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methods compared to
hispatology in dyspepsia patients
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DIAGNOSTIC VALUES OF HELICOBACTER PYLORI STOOL ANTIGEN IMMUNOCHROMATOGRAPHIC METHOD COMPARED TO HISTOPATHOLOGY IN DYSPEPSIA PATIENT

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ABSTRACT

Background: *Helicobacter pylori* infection often leads to complaints of dyspepsia. Enforcement of infection still relies on invasive histopathological methods through endoscopic and biopsy procedures. *Helicobacter pylori* stool antigen (HpSA) is a method of rapid immunochromatography that is not invasive and relatively inexpensive. We determined the diagnostic value of HpSA examination of immunochromatographic methods compared to histopathological examination as the gold standard for diagnosing *H. pylori* infection.

Methods: HpSA examination was used to identify *H. pylori* infection by its ability to detect *H. pylori* antigen from stool of dyspeptic patients. Its diagnostic values including sensitivity, specificity, positive predictive value and negative predictive value was determined by comparing them to those of histopathologic examination as gold standard.

Results: From 93 dyspeptic patients, pre-test probability of *H. pylori* infection using histopathologic examination showed result as much as 17.2%. The sensitivity, specificity, positive predictive value and negative predictive value of HpSA immunochromatographic methods were 38%, 94%, 55% and 88%, respectively. A positive probability ratio of 5.78 increased the post-test probability for *H. pylori* infection by 37.8%. A negative probability ratio of 0.68 increased the post-test probability of not being infected with *H. pylori* by 5.4%.

Conclusion: The diagnostic value of HpSA examination of immunochromatographic methods was not good enough to exclude or diagnose *H. pylori* infection in dyspeptic patients.

KEYWORDS: dyspepsia, *H. pylori* stool antigen, Immunochromatography, Histopathology

INTRODUCTION

Helicobacter pylori infection is often the cause of dyspepsia. There are many methods to diagnose *H. pylori* infection, including invasive method using gastric biopsy through endoscopy and non-invasive (urea breath test (UBT) method, *Helicobacter pylori* stool antigen (HpSA) and serological examination) (1). Until now the enforcement of *H. pylori* infection diagnosis still remains a problem because it still relies on invasive methods and not all patients are willing to go to endoscopy.

The prevalence of *H. pylori* infection in develop-

ing country populations can reach up to 80-90%. In Indonesia, the prevalence ranges from 2-68% (2-4). Not all patients are willing to do endoscopy for *H. pylori* diagnosis. As the result, many people with *H. pylori* infection cannot be detected. It brings clinical consequences, such as an unresolved disease of dyspepsia, progressive upper gastrointestinal bleeding, malignancies such as mucosal-associated lymphoid tissue (MALT) lymphoma and gastric cancer. Therefore, early detection and eradication are expected to prevent gastric cancer (5-7).

The gold standard commonly used for *H. pylori* infection research is histopathology, UBT, rapid urea test, culture and polymerase chain reaction (PCR) (8, 9). The recommended non-invasive method for *H. pylori* infection is urea breath test and SA-based monoclonal antibody examination. The urea breath test requires a special tool and a reagent containing radioactive material. Rapid

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monoclonal antibody-based immunochromatography test is the latest examination of *H. pylori* antigens in feces. This method was reported to be better than the polyclonal antibody-based method (10, 11).

Among noninvasive method, HpSA examination for *H. pylori* detection is a preferred alternative. HpSA examination has the same sensitivity as UBT (95%) and a slightly lower specificity than UBT (94% vs 96%). HpSA examination procedure is faster, easier, relatively inexpensive without requiring special tools. In addition, HpSA examination results were reported more stable than UBT in patients who received proton pump inhibitor therapy (8, 12, 13).

Several laboratories in Surabaya, currently have provided HpSA examinations using the latest method of monoclonal antibody-based rapid immunochromatography. Qualitative noninvasive test for of *H. pylori* detection with relatively inexpensive is expected to help establish the diagnosis of *H. pylori* infection more quickly, easily and accurately and become an alternative examination. This study was to analyze the diagnostic value (sensitivity and specificity) of HpSA examination of rapid monoclonal antibody-based rapid immunochromatography compared to histopathology examination as the gold standard to detect *H. pylori* infection in the dyspeptic patient.

