HELICOBACTER PYLORI DENSITY AND EXPRESSION OF GASTRIC MUCOSAL INTERLEUKIN-8 IN DYSPEPTIC PATIENTS

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ABSTRACT

Background: The density of the *H. pylori* colonies is associated with more severe clinical manifestations, chronic infections, ineffective therapy responses and malignant events. Interleukin-8 (IL-8) is one of cytokines that plays a role in the inflammatory process that results in gastric mucosal damage. We determined the association between *H. pylori* density and gastric mucosal IL-8 expression in dyspeptic patients.

Methods: *H. pylori* density was determined using histopathology based on Updated Sydney System. IL-8 levels were measured using immunohistochemistry from the gastric biopsy.

Results: There was significant difference between gastric mucosal IL-8 expression obtained in gastric mucosal gland epithelium ($p = 0.028$) at mild and high density of *H. pylori* ($p = 0.013$). There was a correlation between *H. pylori* density with gastric mucosal IL-8 expression of glandular epithelium ($r = 0.622$; $p = 0.004$), surface epithelium ($r = 0.510$; $p = 0.026$), and inflammatory cells ($r = 0.054$; $= 0.028$).

Conclusion: We found a significant positive correlation between *H. pylori* density and gastric mucosal IL-8 expression in dyspeptic patients

INTRODUCTION

Helicobacter pylori infection is an infection of the gastrointestinal system with various manifestations (1). A total of 10% of patients with *H. pylori* infection develop into gastric or duodenal ulcers, and about 0.5 to 2% develop into gastric adenocarcinoma or MALT lymphoma with a mortality rate of 20% in western countries and 60% in Asian countries (2). In Indonesia, the prevalence of gastric malignancy was 0.0028% (3).

The various manifestations were determined by the degree of *H. pylori* density associated with more severe manifestations, chronic infections, ineffective therapeutic responses, and occurrence of malignancy (4-7). The condition is caused by high inflammatory activity in the gastric mucosa, with various cytokines that play a role including interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α). IL-8 plays a role in inflammation and causes damage to gastric mucosa (8-10) and IL-8 in gastritis caused by *H. pylori* infection that can trigger the development and progressivity of gastric malignancy (2, 12). However, the presence of ethnic differences from the host and different *H. pylori* strains can lead to different clinical manifestations (13), *H. pylori* strains in Indonesia have lower virulence (14).

The density of the *H. pylori* colonies is determined by the adherence ability of the mucosal surface. Bacteria with high adherence ability will produce colonies with high density. The adhesion and membrane components of the outermost membrane of *H. pylori*, among which are BabA, Saba, and

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OipA, play an important role in the adherence process on the surface of the gastric mucosa. The density of the *H. pylori* colonies was significantly associated with active OipA (15, 16). OipA may induce IL-8 secretion from gastric epithelial cells (17-19). The suggested OipA mechanism to induce IL-8 is by the phosphorylation of the STAT-1 (Signal Transducers and Activators of Transcription-1) pathway in the gastric antigen mucosa (17, 20). Targeted IL-8 therapy may be a promising strategy for gastric malignant therapy caused by *H. pylori* infection (2).

H. pylori bacteria could induce IL-8 secretion through OipA protein on the surface of *H. pylori* bacteria, so it appears that the higher the density of *H. pylori* the higher the OipA protein that can induce IL-8 secretion. Active Oipa proteins have been associated with severe gastritis and gastric malignancy (16). Accordingly, IL-8 levels may be used as a marker of OipA activation, leading to a tendency to cause severe gastritis and malignancy. Given the differences in *H. pylori* and ethnic strains in Indonesia with other countries that may influence clinical manifestations, therefore, the authors wanted to know the correlation between *H. pylori* density and gastric IL-8 expression in dyspeptic patients.

