Helicobacter pylori Antral Density More Valuable than Corporal Density in Chronic Gastritis Patients

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ABSTRACT

Background: *Helicobacter pylori has the capacity to colonize in stomach. In countries with a high prevalence of H. pylori, biopsy specimens must be obtained from corpus and antrum to elevate the accuracy. However, in a country with low prevalence of H. pylori such as Indonesia, the sensitivity of antrum specimen only is still questionable. We compared the density of H. pylori in the antrum and corpus of Indonesian gastric biopsy.*

Methods: *We conducted a prospective, cross-sectional, and observational study in thirty-two samples of corpus and antrum biopsy tissues from Pathology Anatomy Laboratory. The samples were stained by Modified Giemsa or Diff Quik. Updated Sydney System is utilized to classify the density of H. pylori.*

Results: *Among 32 biopsy specimens from corpus and antrum, it has been statistically proven that H. pylori density in antrum and in corpus has a significant difference (65.5% vs. 15.6%, p <0.001). The density of H. pylori in antrum is mild predominant (43.8%), while the density in corpus is normal predominant (87.5%). Thus, the antral H. pylori infection was the predominant site. In 53.12% case, H. pylori was found in antrum but was not found in the corpus. In 6.24% case, H. pylori was found in both sites, but the density was higher in antrum. Importantly, no case with H. pylori infection in corpus only was found..*

Conclusion: *H. pylori density in antrum is higher than in corpus. Only a small advantage to use additional biopsy from corpus to detect H. pylori in Indonesia.*

Key words: density, gastric antrum, gastric corpus, Helicobacter pylori

INTRODUCTION

Gastritis is a term for inflammation that occurs in gastric mucosa and is dominantly caused by infection of *H. pylori*.[1,2] The prevalence rates of *H. pylori* is 10-80%. The prevalence rates corelates with geographical location, age, and social and economic status.[4] The predisposition factor of *H. pylori* is hygiene. Therefore, the prevalence of *H pylori* in Indonesia was forecasted to be high. However, the prevalence compared to the Indonesia population is only 11.2% in Mataram, 36.5% in Purwokerto, 8% in Jakarta, and 11.5% in Surabaya.[5–8]. A study conducted in North Jakarta to assest the prevalence of *H. pylori* in the area culminated a low prevalence rate of infection result, especially in the Javanese, Betawi, and Sunda tribes. Washing hand routine is predicted to be fundamental in resulting the low prevalence *H. pylori* infection in Indonesia.[9]

H. pylori is capable to colonize corpus and antrum.[10] However, the environment in antrum is more suited for *H. pylori* survival. The histologic component of antrum has less parietal cell and more foveolar cell compared to the corpus. In corpus, it is the opposite as there are plenty of parietal cell. Foveolar cell in antrum contributes the mucous that might benefit *H. pylori* for its survival in the stomach. H. pylori survival chance in corpus is diminished by the acidic environment. [10–13] *H. pylori* also use its flagella to retain its place in the gastric mucous.[14]

As one of the predisposition factors of gastric cancer, it is imperative for the diagnosis of *H. pylori* to be done in prompt and precise diagnostic method. The diagnosis is executed by taking one gastric biopsy from incisura angularis, two from corpus, and two from antrum. The density of *H. pylori* will be evaluated by Updated Sydney System.[15–18] Evaluating and interpreting multiple biopsy specimen is very crucial in order to increase the accuracy of histological diagnosis. By using an additional biopsy specimen from corpus, there will be an enhancement of the detection rate by 1-6% compared to

using antral biopsy specimens only. The optimal biopsy site in patient with extensive atrophy is portrayed by greater curvature of the corpus because it yield a better sensitivity than the lesser curvature of the corpus or the antrum. In country with high prevalence of *H. pylori* such as Japan, this is especially important as a higher sensitivity was observed in the upper corpus gastric curvature, but not in antral biopsy. The Japanese guidelines recommends that biopsies should be performed on two sites. The sites are in the upper to middle part of the gastric corpus and the greater curvature of gastric antrum. One of the reason of this suggestion is the distribution of *H. pylori* in the stomach might be uneven. Another reason is that intestinal metaplasia might cause a false negative on the antral specimens. [19–22] This arise question in low prevalence of *H. pylori* countries such as Indonesia[23] whether it is important to investigate multiple biopsy sites or it is enough to evaluate the antrum only. We analyzed the difference of the *H. pylori* density in the gastric antrum compared to the gastric corpus.

