia and *H. pylori* in Giemsa and Immunohistochemistry Staining

	Giemsa		Immunohisto-chemistry		p
	H. pylori (+)	*H. pylori* (-)	*H. pylori* (+)	*H. pylori* (-)	
Inflammation					
Mild	5	17	13	9	
Moderate	12	12	19	5	0.026
Severe	13	1	14	0	
Atrophy					
Mild	15	25	29	11	
Moderate	13	4	15	2	0.556
Severe	2	1	2	1	
Metaplasia					
Absent	27	28	43	12	0.582
Present	3	2	3	2	

Table 4. Evaluation Results of Active Chronic Gastritis Based on Inflammation, Atrophy, and Metaplasia and Shape of *H. pylori* in Immunohistochemistry Staining
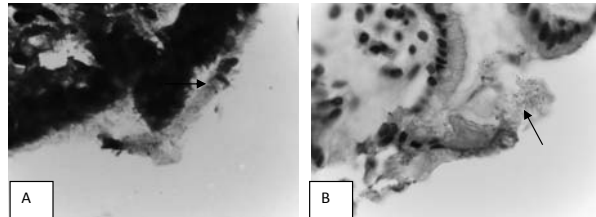
	Immunohistochemistry		p
	Rod-shaped *H. pylori*	Coccoid-shaped *H. pylori*	
Inflammation			
Mild inflammation	14	8	0.086
Moderate inflammation	17	7	
Severe inflammation	13	1	
Atrophy			
Mild atrophy	26	14	0.558
Moderate atrophy	15	2	
Severe atrophy	3	0	
Metaplasia			
Absent	39	16	0.311
Present	5	0	

Sensitivity and specificity tests had been conducted with the results of sensitivity = 30/46 = 65%; specificity = 14/14 = 100%.

In this study, we also performed staining to 30 biopsy samples of non-active chronic gastritis with results of 1 sample which Giemsa staining was positive *H. pylori*, but the immunohistochemistry staining turned out to be negative *H. pylori* (Table 5).

**Table 5. Staining Results in non-active chronic gastritis**

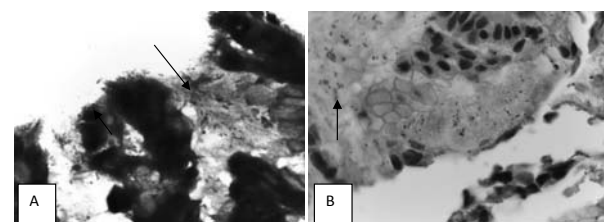|	Giemsa		Immunohistochemistry	
	+	-	+	-
Non-active	1	29	0	30

Figure 1. Evaluation of *H. Pylori*: (A) Giemsa staining, negative *H. Pylori*; **(B)** Immunohistochemistry staining, positive coccoid-shaped (1000 x magnification).
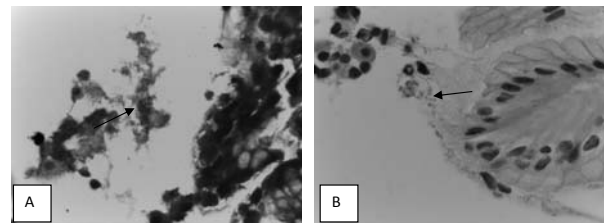
Figure 2. Evaluation of *H. Pylori*: (A) Giemsa staining, negative *H. Pylori*; **(B)** Immunohistochemistry staining, positive coccoid-shaped (1000 x magnification).
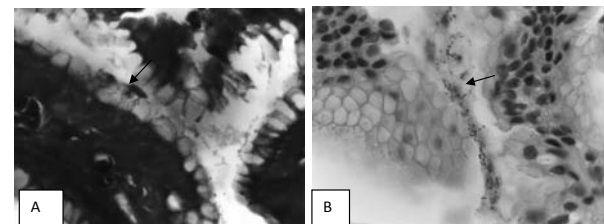
Figure 3. Evaluation of *H. Pylori*: (A) Positive *H. pylori* in Giemsa staining; **(B)** Positive *H. pylori* in immunohistochemistry staining (1000 x magnification).

Figure 4. Evaluation of *H. Pylori*: (A) Positive *H. pylori* in Giemsa staining; **(B)** Positive *H. pylori* in immunohistochemistry staining (1000 x magnification).