Methods

This study used cross-sectional analytic observational study. The sample unit was all adults who met the inclusion criteria and no exclusion criteria were obtained. The subjects of this study were in accordance with the inclusion criteria, male and female aged 18-60 years, outpatients with dyspepsia complaints lasting at least in the last 3 months, and willing to participate this study by signing informed consent, histopathologic results obtained an *H. pylori* positive.

Taking medications used for the eradication of *H. pylori* infection, PPI, H2 receptor antagonists, NSAIDs or steroids within 2 weeks, patients with upper and lower GI bleeding, patients with a history of smoking in the past 1 month, patients with a history of alcohol, patients with impaired renal function, there were contraindications of endoscopic examination and gastric biopsy, patients in infectious conditions, and patients with malignant conditions excluded.

The procedure of this study was as follows; the eligible patient was given an explanation of the purpose and benefits of the examination and asked to participate the study by signing informed consent, then general data other data according to the data collection form, preparation, and endoscopic action were recorded. Endoscopes used were Olympus type CLV - U20 (Olympus Optical Co. Ltd. Japan). Implementation of endoscopy in accordance with endoscopic procedures along with all abnormalities were noted. The biopsy was taken using a sterilized biopsy forceps. The biopsy material was immersed into the formalin solution, then histopathological examination of tissue and *H. pylori* density, gastric mucosal biopsy material fixed in 10% formalin, for the specimen-cutting process for preparation of preparations with HE and Diff Quik staining, and partially stored as paraffin blocks which is fixed with formalin, and stored at room temperature at the Anatomical Pathology Laboratory of Dr.Soetomo General Hospital, a positive histopathologic outcome for *H. pylori* bacteria that was examined to detect IL-8 from gastric mucosal paraffin blocks using immunohistochemical techniques. The results of the examination in the form of figures showing the percentage of total area (μm2) detected IL-8 with% unit. Spearman Rank Test was used to analyze the correlation.

Results

This study was conducted on December 2014 to May 2015. The total number of samples in this study were 19 chronic dyspepsia patients who met the inclusion and exclusion criteria, who underwent endoscopy in the Endoscopic Unit of Gastroenterol-Hepatology Dr.Soetomo General Hospital, Surabaya. Characteristics of study subjects as mentioned in table 1.

In this research, it was obtained 11 female patients (57.9%), and 8 male patients (42.1%). The largest number of patients was obtained in the 40-49 years age range (10 patients (52.6%), followed by 7 patients (36.8%) in the 50-59 years age range, and 2 patients (10.5%) in the age range 30-39 years old. The level of education does not seem to have much effect on the density of *H. pylori*, the primary and high school education levels predominate at mild *H. pylori* density, the least amount obtained at the junior secondary education level (10.5%). Clinical symptoms of heartburn were obtained in all patients (100%), followed by nausea in 15 patients (78.9%), vomiting in 8 pa-

TABLE 1.

Characteristics of research subjects

Variable	Category	H.pylori Density n (%)			Total n (%)
		Mild	Medium	High	
Sex	Male	6 (31.6)	0 (0)	2 (10.5)	8 (42.1)
	Female	6 (31.6)	2 (10.5)	3 (15.8)	11 (57.9)
Age (years)	30-39	1 (5.3)	1 (5.3)	0 (0)	2 (10.5)
	40-49	7 (36.8)	0 (0)	3 (15.8)	10 (52.6)
	50-60	4 (21.1)	1 (5.3)	2 (10.5)	7 (36.8)
Education	Elementary school	5 (26.3)	1 (5.3)	0 (0)	6 (31.6)
	Junior high school	0 (0)	0 (0)	2 (10.5)	2 (10.5)
	Senior high school	6 (31.6)	1 (5.3)	1 (5.3)	8 (42.1)
	Bachelor degree (S1)	1 (5.3)	0 (0)	2 (10.5)	3 (15.8)
Clinical symphtoms	Heartburn	12 (63.2)	2 (10.5)	5 (26.3)	19 (100)
	Puffed-up	3 (15.8)	0 (0)	4 (21.1)	7 (36.8)
	Nausea	9 (47.4)	2 (10.5)	4 (21.1)	15 (78.9)
	Vomit	4 (21.1)	1 (5.2)	3 (15.8)	8 (42.1)
Endoscopic Results	Gastritis superfisialis	9 (47.4)	0 (0)	3 (15.8)	12 (63.2)
	Gastritis erosiva	3 (15.8)	2(10.5)	2(10.5)	7 (36.8)