METHODS

This research used the Cross-sectional diagnostic test. The research took place in endoscopic unit of Dr. Soetomo General Hospital Surabaya from December 2014 to December 2015. The inclusion criteria were: outpatients with complaints of at least 3 months, at least 18 years old, willing to sign Informed Consent for endoscopic examination of the upper feeding tube, gastric biopsy and provide the first stool sample after endoscopy. Patients who received antibiotic drugs and/or H2 inhibitors/proton pump inhibitors within two weeks before endoscopy, patients with diarrhea or constipation, patients with a history of gastrointestinal surgery, patients with upper or lower line bleeding, patients with chronic renal failure, cirrhosis hepatitis and diabetes mellitus, patients whose contraindications to endoscopic examination such as pregnancy, lactation, and gastric biopsy were excluded.

The research procedures were as follows: patients who met inclusion but not exclusion criteria were given explanation and their willingness to participate in this research were asked and stated by signing informed consent. Data were collected and recorded according to data collection form,

endoscopy was performed to collect gastric mucosal biopsy for histopathology examination. HpSA was detected using rapid immunochromatography method. Patients were requested to collect feces after endoscopy to be sent immediately to a designated laboratory within less than 1 hour.

The histopathological examination of *H. pylori* was performed by making 1 slide of biopsy with special staining using modified Giemsa (Diff-Quik). In this study, histopathologic readings were performed by the Anatomical Pathologist. Histopathological readings was performed using the Olympus type CX-41 electric microscope made in Japan with magnification 400 to 600 times. Positive *H. pylori* infection was determined based on finding of *H. pylori* histopathologically, vice versa.

HpSA examination using rapid immunochromatography was performed as follows: Feces was taken as much as 5-10 cc for antigen test examination. On-Site *H. pylori* Ag Rapid Test-cassette (CTK Biotech, Inc; San Diego-USA) was used. The device contains an antibody to *H. pylori*. If stool *H. pylori* antigen, a reaction between the antigen-antibodies and the coloring agent will appear as a red (stem line) line in the instrument test zone. Antigen on specimen will be detected in 15 minutes. Result is reported positive if two red lines in the control zone (C) and test line (T) appear, while it is reported negative if a visible red line in control zone appears and it is invalid if there is no red line that appears in the test zone or control while the control zone is not red. If the result is invalid, then the examination must be repeated using a new tape.

The data that were collected was processed in the form of textual and tabular. The results were presented in 2X2 table to calculate diagnostic values such as sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and accuracy. The data were analyzed using Catmaker program.

RESULTS

There were 100 patients who met the inclusion and exclusion criteria. The demographic characteristics of the subjects were presented in Table 1. The subjects of the study were female by 65.6% and 34.4% by the male. The mean age of the subjects was 49.97 ± 11.52 years, with an age range between 19-75 years. Most of the dyspepsia patients in this study had senior high school education (equal to 38.7%) and housewives (48.4%). While, 3.2% of study subjects admitted not attending school.

In Table 2, the most common complaints of

TABLE 1.

Characteristics of subject			
Characteristics	Samples	<i>H. pylori</i> -infected	<i>H. pylori</i> -uninfected
Number of patients (%)	93	16 (17.2)	77 (82.8)
Sex (%)			
Male	32 (34.4)	3 (18.8)	29 (37.7)
Female	61 (65.6)	13 (81.2)	48 (62.3)
Age (y.o)			
Mean ± SD	49.97±11.52	53.13 ± 2.27	19-75
Range	19-75	40-71	49.31 ± 1.36
Education – n (%)			
Employee	3 (3.2)	0	3 (3.9)
Elementary School	21 (22.6)	5 (31.3)	16 (20.8)
Junior High School	13 (14)	3 (18.8)	10 (13)
Senior High School	36 (38.7)	7 (43.8)	29 (37.7)
Bachelor	20 (21.5)	1 (6.3)	19 (24.7)
Occupation – n (%)			
Unemployee	8 (8.6)	1 (6.2)	7 (9.1)
Housewife	45 (48.4)	11 (68.8)	34 (44.2)
Private worker	25 (26.9)	4 (25)	21 (27.3)
Civil servant/retiree	11 (11.9)	0	11(14.4)
Farmer	4 (4.3)	0	4 (5.2)

dyspepsia patients were heartburn, which was complained by 84 patients (90.3%). The complaints were nausea (78.5%), feeling full (67.7%), susceptible (66.7%), bloating (57%), burning sensation (48.4%) and vomiting (35.5%). The dominance of liver pain was more prevalent in infected patients (93.75%) than uninfected (89.61%).