MATERIAL AND METHODS

Population and sample

A cross-sectional, observational, and prospective study was performed in the Pathology Anatomy Laboratory in Dr. Soetomo General Hospital. The study was conducted between 1st January 2017 until 31st December 2017.

The population of the sample is the gastric biopsy of gastritis patients. Purposive sampling was chosen as the sampling method. The biopsy sample that have been examined for *H. pylori* were included. The sample have not underwent *H. pylori* examination were excluded. Using the Taro Yamane equation, the sample size is 32 with confidence interval (CI) 83%. This study has obtained approval of ethical clearance from

ethics commission of Faculty of Medicine Universitas Airlangga/Dr. Soetomo General Hospital Surabaya (No. 0408/KEPK/VII/2018).

Counting of *H. pylori* density

The independent variable is the biopsy of antrum and corpus that has been differentiated by its histologic structure. The dependent variable is *H. pylori* density in the biopsy sites. The biopsy sites include both antrum and corpus. Using the Updated Sydney System, the density of *H. pylori* was graded ordinally by semiquantitative visual analogue scale as 0 "normal", 1 "mild", 2 "moderate", and 3 "marked".

The staining method that was used is Diff Quik or Modified Giemsa. The biopsy sample that previously had been stained with Modified Giemsa was deparaffinized with xylol for 2 x 15 minutes. The paraffin block was then inserted to methanol for 1 minutes. The paraffin block then was dried before stained by the red reagent for about two minutes. After drying it, the paraffin block was stained by the blue reagent for about one minute. The paraffin block was inserted to clean water before being dried. The mounting medium was given after drying before closing the paraffin block with cover glass. The slide was air dried for about two hours. After that, the density of *H. pylori* was evaluated using Updated Sydney System through light microscope using x40 objective lens. *H. pylori* will be appeared in spiral form and blue-greyish colored. All high-power fields were examined.

Statistical analysis

The data of this study was analyzed with SPSS Statistics 17.0. Wilcoxon Signed Rank Test was selected to analyze the difference of *H. pylori* density between antrum and corpus.