tients (42.1%) and bloating in 7 patients (36.8%). Overview of superficial gastritis endoscopy was obtained in 12 patients (63.2%), erosive gastritis in 7 patients (36.8%).

Degree of *H. pylori* Density and Histopathology in *H. pylori*-Infected Patients

The degree of *H. pylori* density and histopathologic features of dyspeptic patients with positive *H. pylori* are shown in table 2. In this study, *H. pylori* patients with mild *H. pylori* density showed most histopathologic features of inactive chronic gastritis, which were 10 patients (83.3%), and 2 patients (16.7%) showed the active picture. The density of *H. pylori* was showing an inactive and active portion of histopathology with each number being 1 patient (50%). While, the heavy *H. pylori* density showed the more active histopathologic

TABLE 2.

Density levels of *H. pylori* and histopathologic patients infected with *H. pylori*

H. pylori density	Histopathology, n(%)	
	Chronic Gastritis	
	Inactive	Active
Mild (n=12)	10 (83.3)	2 (16.7)
Medium (n=2)	1 (50)	1 (50)
High (n=5)	2 (40)	3 (60)

picture of 3 (60%) patients than inactively i.e., by 2 patients (40%).

Gastric Mucosal IL-8 Expression in *H. pylori*-Infected Patients

The expression of gastric mucosal IL-8 by immunohistochemical examination was determined by calculating the percentage of gastric mucosal areas from several fields of view and then determined for the mean. Figures 1 and 2 show the area of the gastric mucosa that expresses IL-8. The gastric mucosal area expressing IL-8 was shown as a brownish area. While mean and median expression of gastric mucosal IL-8 in patients with *H. pylori*-infected dyspepsia was seen in each density group, shown in Table 3.