The endoscopic features of this study were grouped by the heaviest type of lesions visually as well as gastric and duodenal involvement. Most endoscopic images (48.4%) have only erythema features, which endoscopically concluded as superficial gastritis (Table 3). The presence of erosion in the gastric mucosa was found in 33.3% and

the presence of duodenitis accompanying erosive gastritis was present in 4.3% of patients. There were 9 patients (9.7%) found in peptic ulcer (4 patients with gastric ulcer and 1 patient with duodenal ulcer), 3 patients (3.2%) were obtained polyps in gastric mucosa. The mass in the gastro-esophageal valve, suspected as early gastric cancer was found in 1 patient (1.1%).

In infected patients, most of the endoscopic features showed erosive gastritis (37.5%), whereas in uninfected patients most (54.5%) found superficial gastritis. Similarly, peptic ulcer was found more common in the *H. pylori*-infected group (18.8%) than the uninfected group (7.8%).

The results of the histopathologic examination in most patients were inactive chronic gastritis (73.1%). Next consecutive chronic gastritis was obtained active (15.1%), gastroduodenitis (10.8%). Patients who on endoscopic examination was obtained a mass, histopathology results show images Non-Hodgkin's lymphoma, which with Giemsa modified special staining found *H. pylori*.

In this study, both groups showed that most chronic inactive gastritis was obtained in the histopathologic examination (Table 3), but the frequency decreased in the infected group (50% vs. 77.9%). Another difference lies in the description of active chronic gastritis that was more prevalent in the infected group than in the uninfected group (31.3%

TABLE 2

Characteristics of complaints			
Symptoms	Samples n (%)	<i>H. pylori</i> -infected n (%)	<i>H. pylori</i> -uninfected n (%)
Epigastric pain	84 (90.3)	15(93.75)	69 (89.61)
Nausea	73 (78.5)	13 (81.25)	60 (77.92)
Gastric fullness	63 (67.7)	9 (56.25)	54 (70.13)
Satiated	62 (66.7)	9 (56.25)	53 (68.83)
Bloating	53 (57)	10 (62.5)	43 (55.84)
Heartburn	45 (48.4)	7 (43.27)	38 (39.35)
Vomiting	33 (35.5)	8 (50)	25 (32.47)

TABLE 3

Endoscopic and histopathological reading			
Characteristics	Samples	Positive <i>H. pylori</i>	Negative <i>H. pylori</i>
Endoscopic reading			
Superficial Gastritis	45 (48.4%)	3 (18.8%)	42 (54.5%)
Erosive gastritis	31 (33.3%)	6 (37.5%)	25 (32.5%)
Erosive gastritis + duodenitis	4 (4.3%)	1 (6.3%)	3 (3.9%)
Peptic ulcer	9 (9.7)	3 (18.8%)	6 (7.8)
Gastric polip	5 (5.4%)	2 (12.5%)	1 (1.3)
Mass	1(1.1%)	1 (6.3%)	0
Histopatological reading			
Inactive chronic gastritis	68 (73.1%)	8 (50%)	60 (77.9%)
Active chronic gastritis	14 (15.1%)	5 (31.3)	9 (11.7)
Gastroduodenitis	10 (10.8%)	2 (12.5%)	8 (10.4)
Non-Hodgkin Lymphoma	1 (1.1%)	1 (6.3)	0

v.s 11.7%). Similarly, gastroduodenitis was higher in the infected group (12.5% v.s 10.4%). The presence of malignancy (Non-Hodgkin's lymphoma) was also obtained only in the infected group.

Sensitivity and Specificity of HpSA Examination Compared to Histopathology

Based on the 93 patients who participated in the study, the histopathologic examination found in 16 patients infected with *H. pylori*. On examination of *H. pylori* in feces by using rapid immunochromatography method, we found 11 patients who showed positive results. Then, the 11 patients with positive HpSA, there were 6 patients (55%) with histopathologic results showing positive results. Eighty-two patients obtained negative HpSA, 72 patients (88%) showed negative histopathologic results.