DISCUSSION

Gastritis cases in this study were found in patients aged 7 years old to 86 years old with the peak incidence found in 51-60 years old age group with the mean age of 49 years old. This is in concordance with the literature which state that the incidence of chronic gastritis may happen in all age group from childhood to adulthood.[4] The results of this study was not so different from the study conducted by Dhakwa et al which revealed that the average age of gastritis patients were 41.5 years old. This was also in agreement with the study performed by Kalebi et al which concluded that the mean age was 43 years old with variation of 18-86 years old.[14,15] The incidence of gastritis is higher in female (57.8%) as compared to male. This was in accordance with the study done by Al Ammar et al which reported 58.19% female, and different from the study conducted by Capelle et al that found 55% chronic gastritis incidence were male.[27,16]

Gastritis cases with mild inflammation were found in 41 cases (45.6%), moderate inflammation in 33 cases (36.7%) and severe inflammation in 16 cases (17.8%). There was no atrophy in 6 cases (6.7%), mild atrophy in 57 (63.3%), moderate atrophy in 22 cases (24.4%), and severe atrophy in 5 cases (5.6%). Metaplasia was found only in 6 cases (6.7%). Hashemi et al found gastric histopathological appearance of normal mucosa in 8.7%, inactive chronic gastritis in 37.7%, active chronic gastritis in 47.1%, atrophy changes in 25%, and intestinal metaplasia in 8.9%. Zhang et al reported gastric histology appearance of patients with chronic gastritis (non-ulcer dyspepsia) *H. pylori* infection was found in 55.0%, inflammation in 90.3%, atrophy of the mucosa in 36.8%, and intestinal metaplasia in 37.0%.[17,18]

Active chronic gastritis cases with negative *H. pylori* Giemsa staining, but with positive *H. pylori* in immunohistochemistry staining were found in 53.3% cases with coccoid-shaped morphology. Study performed by Tajalli et al towards 54 samples found that the positivity of *H. pylori* with immunohistochemistry method was as many as 43 cases (79.63%), while the positivity with Giemsa method was as many as 24 cases (44.44%) and 18 (33.33%) with H & E staining. The results of this study revealed that classical method was not sensitive enough to identify *H. pylori* particularly the coccoid-shaped.[43] Key et al detected 37% *H. pylori* with H & E staining, 55% with Giemsa staining, 62% with Warthin starry, 66% with immunohistochemistry and 45% were detected using PCR. Immunohistochemistry staining was positive in all cases where *H. pylori* was detected using other methods.[19]

Study performed by Orhan et al revealed that with immunohistochemistry method, low-density coccoid-shaped *H. pylori* could be observed easily. Positive *H. pylori* with immunohistochemistry staining were found in 3 from 10 cases of negative urea breath test (UBT). This study concluded that immunohistochemistry staining was more specific compared to Giemsa and UBT in detecting *H. pylori* infection.[20] Immunohistochemistry examination statistical test was performed towards the coccoid-shaped *H. pylori* obtained that there was significant difference between the two (Appendix 1).

Results of statistical test showed that there was significant difference (p < 0.05) between *H. pylori* in active and non-active chronic gastritis with Giemsa staining. Statistical test was also performed towards *H. pylori* in active and non-active chronic gastritis towards immunohistochemistry staining; fisher exact test was performed and found that there was significant difference (p < 0.05) between *H. pylori* in active and non-active chronic gastritis towards immunohistochemistry staining (Appendix 2-3). Statistical test towards *H. pylori* in active chronic gastritis with immunohistochemistry staining with inflammation was performed and found that there was significant difference (p < 0.05) between active chronic gastritis with immunohistochemistry staining and inflammation (Appendix 4). This was in accordance with the literature that stated that the density of mononuclear cells and activation of polymorphonuclear cells in general were proportional with the density of *H. pylori*.[5,19,20,21,22] Study conducted by Yakoob et al on 176 cases of chronic gastritis concluded that there was significant association (p = 0.002) between infection and activity of *H. pylori*. Aggregated lymphoid was significantly associated with active chronic gastritis.[23]