In this study, IL-8 was well expressed in the antrum mucosa, whereas in the corpus there was a very weak expression, thus no reading was possible. The IL-8 expression was obtained either in the epithelial mucosal surface, glandular epithelium or in the infiltration of inflammatory cells below the surface. The strongest expression was obtained in the glandular epithelium, followed by epithelial mucosal surfaces and inflammatory cells. There was an increased expression of the surface epithelium of mucosa and gland epithelium according to the degree of density of *H. pylori* ranging from
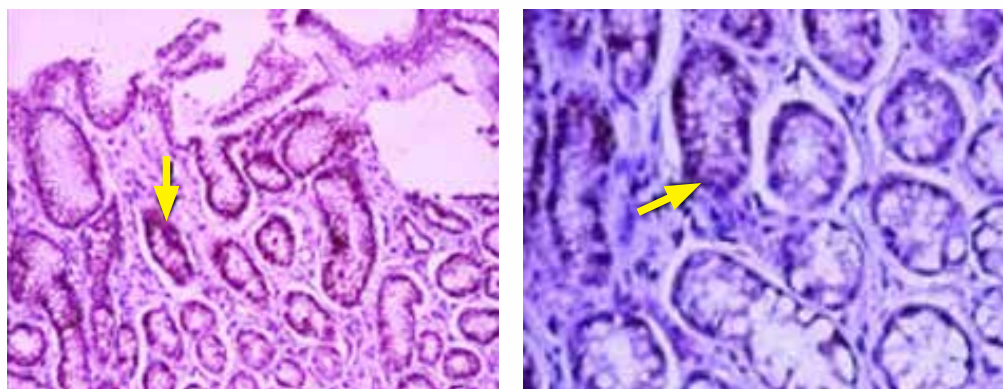
Figure 1. *The area of the gaster mucosa that expresses IL-8 as a brownish-colored area visible on the entire mucosal (left) slice and in the gland mucosal (right) glandular epithelium*
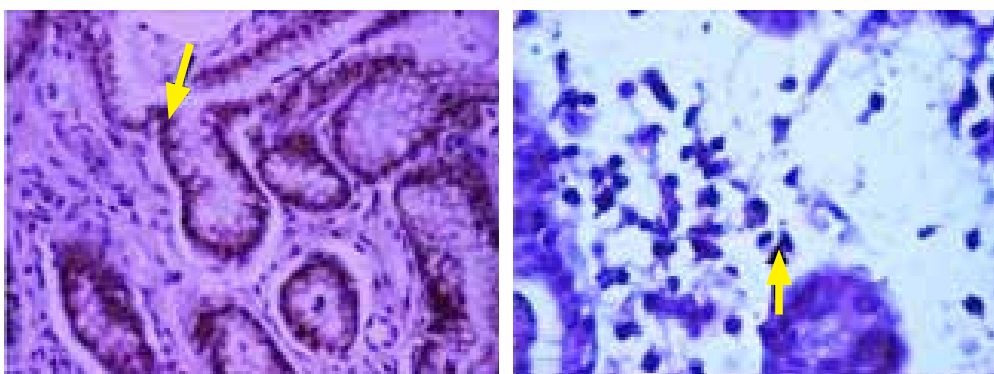

Figure 2. *Area of the gaster mucosa that expresses IL-8 is shown as a brownish-colored area visible on the mucosal surface epithelium (left) and in the infiltration of inflammatory cells in the gastric (right) mucosal lamina propria.*

mild, moderate and severe. However in the inflammatory cell was slightly different, with expression rising from mild to moderate density, but then down at heavy density.

In this study, Kruskal-Wallis test was used to determine the difference of IL-8 expression in the epithelial surface of the mucosa, gland and inflammatory cells in each group of *H. pylori* density ranging from mild, moderate and severe. The Kruskal-Wallis test showed p = 0.028 on the glandular epithelium, followed by p = 0.087 on the surface epithelium and p = 0.088 in the inflammatory cell. The result of p = 0.028 showed the significant difference from the IL-8 epithelial expression of the gastric mucous gland at mild, moderate and severe *H. pylori* density. There was no difference in the expression of IL-8 in each group of *H. pylori* density (mild, moderate, severe). The difference in the expression of IL-8 in the gastric mucosal gland epithelium was significant in the mild and severe

Table 3.

Mean and median IL-8 expression of gastric mucosa in patients infected with *H. pylori*

IL-8 Expression (%)	*H. pylori* Density (n)		
	Mild (n=12)	Medium (n=2)	High (n=5)
Surface Epithelial			
Mean±SD	25.6 ± 16.5	47.5 ± 51.9	57.4 ± 20.8
Median (min-max)	23(4.8-58.8)	47.5(10.8-84.2)	60.4(31.6-80.8)
Epithelial Glands			
Mean±SD	64.9 ± 16.6	76.3 ± 2.1	81.8 ± 4.5
Median (min-max)	70.9(40.0-89.2)	76.3(74.8-77.8)	81.6(75.8-88.4)
Inflammatory cells			
Mean±SD	7.2 ± 3.7	14.9 ± 11.5	11.4 ± 3.5
Median (min-max)	5.5(2.6-14.2)	14.9(6.8-23)	12.4(5.4-14.4)

H. pylori density known through the Mann-Whitney test with p = 0.013.

Correlation of *H. pylori* Density and IL-8 Gastric Mucosal Expression in *H. pylori*-Infected Patients