The results of the diagnostic test of HpSA examination of rapid immunochromatography method compared with histopathology using special staining (modified Giemsa) as the gold standard, obtained sensitivity and specificity of HpSA respectively 38% and 94%. Calculation of diagnostic test in this study was conducted by using the Catmaker program. There were 5 patients (6.5%) who showed false positives. Ten patients have not detected *H. pylori* on faces examination, although on histopathologic examination was detected. If calculated, then get a false negative value of 62.5%.

Based on Updated Sydney Systems, there were three levels of *H. pylori* germination i.e., mild, moderate and severe. In this study, most patients showed mild density (81.25%) and the rest of them (18.75%) showed moderate density. Then, no *H. pylori* density was found with severe intensity in this study (Table 4).

Positive predictive value (NDP)/positive predictive value (PPV) was also called positive predictive

value or positive post-test probability. HpSA positive predictive value of rapid immunochromatography method in this study was 55%. However, the negative predictive value (NDN)/negative predictive value (NPV) in this study was higher, at 88%.

Likelihood Ratio (LR +) is the ratio between positive outcomes in patients compared to positive outcomes in negative groups. In this study obtained RKP of 5.78. The negative likelihood ratio (LR-) ratio shows the comparison between the negative results in the positive patients compared with the negative results in the negative group. In this study, the obtained of possibility value was 0.67.

After knowing the value of positive ration and negative ratio then performed a Pretest Odds calculation which will ultimately be used for counting probability value post-test. Pretest Odds in this study obtained 0.21. Posttest Odds on the results obtained 1,2. From Posttest Odds result, calculated probability after test result obtained 55%. The value was the same as that obtained in positive predictive value calculation, so it was said to be equal to the positive post-test probability.

The positive ratio value was used to determine the probability of a negative post-test. After the value of Pretest Odd, the value of Posttest Odds was calculated, the result of this research was 0.134. From the Posttest Odds result, the post-test proba-

TABLE 4

<i>H. pylori</i> density of histopathological reading	
Degree of density	Amount (%)
Mild	13 (81.25%)
Moderate	3 (18.75%)
Severe	0
Total	16 (100%)

bility calculation is negative, the result was 88.2%. The value was the same as that obtained on the positive predictive value calculation, so it was said to be equal to the post-test negative probability.

Discussion

H. pylori infection is one of the causes of dyspeptic complaints (14). In this study the gold standard is a histopathological examination with Giemsa that modified staining through a gastric biopsy procedure by using an endoscopy device. The value of Kappa 0.68 shows that the level of conformity between two readers was good and strong. In addition, no difference was obtained in the histopathologic reading results between the two readers (McNamara test = 1). This was in accordance with the study that the suitability of inter-observer on histopathology examination *H. pylori* using the classification of Updated Sydney System obtained Kappa value ranged from 0.38-0.56 (15-17).

Based on histopathological examination as the gold standard, in this research, the prevalence/preoperative probability of *H. pylori* infection was 17.2%. This was not much different from the research in 2014 to get the frequency of infection was 11.5%. This difference in frequency can be caused by differences in methods and criteria used in detecting *H. pylori* infection, whether there was a history of bleeding and the use of drugs such as antibiotics (18).

Ninety-three patients in this study had an age range of 19-75 years. Average age 49.97 ± 11.51 years. In the previous study obtained a younger average age of 32.4 ± 13.1 years (19) and 34.2 ± 14.3 years (20). The difference was due to the lack of age restrictions. In this study, it obtained that most dyspeptic patients were female (65.6%). Epidemiological data suggest dyspepsia more common in women. The causes include differences in hormones, lifestyle, and habits. In this study, most of the subjects studied high school (38.7%) and housewives. In the infected group, most have high school education and housewives. Environmental factors play a role in the occurrence of *H. pylori* infection (21).

Overview of erythema in gastric dominates the endoscopic picture in this study (48.4%). The presence of gastric erosion involving duodenum was obtained in 4.3% of patients and peptic ulcer was obtained in 9 (8.6%) patients. Only 1 patient found the mass of the gastroesophageal valve. There are differences in endoscopic features between infected and non-infected *H. pylori*. In the infected group, erosion profile (37.5%), whereas in the uninfected

group only erythema was obtained in the mucosa. Similarly, more gastric ulcers were found in the infected group than not infected (18.8% vs. 7.8%).

