Detection Of Helicobacter Pylori Infection With Stool Antigen: Comparison With Other Techniques

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ABSTRACT

Helicobacter pylori has been known as a cause of chronic gastritis, a predisposition to gastric and duocenal ulcers, and a class I gastric carcinogen. Throughout the world, H. pylori infection is very common, reaching 40% -50% of the population in developed nations and 80% – 90% of the population in developing nations.

Several techniques have been used to detect H. pylori infection, such as the urea breath test, rapid urease test, serological test, as well as biopsies of gastric or duodenal tissues for culture and histopathology. In this review article, we will discuss a relatively new method to detect H. pylori antigen in stools with enzyme immunoassay, and comparisons with other standard techniques. However, the H. pylori stool antigen test is not yet commercially available in Indonesia.

***Key words:** Helicobacter pylori - stool antigen - enzyme immunoassay*

INTRODUCTION

Helicobacter pylori is a bacteria that infects over 50% of the human population.[1] H. pylori infection particularly occurs during childhood, and resides in the gastric mucosa for long periods of time, or even eternally, if no erradication measures with specific a ntibiotics are taken. In South and Central America, Asia and Africa, approximately 80% of all children are infected by H. pylori at the age of 10 years. Worldwide, H. pylori is very common, reaching 40- 50% of all populations in developed nations and 80-90% of all population in developing nations. In Indonesia, seroepidemiologic studiens at several centers found a prevalence rate of 36-64,3%, with the youngest age of infection at 5 months.[2]

Warren and Marshall were the first to report H. pylori bacteria and its histological correlation with gastritis. This bacteria is associated with acute and active chronic gastritis, peptic ulcer, and atrophic gastritis.[1,3] H. pylori is considered as a class I gastric carcinogen, since its involvement is suspected in gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma.[4,5,6,7,8] H. pylori erradication in patients with low-grade lymphoma produces a remission rate in approximately 80% of all patients.[9]

Presently, several invasive as well as non-invasive methods are available to detect H. pylori infection, such as the urea breath test, the rapid urease test, serologic testing, and gastric or duodenal tissue biopsy by endoscopy for culture and histopathological evaluation. Nonetheless, for the patient's comfort, diagnostic acuracy, and evaluation of erradication treatment, various new methods have been developed in the hope to find a better diagnostic test. Recently, the non-invasive enzyme immunoassay (EIA) technique to detect H. pylori antigen in the patient's stool (H. pylori stool antigen (HpSA) has been developed. This method has been used in various countries to diagnose H. pylori infection, and even as a follow-up/evaluation of erradication treatment.

CHARACTERISTICS OF HELICOBACTER PYLORI

H. pylori is a negative-Gram bacteria, shaped as spiral, the letter S or curved, is micro-aerophillic, 0.5 x 3 mm in size, and has 4 to 7 coated flagels at one end. When dormant, the bacteria is shaped like a cocoid, and thus can survive in difficult environments. Its natural habitat is the human gaster. However, H. pylori can also

be found in primates and cats.[10]

H. pylori can produce a number of important virulence factors to be able to colonize the gaster and survive in unfriendly environments. The factors include:[1]

1. Urease, which plays an important role in neutralizing gastric acid secretion.
2. Flagel, to assist H. pylori in swimming in the mucous layer.
3. Superoxide dysmutase.
4. Several molecules involved in the specific adhesion to gastric superficial epithelial cells.
5. Cytotoxin-associated gene (CagA) and vacuolating cytotoxin (VacA) proteins, only produced by certain types of H. pylori.

To detect the presence of a microorganism, it is also important for us to know the mechanism for its transmission, to be able to determine the best method of evaluation for certain specimens.

H. Pylori transmission can occur through the following three (3) pathways:

1. Fecal-oral; H. pylori has been isolated in feces.[11]
2. Oral-oral; H. pylori has been isolated from the mouth cavity.
3. Iatrogenic; transmission from instruments for endoscopy, biospy forceps, or pH electrode contaminated with H. pylori.

