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THE RELATIONSHIP OF *HELICOBACTER PYLORI CYTOTOXIN*-ASSOCIATED GENE A (Cag A) POSITIVE AND NEGATIVE WITH THE DEGREE OF GASTRITIS SEVERITY BASED ON THE HISTOPATHOLOGY

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ABSTRACT

Introduction : *Helicobacter pylori* (*H.pylori*) is the commonest cause of chronic active gastritis in the world that is around 80%. Risk of prolonged chronic gastritis into atrophy and metaplasia in gastric mucosa which leads to the occurrence of gastric cancer. *H.pylori* carry different virulence factors such as urease, flagellar, VacA, and CagA, which plays an important role in the invasion, colonization and proliferation. CagA can activate a number of signal transduction pathways that resembles released by growth factor receptors, occurs continuously, involved in binding and disrupting the epithelial junction resulting in abnormalities in the tight junctions, cell polarity and cell differentiation. Objective to identify the relationship *H.pylori* CagA(+) and CagA(-) to the severity of gastritis by histopathology.

Methods: The study was conducted with a cross-sectional design of the 30 patients with dyspepsia based on Rome III criteria, using PADYQ scores. Furthermore, endoscopy and biopsy to see gastritis, CLO checks for the presence of *H.pylori*, PCR for virulence examination CagA and Histopathological examination to examination of chronic inflammatory infiltration neutrofi, atrophy and metaplasia

Results: Of the 30 subjects, 18 men and 14 women with a mean age of 53.3 years, were found more subjects with gastritis *H. pylori* CagA(+) 21(70%) and CagA(-) 9(30%). Then found a significant association between CagA status with the degree of chronic inflammation (p=0.032) in which patients with *H.pylori* CagA(+) at risk of 3.43x experiencing gastritis with chronic inflammation, the degree of infiltration of neutrophils (p=0.037) with the risk of 4.5x experience gastritis with infiltration of neutrophils, and the degree of atrophy (p=0.041) risk of atrophic gastritis 2.17x experiencing moderate + severe. But the absence of statistically significant relationship between CagA status with the degree of intestinal metaplasia (p = 0.077).

Conclusion: There is a significant relationship between *H.pylori* CagA status with the degree of chronic inflammation, neutrophil infiltration degree and the degree of atrophy. There is no significant relationship between CagA status with the degree of intestinal metaplasia.

KEYWORDS :

INTRODUCTION

Gastritis is an inflammatory process in the gastric mucosa and submucosa in response to injury which can be acute or chronic where proinflammatory factors, or cytokines, are activated and cause mucosal inflammation. Infection with Helicobacter pylori bacteria is the most common cause of chronic gastritis active throughout the world.[1]

Prolonged chronic gastritis is at risk of atrophy and metaplasia of the gastric mucosa leading to gastric carcinoma. Gastroscopy and biopsy of gastric tissue is the most accurate screening method in looking at the severity of gastritis and assessing the risk of metaplasia. H.pylori carries different virulence factors such as urease, flagellar, vacuolating cytotoxin A, and cytotoxin associated gene A (CagA), which play an important role in invasion, colonization and proliferation. This protein we know as CagA is also associated with increased inflammation, proliferation cells, and gastric mucosa metaplasia. CagA can activate a number of signal-like transduction pathways released by growth factor receptors, occur continuously, engage in bonding and interfere with the epithelial junction resulting in abnormalities in the tight junction, cell polarity and cell diffraction. [3,4,5]

Previous studies reported a high prevalence (93.9%) of CagA(+) H.pylori in China. According to Hou et al., H.pylori CagA has a high prevalence of 93.2% in Shanghai (southern China). China's neighboring countries are also reported to have a high prevalence of CagA, for example India has a high CagA prevalence of 96.2%. Rasheed et.al. reported that 52% of H. pylori strains carried out CagA (+) genes of 80% in GC, 74% in GU, 63% in duodenal ulcer (DU), and 11% in normal cases from Pakistan. In contrast, in Western Europe, CagA (+) strains are less prevalent and are more often found in GU or GC patients.

Based on the information above, this study was prepared to determine the relationship of CagA virulence status to H. pylori with histopathological severity of gastritis.