Most of the histopathologic features in this study were inactive chronic gastritis (73.1%). Inactivation of inactive chronic gastritis, in the infected group the percentage was smaller (50% v.s 77.9%). In addition, the presence of active chronic gastritis and gastroduodenitis was more prevalent in infected patients. According to the study, there was a significant difference between the degree of neutrophil activity between the infected and non-infected *H. pylori* (22). One patient in the endoscopy was obtained a mass picture, on histopathologic examination results showed non-Hodgkin's lymphoma. The prevalence of Lymphoma (MALT) in *H. pylori* patients is quite rare, about 1% (23).

This study obtained sensitivity examination of HpSA method of rapid immunochromatography compared to histopathology as the gold standard about 38%. The ability of HpSA examination to produce positive examination results, only 38%, so that method cannot be used for *H. pylori* infection screening. Specificity of HpSA examination of rapid immunochromatography method compared to histopathology as the gold standard was 94%. If the patient was not infected with *H. pylori*, then the ability of HpSA examination to produce a negative examination was 94%, then the examination in this study was specific for *H. pylori*. This means that it can be used to diagnose the presence of *H. pylori* infection in the sample population.

There were several high possible causes of false negative rate (62.5%) in an examination of HpSA method of rapid immunochromatography. The first cause was the existence of different strains of *H. pylori* germ, a tool used by the United States, so the *H. pylori* strain used is different from the bacteria strain in Indonesia. The second cause was the failure of a device to detect the presence of antigens in the feces, that may occur due to several factors such as when collecting the specimen, feces was too long to be accommodated so that *H. pylori* was not detected. In this study most of the infected cases were obtained the density of with a mild intensity (81.25%), it means that the number of on the mucosal surface of gaster only 1-3. The third cause was the likelihood of the effect of reading time. In this study as directed that the reading of the results was performed after 15 minutes and not recommended to reread after 15 minutes.

The false-positive values in this study were relatively small (6.5%). The several causes were sus-

pected to result in false positives on HpSA examination of rapid immunochromatography method that was the cross-reaction between the tool with other *Helicobacter* spp in the gastrointestinal tract and *H. pylori* in the form of a coracoid. HpSA positive predictive value of rapid immunochromatography method in this study was 55%, then the probability of patients with HpSA positive results for *H. pylori*-infected was only 55%. However, the negative predictive value (NPV) in this study was higher by 88% that if doctors got negative HpSA test results, then the negative result was completely negative by 88% (24). Based on the results of positive and negative value, in this study examination of HpSA method of rapid immunochromatography, better in ridding (ruling out) the infection of *H. pylori* bacteria.

Factors that affect false-positive and false-negative values also affect the outcome of positive and negative value. Then, for clinicians, positive and negative value were more important than sensitivity and specificity. A clinician should be able to interpret the results of the examination that has been performed. The positive and negative value was affected by the prevalence of disease. Both of these values will be different if done on the prevalence of different diseases. There were other parameters that uninfluenced by the prevalence, the positive likelihood ratio (LR+) and the negative likelihood ratio (LR-) (17).

The positive and negative values were useful for knowing the probability value of post-test. In this study, the probability of pre-test was 17.2%

and the probability of post-test 55%. This means that dyspepsia patients were performed by HpSA examination of rapid immunochromatography method will increase the probability from 17.2% to 55%, only increase by 37.8%. The higher the probability increase (up to close to 100%) then the method of examination was getting better (25).

In this study, low sensitivity (38%) and the positive value (not too high) (5.78) indicated that if HpSA examination results of the rapid immunochromatography negative method, it was not good enough to rid off an infection of *H. pylori*. The high specificity (94%) in this study was not supported by a relatively low negative value (0.67) indicating that if positive results obtained for HpSA examination the rapid immunochromatography method was not good enough in diagnosing (ruling in) *H. pylori* infection.

CONCLUSION

Based on a positive likelihood ratio and a negative likelihood ratio, *H. pylori* stool examination The immunochromatographic immune method was not good enough to improve post-test probability in patients. The overall examination of *H. pylori* Stool Antigen immunochromatography method is not good enough used to enforce (ruling in) and riddled (ruling out) the diagnosis of *H. pylori* infection in adult patients in Endoscopic Unit of Dr. Soetomo General Hospital, Surabaya.

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