Among 19 samples examined, the *H. pylori* density correlation analysis with the highest IL-8 expression was obtained in glandular epithelium followed by epithelial mucosal surfaces and inflammatory cells. The line diagram of Fig. 3 illustrates the *H. pylori* density correlation with gastric mucosal IL-8 expression seen by *H. pylori* density (mild, moderate and severe). The diagram shows an increasing line from mild to the severe density of the glandular mucosal epithelium followed by surface epithelium.
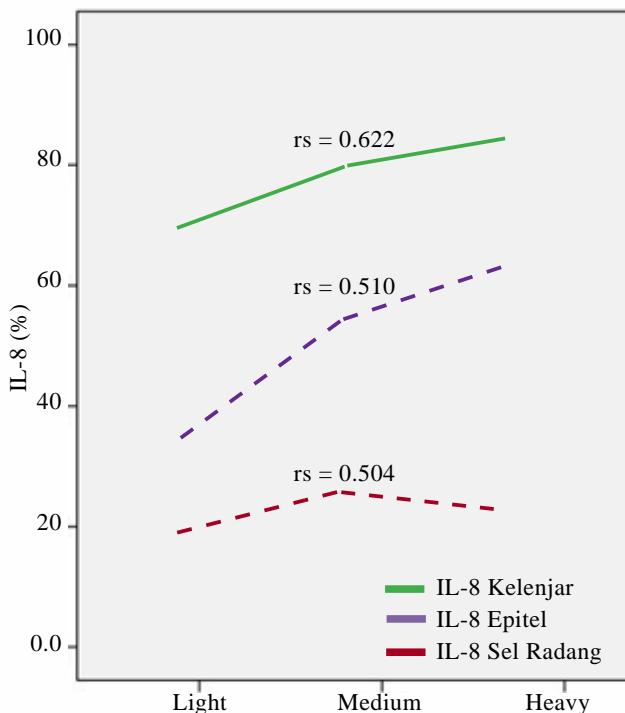

FIGURE 3. *Line diagram of Helicobacter pylori density correlation and expression of gastric mucosal IL-8*

DISCUSSION

In this study, we revealed that the expression of IL-8 gastric mucosa varied considerably. IL-8 was well expressed in the gastric mucosal epithelium of the antrum area, but expressed weakly in the corpus, so that it could not be assessed. The expression of cytokine on the antrum was mentioned to correlate with the degree of histology compared within the corpus. In this study, IL-8 expression quite varied. The difference of expression of IL-8 according to group of density, at gland epithelium

was obtained a significant difference.

The comparison of IL-8 expression on the glandular epithelial between density groups that obtained a significant difference between mild and severe *H. pylori* density with p = 0.013. In this study, the number of patients with *H. pylori* densities was moderate, thus affecting the different test results when compared to moderate *H. pylori* density (mild vs moderate with p = 0.273, moderate vs weight with p = 0.121). It could be concluded that there was difference of IL-8 expression in gland mucosal of gland epithelium between mild and severe *H. pylori* density.

In this study, IL-8 expression was highest in gland mucosal epithelial, with significant expression difference between mild and severe *H. pylori* density. The results of gastric mucosal IL-8 expression in *H. pylori* infection reflects the pathogenesis of *H. pylori* in causing ulcers and malignancies. However, in this study there was no ulcer manifestation, this may be influenced by host factors that also play a role in the regulation of acid secretion.

The density of *H. pylori* was determined by various factors including the motility of *H. pylori*, adhesion and the outer membrane proteins, lipopolysaccharides, and virulence factors (cagA)(16). The density of *H. pylori* has been associated with increased inflammatory activity in the gastric mucosa. Cytokines play an active role in the inflammatory process, and it is reported that the most important role was IL-8. Various studies have mentioned the relationship between *H. pylori* infection with IL-8 and obtained a positive correlation. In additiion, we determined a strong correlation with r = 0.622 for the expression on the gland mucosal epithelial epithelium. The correlation was obtained by r = 0.510 for correlation with the expression on the epithelial surface of the gastric mucosa, and r = 0.504 with expression in inflammatory cells in the gastric mucosal lamina propria.