TESTS TO DETECT H. PYLORI

The Urea Breath Test

The urea breath test (UBT), which uses the isotop ^{13}C or ^{4}C is an easy to perform non-invasive examination. Unlike the serological test, a positive result on the UBT signifies current H. pylori infection with a high sensitivity and specificity rate.[12] The goal of the UBT is to evaluate H. pylori urease activity. The UBT is able to prevent errors due to incorrect endoscopic biopsy sampling in cases of patchy gastritis. The UBT can be used to evaluate H. pylori 4 weeks after the administration of antibiotics, when serologic testing still produces a positive result.[2,13] False negatives occur if H. pylori has been suppressed by treatment with antibiotics or proton pump inhibitors.[12] UBT has a sensitivity rate of 95-99% and a specificity of 94-99%, while for the evaluation of post-H. pylori erradication treatment, it has a sensitivity rate of 94% and a specificity rate of 95%.[9] Cutler et al (1995) in his study compared the sensitivity and specificity of several diagnostic procedures for H. pylori and found the non-invasive UBT procedure as acurate as the campylobacter like organism test (CLO test) and Warthin-Starry staining to determine the H. pylori status in patients that have not undergone treatment.[14] However, the cost to perform this test and the complecity of the instruments cause this test to be rarely used.

Serological Testing

The objective of serological testing is to detect IgG anti-Hp antibodies. This test is non-invasive and is not too expensive compared to the UBT. However, since it requires blood sampling, it sometimes becomes a problem, particularly in children. After treatment for H. pylori, the serological titer for anti-Hp antibodies is slowly reduced within several months to several years, but does not always become negative. In addition, within 6 weeks after treatment for the erradication of H. pylori, the level of H. pylori antibodies only falls to 50-60% from the level before treatment, thus making it more difficult to evaluate the success of treatment.[15] A positive serological test result does not always determine cuurent infection with life bacteria. Serological testing is recommended for epidemiological surveys.[2,13] It has a sensitivity and specificity rate of only 82% and 81%, respectively.[9] A meta-analysis study on 11 commercial enzyme-linked immunoadsorbent assay (ELISA) kit and 1 latex agglutination kit found a sensitivity rate of 85% and a specificity rate of 79%.[16]

Polymerase Chain Reaction

Detection of H. pylori using the polymerase chain reaction (PCR) method was first introduced in early 1990. Because of its sensitivity, PCR is the method of choice to evaluate treatment response, if treatment has been able to reduce the number of bacteria. PCR can be performed from samples from saliva, dental plaques, gastric aspiration, and feces. PCR evaluation from gastric aspiration fluid has a sensitivity and specificity rate of 96% and 96% respectively compared to culture and histological evaluation from antrum biopsy specimens.[17] For H. pylori detection from fecal samples, the PCR has a specificity and sensitivity rate of 93,7% and 100% respectively.[3] Up to now, the PCR has not been frequently used for daily clinical purposes, and is instead more commonly used for research purposes.

Culture

H. pylori can be cultured from gastric biopsy, but the process is slow and requires a specific culture media, thus causing this method to be rarely used.[12] The sensitivity rate can reach over 95%, but other methods to diagnose H. pylori are relatively easier and produces more rapid results. The advantage of performing a culture is the

ability to determine antibiotic resistance. In the case of patchy H. pylori infection, it is difficult to obtain a representative sample for this method of evaluation.[14]

Histopathology

The gold standard for the diagnosis of H. pylori is to detect the organism from gastric biospy that has been processed with histological staining. The sensitivity rate for biopsy reaches 100% for biopsy of the angulus, 96-97% for antrum biopsy and 91-94% for corpus biopsy. If biopsy is taken from the antrum and corpus, the sensitivity rate can reach 100%. Specimen staining methods can influence the acuracy of H. pylori detection.[14] If examined by an experienced pathologist, routine staining with hematoxyllin and eosine can demonstrate the presence of H. pylori. The presence of polymorphonuclear leukocytes in the inflammed gastric mucosa supports H. pylori gastritis due to other causes (such as alcohol and non-steroid anti-inflammatory agents), and does not produce significant PMN infiltration.[12] Other staining include Giemsa, Warthin-Starry and Genta.

Rapid Urease Test

The rapid urease test, also known as the CLO test, was developed by Marshall and specifically designed to detect H. pylori. It has a sensitivity rate of approximately 90%, but varies from one study to the next, even within each institution. Its specificity reaches 95-100%. Gastric biopsy is placed in a media containing urea and phenophtaline. With the presence of H. pylori urease, urea is conversed into ammonium hydroxide, which changes the color of the indicator from yellow to red. A positive CLO test in patients with peptic ulcer is a strong proof of H. pylori infection. If the CLO test proves negative, further histological evaluation is required. As in the UBT, the CLO test and result in a false negative in patients whose infection is suppressed by antibiotics or proton-pump inhibitors.[12] Due to its high cost, the CLO test has become more difficult to obtain in Indonesia.