METHOD

The design used was cross sectional with the independent variables were H.pylori CagA (+) and CagA (-), and the dependent variable was the degree of H.pylori gastritis based on histopathology. The research will be conducted at the Endoscopic Unit of the H. Adam Malik Hospital in Medan and the RS USU FK network after obtaining approval from the Health Research Ethics Commission and related agencies.

The subjects of this study were taken from a population of patients with H.pylori gastritis who met the inclusion criteria, namely nonpregnant men and women aged 18-70 years who were diagnosed with H.pylori gastritis and received voluntary and written information and participation approval for a physical examination, laboratory, gastrocopy and biopsy that is known and approved by the Health Research Ethics Committee.

And exclusion of patients who had received H.pylori eradication in the past 6 months or were on antibiotic therapy commonly used in eradication therapy, taking Proton Pump Inhibitors, H2 receptor antagonists, NSAIDs, steroids, alcohol over the past 48 hours, people with systemic diseases, and the patient is not cooperative

Patients were interviewed about the characteristics of respondents (including gender, age, ethnicity, religion, level of education, occupation) and using The Porto Alegre Dyspeptic Symptoms Questionnaire (PADYQ) which is a quantitative analysis instrument of dyspeptic symptoms. Next is endoscopy and biopsy to see gastritis, CLO examination to see the presence of H.pylori, PCR examination for CagA virulence examination and Histopathological examination for examination of chronic inflammation, neutrophic infiltration, atrophy and metaplasia.

To determine the size of the study sample used a large sample calculation formula with unpaired categorical comparative analytics 2 groups. Data analysis used the chi square test, where the dependent and independent variables were categorical data, to

assess the relationship between H.pylori cagA (+) and cagA (-) with histopathological degrees, namely normal + mild, moderate + severe chronic infiltration; normal neutrophil infiltration + mild, moderate + severe; Normal atrophy + mild, moderate + severe; normal metaplasia + mild, moderate + severe. If it does not meet the chi square test requirements, where tables 2 x 2, namely B x K are not feasible to be tested because the cell with an expected value of less than 5, an alternative test is used, that is fisher test. Probability value (p> 0.05).

RESULTS

From the total number of samples using a pilot study, the minimum number of samples for ni1 = na2 was 12 people. Then the study was followed by 30 patients who had met the inclusion criteria and did not meet the exclusion criteria.

Table 1. Baseline Characteristic Clinical Demographi and clinical of the research subjects

Variabel	n = 30
Gender	
Man	18 (60%) a
Women	12 (40%)
Age	53.5 (20 – 68) b
Tribe	
Batak	16 (53.3%) a
Java	6 (20%)
Aceh	5 (16.7%)
Malay	2 (6.7%)
India	1 (3.3%)
Religion	
Islam	23 (76.7%) a
Christian	6 (20%)
Hindu	1 (3.3%)
Level of education	
Elementary school	3 (10%) a
Junior high school	4 (13.3%)
High school	20 (66.7%)
S1	3 (10%)
Work	
entrepreneur	14 (46.7 %) a
Housewife	12 (40 %)
Employee	3 (10 %)
College student	1 (3.3 %)
CagA	
Positive	21 (70%) a
Negative	9 (30%)

a Categorical data: n (%)

b Numerical data, abnormal distribution: median (minimum - maximum)

Table 2. Chronic Inflammatory Distribution, Neutrophil Infiltration, Atrophy and Intestinal Metaplasia

Variabel	Derajat				Total
	0 (Normal)	1 (mild)	2 (moderate)	3 (severe)	
Inflamasi Kronik	0 (0%)	20 (66.7%)	7 (23.3%)	3 (10%)	30 (100%)
Infiltrasi Neutrofil	15 (50%)	8 (26.7%)	5 (16.7%)	2 (6.6%)	30 (100%)
Atrofi	9 (30%)	10 (33.3%)	8 (26.7%)	3 (10%)	30 (100%)
Metaplasia Intestinal	13 (43.3%)	6 (20%)	11 (36.7%)	0 (0%)	30 (100%)

Table 3. The relationship between CagA status and the degree of chronic inflammation and infiltration of neutrophils