The study included 19 chronic dyspepsia patients who showed positive *H. pylori* results from a histopathologic biopsy examination. The characteristically chronic dyspepsia patients with positive *H. pylori* were mostly female (57.9%), and male (42.1%) (8 patients). In this study, the number of female patients was higher than male. In this study, patients with mild *H. pylori* density of 31.6% (6 patients) were found in male and female, moderate density was not obtained in male while

in female 10.5% (2 patients), and the weight density was 10.5% (2 patients) in men and 15.8% (3 patients) in women.

Age is a factor that affects *H. pylori* infection, infections in children is more difficult to identify, so the infection will latent and appear in adulthood. While in adulthood, of some age groups, *H. pylori* infection is more widely distributed in the productive age, although still may be obtained in old age. In this study, the highest number of patients in the age group 40-49 years by 10 patients (52.6%), age 50-60 years old was 7 (36.8%) patients and age 30-39 years old was 2 (10.5%) patients. *H. pylori* infection increases with age in adults, especially in middle age and then decreases in old age (21).

Education is closely related to the socioeconomic status that affects the risk of *H. pylori* infection. It was mentioned that low socioeconomic conditions will increase the risk of *H. pylori* infection (22). In this study, mild density was obtained by 12 patients, consisting of 11 patients with elementary school and senior high school education, and 1 patient with undergraduate education. At a moderate density, 2 patients were composed of patients with primary and secondary education. At a heavy density, 5 patients were found, consisting of 2 patients with junior high school, 1 senior high school, and 2 bachelor degree. The data obtained cannot be used to represent the population because to obtain epidemiological data required a large number of samples.

Clinical symptoms of *H. pylori* infection can generally be grouped according to functional dyspepsia type, ulcer type, motility, and mixture. In this study, clinical symptom grouped according to complaints of heartburn, nausea, vomiting and bloating. Clinical symptoms of liver pain (epigastrium) predominate in all H. positive Pylori patients. At the density of *H. pylori*, severe symptoms of heartburn were 26.3%, bloating and nausea respectively 21.1%, and vomiting 15.8%. However, in the density of *H. pylori* mild heartburn also quite a lot that equal to 63.2%. The condition of the heartburn that obtained was quite high in *H. pylori* mild density influenced by other factors such as emotional condition and stressor. The pain was strongly influenced by emotional changes, as the epigastric pain that occurs in *H. pylori* patients.

H. pylori infection was often associated with gastric ulcers, but in this study, there was no evidence of ulceration of endoscopy of superficial and erosive gastritis. Clinical features of patients infected with *H. pylori*, determined by host factors, virulence factors of *H. pylori* bacteria, and the environment. In research in India mentioned that the frequency of erosive gastritis was quite high in patients with higher *H. pylori* density (23). However, in this study, the heavy density of *H. pylori* was obtained more superficial gastritis. This may be due to the strain of *H. pylori* in Indonesia that different from other Asian countries.

In this study, we obtained the histopathology of inactive and active chronic gastritis. Almost the majority of patients are inactive chronic gastritis, especially in mild *H. pylori* density, although some patients show an active chronic gastritis picture. The obvious difference between active and inactive chronic gastritis from *H. pylori* density from mild to severe does not seem so obvious. It was also mentioned in a study that there was no significant difference between the degree of gastritis activity and colonization of *H. pylori*. It was assumed that patients with increasingly severe *H. pylori* density, histopathologic features show more active chronic gastritis than inactively. In this study, most patients with mild *H. pylori* density were present, so it was only natural that the chronic histopathology of inactive chronic gastritis was greater than that of active chronic gastritis. However, in patients with severe *H. pylori* density, an active chronic gastritis (60%) was present more than inactive chronic gastritis (40%). A history of use of proton pump inhibitors and previous eradication may affect *H. pylori* density and change the morphology of *H. pylori* to coccoid formation. In this case required imunohistokimia checks to determine the presence of *H. pylori* germs (10). In this study, proton pump inhibitors and antibiotic drugs have been used for *H. pylori* therapy for 2 weeks before endoscopy.