Enzyme Immunoassay Test for HpSA

It has been proven that patients infected with H. pylori can excrete H. pylori bacteria in their feces, thus allowing the bacteria to be detected in fecal specimen using PCR or culture.[3,11] However, culture of H. pylori from the feces is very difficult, since it is few in number, and PCR is costly. Thus, these two methods cannot be used as routine diagnostic procedures. Thus, a new method using the EIA, which is able to detect H. pylori antigen in human feces was developed (HpSA).

The method of evaluation is roughly as follows. The fecal sample is mixed with 200ml of the sample solvent. The fecal sample is then dissolved with peroxidase conjugated polyclonal antibody and inserted into the micro-well, to then be incubated for 1 hour in room temperature. After the discs are washed to eliminate materials that are not bound, add 2 drops of substrate liquid and incubate for 10 minutes in room temperature. The reaction is terminated by dropping 1 drop of terminating agent. The result is then read using a spectrophotometer.[18]

Makristathis *et al*. (1998) in his prospective study, found that EIA has a sensitivity of 88,9% and a specificity of 94,6% to detect HpSA prior to eradication treatment. Thus, we could conclude that EIA is a satisfactory method to detect H. pylori infection in the feces, since it is just as sensitive as PCR, histology, and gastric biopsy culture.[3]

Fanti *et al.* (1999) in his study to evaluate EIA for HpSA found that this method has a sensitivity of 98,2%, with a negative prediction value of 96,4% and a specificity rate of 93,1% with a positive prediction value of 96,4%. Fanti *et al* concluded that this test has a high and specific sensitivity for the detection of H. pylori infection. Nevertheless, the acuracy of EIA in detecting antigen after erradication treatment requires further evaluation. The most recent reports demonstrate conflicting results, even though most studies report a satisfactory sensitivity and specificity even for HpSA testing after erradication treatment. Likewise, the precise point for the monitoring of H. pylori erradication treatment needs further evaluation.[15]

Vaira *et al.* (1999), in a multi-center prospective study, found a sensitivity rate of 94,1% and a specificity rate of 91,8% for HpSA testing. The HpSA test and the UBT conducted 4 weeks after erradication treatment also found a sensitivity and specificity rates of 90% and 95,3% respectively for HpSA testing, and 90% and 98,9% respectively for the UBT. Thus, unlike serologic testing that requires several months to achieve significant reduction in antibody titer, the HpSA and UBT with ^{13}C can be used not long after treatment (4 weeks).[4]

Forné *et al.* (2000) compared HpSA testing with histological methods, , UBT ewith ^{13}C and the rapid urease test for the diagnosis of H. pylori infection and to evaluate the use to determine H. pylori state after treatment. To diagnose H. pylori infection, the HpSA test has a sensitivity and specificity rates of 89,5% and 77,8% respectively. The specificity is lower than that of UBT, histological evaluation and rapid urease test. Within 24 hours after treatment, the sensitivity test for HpSA is 0%. Within 6 weeks after treatment, the sensitivity falls to 70,4% and 81,6%.

Six months after treatment, the sensitivity and specificity is further reduced to50% and 79,3%. Thus, we can conclude that the HpSA test is beneficial for the primary diagnosis of H. pylori, with a similar sensitivity with other standard tests, but with a lower specificity. HpSA testing is not useful for early monitoring to determine the efficacy of treatment. Within 6 weeks and 6 months after treatment, for further evaluation of the result of erradication treatment, HpSA testing is not very accurate compared to the UBT.[18]

Rani (2001) in a study on 44 dyspepsia cases found that the HpSA is quite sensitive (up to 100%), but has a lower specificity (57,9%). In cases of duodenal ulcer and gastric carcinoma, H. pylori detection by this means can reach a rate of 100%.[19]

CONCLUSION

- There are several evaluation methods for the detection of H. pylori infection, which are as follows: the urea breath test, serological testing, the rapid urease test, histopathological evaluation, polymerase chain reaction and enzyme immunoassay for fecal H. pylori antigen, with various advantages and disadvantages.
- The enzyme immunoassay for fecal H. pylori antigen (HpSA) is expected to be an alternative for the detection of H. pylori and for further monitoring of erradication treatment in daily practice.
- Due to inadequate data on the use of HpSA testing, further studies are required to evaluate the sensitivity and specificity of HpSA testing, both to detect H. pylori as well as to monitoring the success of erradication treatment.

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