RESULTS

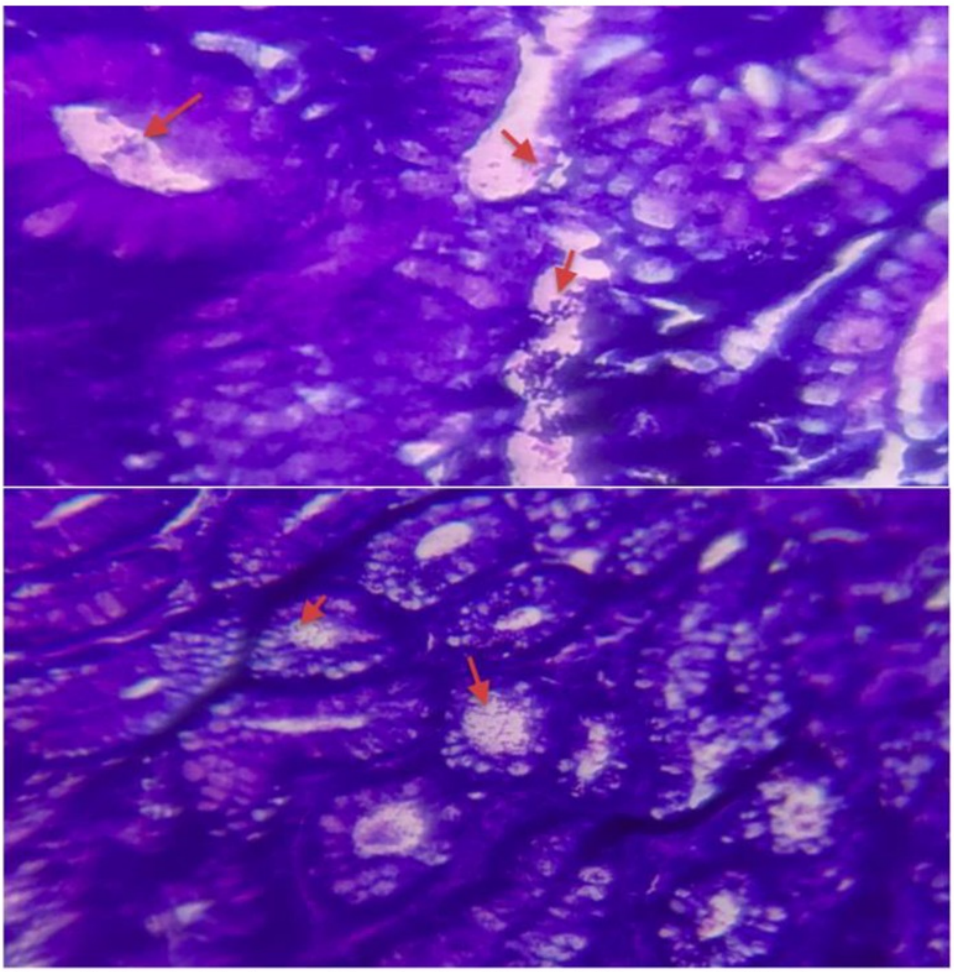

Figure 1. *H. pylori* (red arrow) is stained by Modified Giemsa or Diff Quik

From 32 samples, we found that 65.6% antrum and 15.6% of corpus was infected by *H. pylori*. It is also found that 34.4% antrum and 84.4% corpus was not infected. (Table 1)

Table 1. Sample Infected with *H. pylori*

	Infected		**Uninfected**	
	Antrum	**Corpus**	**Antrum**	**Corpus**
Frequency	21	4	11	27
Percentage (%)	**65.6**	**15.6**	**34.4%**	**84.4**

From 32 samples of antrum, the score of *H. pylori* density that was found was mainly "1" or *mild*, followed by "0" or *normal*, "2" or *moderate*, and "3" or *marked*. (Table 2)

Table 2. *H. pylori* density in Antrum

Scale	**Frequency**	**Percentage (%)**
0 (*normal*)	11	34.4
1 (*mild*)	14	43.8
2 (*moderate*)	5	15.6
3 (*marked*)	2	6.3
Total	**32**	**100**

From 32 samples of corpus, the score of *H. pylori* density that was found was mainly "0" or *negative*, followed by "1" or *mild*, and "2" or *moderate*. (Table 3)

Table 3. *H. pylori* density in Corpus

Scale	Frequency	Percentage (%)
0 (*negative*)	28	87.5
1 (*mild*)	3	9.4
2 (*moderate*)	1	3.1
3 (*marked*)	0	0
Total	**32**	**100**

Using ordinal scale and semi-quantitative research, the density between antrum and corpus was analyzed using non-parametric statistic, using the Wilcoxon Signed Rank Test. The test revealed that there was a significant difference between *H. pylori* density in antrum and corpus with p value 0.000 ($p<0.05$) and Z score -4.017.

Table 4 Predominance of *H. pylori*

No.	Density in Antrum	Density in Corpus	Frequency	Percentage (%)
I	Not found	Not found	11	34.4
II	Found	Not found	17	53.12
III	Higher	Lower	2	6.24
IV	Same	Same	2	6.24
V	Lower	Higher	0	0
VI	Not found	Found	0	0
Total Case			**32**	**100**

There are some probabilities of result from observing and comparing the density of *H. pylori* in antrum and corpus. In 34.4% case, we did not found *H. pylori* in antrum and in corpus. In 53.12% case, *H. pylori* was found in antrum but not in corpus. In 6.24%

case, *H. pylori* was found in both antrum and corpus, but the density was higher in antrum. In another 6.24% case the density in antrum and corpus was the same. There is no case of *H. pylori* density was higher in corpus than in antrum. There is also no case of *H. pylori* was found in corpus only. We can conclude that there is an antral predominance of *H. pylori* infection.