This study may provide a notion that the *H. pylori* strain under study may be included in the *cagA* + strain, with functional OipA, and this was in accordance with previous studies suggesting that the *H. pylori* strain studied in Indonesia was a cagA +(25). However, the absence of ulcer manifestation in this study may also suggest that there was a possibility of CagA strains present in Indonesia in contrast to other Asian countries. This was consis-

tent with studies that reporting the *H. pylori* strain in Indonesia included in the *H. pylori* East-Asian type-cagA strain that had a 6-bp deletion in the first pre-EPIYA region resulting in low virulence levels (13). This study also showed that the higher the density of *H. pylori*, the greater IL-8 is expressed. This means that the greater the number of *H. pylori* bacteria, the more virulence factor (CagA, oipA), so that the stimulation of IL-8 production is stronger and with the influence of host genetic factors that play a role in determining the inflammatory response, IL- 8 was increasingly strongly expressed in the gastric mucosa of patients infected with *H. pylori*.

CONCLUSION

In patients infected with *H. pylori*, there was a strong positive correlation for epithelial gland, moderate positive correlation for surface epithelium, and inflammatory cells between *H. pylori* density and gastric mucosal IL-8 expression. *H. pylori* density determines the expression of gastric mucosal IL-8, thereby explaining that *H. pylori* infection leads to chronic inflammation through IL-8.

REFERENCES

1. *Emami MH TH, Tavakoli H, Esmaelli,.* Are endoscopic findings predictive for the presence of H. pylori infection? What about indirect histologic findings? Journal of Research in Medical Sciences. 2007;12(2):80-5.

2. *Lee KE, Khoi PN, Xia Y, Park JS, Joo YE, Kim KK, et al.* Helicobacter pylori and interleukin-8 in gastric cancer. World journal of gastroenterology. 2013;19(45):8192-202.

3. *WHO. GLOBOCAN2012:* Estimated cancer incidence, mortality and prevalence worlwide in 2012: International Agency of Cancer; 2012. Available from: http://globocan.iarc.fr/.

4. *Khulusi S, Mendall MA, Patel P, Levy J, Badve S, Northfield TC.* Helicobacter pylori infection density and gastric inflammation in duodenal ulcer and non-ulcer subjects. Gut. 1995;37(3):319-24.

5. *Sheu BS, Yang HB, Su IJ, Shiesh SC, Chi CH, Lin XZ.* Bacterial density of Helicobacter pylori predicts the success of triple therapy in bleeding duodenal ulcer. Gastrointestinal endoscopy. 1996;44(6):683-8.

6. *Lai YC, Wang TH, Huang SH, Yang SS, Wu CH, Chen TK, et al.* Density of Helicobacter pylori may affect the efficacy of eradication therapy and ulcer healing in patients with active duodenal ulcers. World journal of gastroenterology. 2003;9(7):1537-40.

7. *Onal IK GH, Benzer E, Bilir G, Oztas E,.* What is the impact of Helicobacter pylori density on the success of eradication therapy: A clinico-histo-pathological study. Clinics and research in hepatology and gastroenterology. 2013;37(6):642–6.

8. *Sharma SA, Tummuru MK, Miller GG, Blaser MJ.* Interleukin-8 response of gastric epithelial cell lines to Helicobacter pylori stimulation in vitro. Infection and immunity. 1995;63(5):1681-87.

9. *Crabtree JE.* Gastric mucosal inflammatory responses to Helicobacter pylori. Alimentary pharmacology & therapeutics. 1996;10 Suppl 1:29-37.