Different result was found in atrophy and intestinal metaplasia, where there was no significant difference (p < 0.05) between positive and negative *H. pylori* in immunohistochemistry staining in active chronic gastritis with degree of atrophy and presence of intestinal metaplasia (Appendix 5-6). Albertus et al in their study with 72 samples found that there was significant difference for *H. pylori* infection in the antrum in superficial gastritis 19.4%, erosive gastritis 26.4%, and gastric ulcer 34.7%. *H. pylori* infection in gland atrophy and intestinal metaplasia were found in superficial gastritis 12.5% and 14.0%, in erosive gastritis 26.3% and 16.6%, and in gastric ulcer 38.9% and 29.3%, respectively; however, statistically, there was no significant difference.[6]

There was no significant difference between the degree of inflammation in active chronic gastritis with rod-shaped and coccoid-shaped *H. pylori* in immunohistochemistry staining (p > 0.05) (Appendix 7)*;* similar result was also found in atrophy and intestinal metaplasia, where there was no significant difference between the degree of atrophy and the presence of intestinal metaplasia in chronic active gastritis with rod-shaped and coccoid-shaped *H. pylori* in immunohistochemistry staining (Appendix 8-9). Soylu et al studied *H. pylori* using immunohistochemistry method found that from positive *H. pylori* samples there was diffuse staining pattern, vaguely in 90.9%, with smooth granules on the surface in 90.9%, granule-like dot pattern in 54.5%, and spiral-shaped in 9.1%.[24]

Evaluation on the sensitivity and specificity between Giemsa and immunohistochemistry staining had been performed; it was found that the sensitivity was 65% and the specificity was 100%. This was in concordance with the study conducted by Monteiro et al who in their study of culture obtained sensitivity value of 93.8%, specificity 100%; the positive value of *H. pylori* culture confirmed the presence of infection but negative culture did not exclude the suspicion of *H. pylori*.[25] Study performed by Dogar et al compared haematoxylin eosin staining with immunohistochemistry staining in the identification of *H. pylori* found 27.2% *H. pylori* were detected using H & E staining, and 31.4% *H. pylori* were detected using immunohistochemistry staining. H & E sensitivity test was performed upon immunohistochemistry showed sensitivity value of 78.3% and specificity value of 97.9%. This study concluded histopathological examination from gastric biopsy was still an accurate and efficient method to diagnose *H. pylori*, immunohistochemistry staining might increase diagnostic results.[26]

Urease examination of biopsy tissue (campylobacter like organism test/CLO test), Monteiro et al obtained sensitivity value of 83%, specificity 96.4%.[25] Histopathology method, Monteiro et al found sensitivity value of 93.8%, specificity 98.2%. While with polymerase chain reaction (PCR) of the biopsy tissue, they found sensitivity 100%, specificity 97%.[25] Rotimi et al (year 2000) evaluated 63 gastric samples to detect *H. pylori*. Observation results using combination of five tests (rapid biopsy urease test, urea breath test, culture, serology, and histology) found that from interobserver consensus the best method from all the four methods were *H. pylori* antibody (98.3%), McMullen modification (90%), Giemsa (86,7%), and silver staining (85%), respectively.[12]

This study also evaluated Giemsa and immunohistochemistry staining in non-active chronic gastritis from 30 samples with the result that 1 case with positive *H. pylori* in Giemsa staining but the immunohistochemistry staining showed negative *H. pylori* results; this is possible that the positive result in Giemsa staining was a false positive result.

CONCLUSION

Morphological appearance of active chronic gastritis in positive *H. pylori* and negative *H. pylori* in immunohistochemistry staining has significant difference with the degree of inflammation. Morphological appearances of active chronic gastritis in the mucosa that are infected with coccoid-shaped and rod-shaped *H. pylori* were not significantly different; probably this coccoid-shaped is the active form of *H. pylori.* Immunohistochemistry staining to detect *H. pylori* is more sensitive compared to the Giemsa staining, particularly in the coccoid-shaped. False positive *H. pylori* was found in Giemsa staining in non-active chronic gastritis case. The results of this study may be extended to study further about coccoid-shaped *H. pylori,* both in terms of coccoid bacteria activity or even the morphological changes it caused. Immunohistochemistry examination is recommended to be used in diagnosing *H. pylori* in active chronic gastritis, particularly the coccoid-shaped.

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