DISCUSSION

We found that *H. pylori* infected majority of the sample. The result of this study contradicts previous studies in Indonesia's result that stated the prevalence of infection was only 11.5% in Surabaya[8], 11.2% in Mataram[5], and 8% in Jakarta[9]. This difference is assumed due to the difference of the staining method. The previous study used Haematoxyllin-eosin as the staining method.[24] In this study, Modified Giemsa or Diff Quik was used as the staining method. Modified Giemsa is the combination between Giemsa and Warthin Starry. However, there is no difference of colour between *H. pylori* and the histologic structure of the gastric. This problem can be solved with an observation done by careful observers. Modified Giemsa staining method is also cheap, easy, and has high sensitivity.[25]

Another important result is that there is an extremely significant difference between *H. pylori* density in antrum and corpus. Previous study concluded that antral lesser curvature or near the incisura have a higher score of *H. pylori* density but there is no significant differences. It was also stated that in order to achieve 100% sensitivity in detecting *H. pylori*, two antral biopsy specimens is required. Those antral specimens are seized from the greater curvature and the lesser curvature. The usage of corpus specimen is for assessment of severity and distribution of gastritis. However, only if major intestinal metaplasia is present in antrum, corpus specimen increased the diagnostic yield.[26] In this

study, we found that there is only small additional percentage of corpus infection. We can conclude that there is only a small advantage to use additional biopsy from corpus to detect H. pylori in Indonesia.

H. pylori is a spiral gram-negative bacterium that produce urease and survive optimally in pH 6.7-7.0. The urease of *H. pylori* produces ammonia and increase the buffer of the acid. In the pH of gastric lumen that revolves around 1.0-2.0, *H. pylori* will not survive.[12] There are four structures of gastric: cardia, pylorus, corpus, and fundus. Antrum is a part of pylorus. Cardia and pylorus are histologically identic and in charge of producing mucus. Fundus and corpus are in charge of producing acid.[27] In corpus, it is found that the gastric pit is deeper. This result in the number of parietal cell in corpus is more than in antrum. However, there are more foveolar cell in antrum than in corpus.[13,28] We can draw a conclusion that *H. pylori* density in antrum is higher than in corpus because mucus in antrum enable *H. pylori* to survive and the acidic situation in corpus disable it survival.

The antral predominance can be assumed because the condition in antrum is more suited for the survival of *H. pylori,*[27-28] the gastritis has progressively moved from antrum to corpus[13], and that atrophy of the antrum had not been occurred because atrophy in antrum lower the colonization of *H. pylori* in antrum and trigger *H. pylori* to colonize corpus.[3,31]

The eradication of *H. pylori* is imperative to be done in all infected individual because of H. pylori infection is one of the predisposition factor of gastric cancer. It is assumed that there is no difference of treatment method based on the density result. The eradication of *H. pylori* will preserve the risk of gastric cancer, escalate the function of the gastric mucous, intercept mucosal damage, resolve the mucosal inflammation, and

reconstruct the normal mechanism of gastric acid secretion. However, maximum advantage of eradication will only be achieved in patient without atrophy.[32]

The limitation of this study is that the confidence interval is only 83% and the sample size is only 32. For the next research, it is suggested to increase the confidence interval until 90% and using the Diff Quik staining method to get more accurate result.

CONCLUSION

The density of *H. pylori* in antrum is higher than in corpus with an extremely significant difference. Only a small advantage to use additional biopsy from corpus to detect *H. pylori* in Indonesia. Further study with a number big samples is needed to ensure the best amount of biopsy specimens to detect *H. pylori* infection in Indonesia.

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CONFLICT OF INTEREST

No conflict of interest is found in this research.

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