Variabel		Inflamasi Kronik				Infiltrasi Neutrofil			
		moderate + severe	Normal + mild	OR (95% CI)	P	Moderate + severe	Normal + mild	OR (95% CI)	p
CagA	(+)	10 (47.6%) (52.4%)	11	3.43 (1.12 – 14.05)	0.032*	8 (38.1%)	13 (61.9%)	4.5 (1.08 – 18.77)	0.037*
	(-)	1 (11.1%)	8 (88.9%)			1 (11.1%)	8 (88.9%)		

*p<0.05

Table 4. Relationship between CagA status and degree of intestinal atrophy and metaplasia

Variabel		Atrofi				Metaplasia Intestinal			
		Moderate + severe	Normal + mild	OR (95% CI)	P	Moderate + severe	Normal + mild	OR (95% CI)	P
CagA	(+)	11 (52.4%)	10 (47.6%)	2,17 (0.98 – 4.79)	0.041*	10 (47.6%)	11 (52.4%)	2 (0.89 – 4.49)	0.077
	(-)	2 (22.2%)	7 (77.8%)			2 (22.2%)	7 (77.8%)		

*p<0.05

DISCUSSION

From the results of the study, it was found that the number of men suffering from gastritis with H.pylori (+) was greater than women, namely 18 people (60%) and 12 people (40%). This result is in accordance with Goh et al .'s study which states that the majority of sufferers of H.pylori gastritis are male, according to the study of Ali et al (2005) which found that 174 patients examined 100 people were men. With the median age of patients suffering from H.pylori (+) being 53.5 (20-68) this is consistent with the study of Deassy et (2014) found in 30 patients with H.pylori (+) as many as 26.7% between the ages of 51-60 years. This is in line with the research conducted by Mahdy et al and Woodward et al. The incidence of chronic gastritis caused by H.pylori in old age is higher compared to young age. This shows that as we age, the gastric mucosa tends to become thinner so it is more susceptible to bacterial infections, especially H. pylori and the etiology of other gastritis such as drugs (NSAIDs), foods that can stimulate stomach acid and stress.

There was a significant association between CagA strains with chronic inflammation (p = 0.032) where H.pylori CagA (+) gastritis had a risk of 3.43 x to experience gastritis with chronic degrees of fallow. This is in accordance with the previous study, Deassy et al (2014) where lymphocyte inflammation cells in chronic gastritis were found to be at most severe degrees. These results are consistent with the research of Qamar et al. and the 2009 Manxhuka et al study of 103 cases found a moderate to severe degree of inflammation of 15% from 26.5% with H.pylori (+) with P <0.05.

Neutrophilic infiltration and the presence of lymphoid follicles with the germinal center are two distinctive features of the histological picture of H. pylori infection, and eradication causes rapid loss of neutrophils. Furthermore these components become permanent and infect the gastric mucosa. This is in accordance with the results of the study, neutrophil infiltration was found to be a significant relationship with CagA (+) patients at risk of 4.5 x having gastritis with moderate to severe degrees P = 0.037.

In the previous study showed mixed results of Garg et al, found that chronic inflammation occurred in 100% of subjects, neutrophil infiltration of 33.33%, atrophy at 12.33%, and intestinal metaplasia at 7%. Whereas Zhang et al. Found 90.3% chronic inflammation, 56.2% neutrophil infiltration, 36.8% atrophy, and 37% intestinal metaplasia. In the study of Hashemi et al., It was found in inflammatory active chronic gastritis 47.1%, 25% atrophic changes, and 8.9% intestinal metaplasia. The results of this study are consistent with Anderson et al. and Weiss et al. who reported a high association between lymphocytes and positive H. pylori.

So it was found a significant relationship between CagA status and degree of atrophy ($p = 0.041$), where patients with H. pylori with CagA (+) are at risk of 2.17x experiencing gastritis with moderate degree of atrophy + weight. There was no significant relationship between CagA status and intestinal metaplasia degree ($p = 0.077$). This is in line with the study of Weck et al. where there is a significant relationship between H. pylori infection and chronic atrophic gastritis.

The presence of virulent factors that infect H.pylori strains is known as a determinant of infection. Infection with CagA (+) is associated with the development of gastric carcinoma, while CagA infection (-) does not increase the risk of carcinoma and is associated with persistence of nonatrophic gastritis.

CONCLUSION

There is a significant relationship between the status of Cag A and the degree of chronic inflammation, the degree of neutrophil infiltration and the degree of atrophy. There was no significant relationship between the status of Cag A and the degree of intestinal metaplasia

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