10. *Andersen LP, Holck S, Janulaityte-Gunther D, Kupcinskas L, Kiudelis G, Jonaitis L, et al.* Gastric inflammatory markers and interleukins in patients with functional dyspepsia, with and without Helicobacter pylori infection. FEMS Immunol Med Microbiol. 2005;44(2):233-8.

11. *Eftang LL, Esbensen Y, Tannaes TM, Bukholm IR, Bukholm G.* Interleukin-8 is the single most up-regulated gene in whole genome profiling of H. pylori exposed gastric epithelial cells. BMC microbiology. 2012;12:9.

12. *Kuai WX, Wang Q, Yang XZ, Zhao Y, Yu R, Tang XJ.* Interleukin-8 associates with adhesion, migration, invasion and chemosensitivity of human gastric cancer cells. World journal of gastroenterology. 2012;18(9):979-85.

13. *Miftahussurur M, Yamaoka Y.* Helicobacter pylori virulence genes and host genetic polymorphisms as risk factors for peptic ulcer disease. Expert review of gastroenterology & hepatology. 2015;9(12):1535-47.

14. *Miftahussurur M, Syam AF, Makmun D, Nusi IA, Zein LH, Zulkhairi, et al.* Helicobacter pylori virulence genes in the five largest islands of Indonesia. Gut pathogens. 2015;7:26.

15. *Dossumbekova A, Prinz C, Mages J, Lang R, Kusters JG, Van Vliet AH, et al.* Helicobacter pylori HopH (OipA) and bacterial pathogenicity: genetic and functional genomic analysis of hopH gene polymorphisms. J Infect Dis. 2006;194(10):1346-55.

16. *Oleastro M, Menard A.* The Role of Helicobacter pylori Outer Membrane Proteins in Adherence and Pathogenesis. Biology. 2013;2(3):1110-34.

17. *Yamaoka Y, Kwon DH, Graham DY.* A M(r) 34,000 proinflammatory outer membrane protein (oipA) of Helicobacter pylori. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(13):7533-8.

18. *Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY.* Importance of Helicobacter pylori oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology. 2002;123(2):414-24.

19. *Shao SH, Wang H, Chai SG, Liu LM.* Research progress on Helicobacter pylori outer membrane protein. World journal of gastroenterology. 2005;11(20):3011-3.

20. *Yamaoka Y, Kudo T, Lu H, Casola A, Brasier AR, Graham DY.* Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in Helicobacter pylori infection. Gastroenterology. 2004;126(4):1030-43.

21. *Haruma K, Kamada T, Kawaguchi H, Okamoto S, Yoshihara M, Sumii K, et al.* Effect of age and Helicobacter pylori infection on gastric acid secretion. Journal of gastroenterology and hepatology. 2000;15(3):277-83.

22. *Moayyedi P, Axon AT, Feltbower R, Duffett S, Crombe W, Braunholtz D, et al.* Relation of adult lifestyle and socioeconomic factors to the prevalence of Helicobacter pylori infection. International journal of epidemiology. 2002;31(3):624-31.

23. *Shah DK, Jain SS, Mohite A, Amarapurkar AD, Contractor QQ, Rathi PM.* Effect of H. pylori density by histopathology on its complications and eradication therapy. Tropical gastroenterology : official journal of the Digestive Diseases Foundation. 2015;36(2):101-6.

24. *Lopes AI, Quiding-Jarbrink M, Palha A, Ruivo J, Monteiro L, Oleastro M, et al.* Cytokine expression in pediatric Helicobacter pylori infection. Clinical and diagnostic laboratory immunology. 2005;12(8):994-1002.

25. *Hapsari P. Hubungan* Seropositivitas Cag-A dengan derajat keparahan gastritis pada pasien dispepsia terinfeksi H.Pylori. Surabaya: 2013.