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Original Article

Evaluation of Bio M *pylori* serologic test and C-13 urea breath test for *H. pylori* infection in children with recurrent abdominal pain: a pilot study

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Abstract

Background Diagnosing *Helicobacter pylori* infection in children is still a problem. Urea breath test is the gold standard for non-invasive diagnostic test, but it is expensive and not available in most hospitals. The Bio M *Pylori* serologic test has good diagnostic value in adults, less expensive and more practical, but had never been evaluated in children.

Objective To determine the prevalence of *H. pylori* infection and the diagnostic accuracy of Bio M *Pylori* serologic test in children with recurrent abdominal pain.

Methods This study was conducted in May – June 2009. Children aged 12-15 years with recurrent abdominal pain were examined with urea breath test and the Bio M *Pylori* serologic test. Sensitivity, specificity, area under the curve (AUC), predictive values, and likelihood ratios were calculated for the Bio M *Pylori* serologic test.

Results Most subjects aged 13 years (83%). Girls outnumbered boys, and most were undernourished. The prevalence of *H. pylori* infection detected by urea breath test and Bio M *Pylori* serologic test was 8% and 52%, respectively. The Bio M *Pylori* serologic test had sensitivity and specificity of 100% and 53%, respectively. Area under the curve (AUC) was 0.764. Positive and negative predictive values were 16 and 100%, whereas positive and negative likelihood ratios are 2.12 and 0. The overall accuracy of this test is 57%

Conclusions The Bio M *Pylori* serologic test has high sensitivity value (100%). This diagnostic kit can be considered as a good pre-endoscopic screening tool in children with recurrent abdominal pain caused by *H. pylori* infection. **[Paediatr Indones. 2010;50:101-104]**.

Keywords: *H. pylori infection, recurrent abdominal pain, Bio M Pylori serologic test, urea breath test*

Helicobacter pylori infection is still a problem in children, with the prevalence ranging from 10 to 80%; however most children show no specific symptoms.[1-4] The most common symptom correlated with *H. pylori* infection is recurrent abdominal pain.[2,3,5-7] *Helicobacter pylori* infection is chronic, always active and tends to spread if not completely eradicated. There are several diagnostic tools to detect *H. pylori* infection with its own advantages. A reliable diagnostic tool is needed to establish diagnosis and evaluate treatment. [8,9]

Problems are found in performing diagnostic test for *H. pylori* infection in children, especially in collecting breath samples and controversies about validation of test.[2,3,10] Urea breath test (UBT) is the most common diagnostic test in establishing *H. pylori* infection in children because proven for its high sensitivity and specificity (with tissue culture as gold standard).[9,11] The basic mechanism of UBT is the breakdown of urea by urease in *H. pylori* into

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ammonia and CO_2.[2,10-14] UBT is considered as the non-invasive gold standard for *H. pylori* infection in children, but this tool is expensive and not available in most hospitals.[9,10] This test also requires good cooperation from patient to collect breath samples. An alternative tool which is more affordable, available in most hospitals and using local antigen is needed in developing countries.[12,13] Bio M *pylori* Serologic test is commonly used to detect *H. pylori* infection, but *H. pylori* is a bacteria with high diversity, that antigen from one strain in our country may differ with other country.[15,16] Besides this high diversity, host immune response towards *H. pylori* infection is different among ethnics.[2,8,14]

The aim of this study was to determine the prevalence of *H. pylori* infection in children with recurrent abdominal pain and to determine the sensitivity dan specificity of Bio M *pylori* Serologic test in detecting *H. pylori* infection compared to UBT as the gold standard.

Methods

This diagnostic study was conducted in May-June 2009 at 5 junior high schools in Jakarta. Purposive sampling was done with 60 minimum subjects needed. This study was approved by the Ethics Committee; written informed consent was obtained from each child's parents or legal guardian prior to enrollment.

There were 60 children aged 12-15 years with recurrent abdominal pain included in this study. Basic data were obtained, including sex, age, weight, height, nutritional status, duration of recurrent abdominal pain, and other complaint.

Each subject underwent urea breath test. Subjects were asked to hold breath for ten second and breath forcefully (with a minimal volume of 150 cc or 1/3 bag volume) into the first UBT bag as a baseline sample. Afterwards, patients were asked to drink 1 glass of water containing one bag of 13C-urea, and lie down on the left side for 5 minutes, then stand in the waiting room. A post-UBT breath was taken 20 minutes later, with the same protocol as the baseline sample. All UBT bags were collected in a beer box and sealed promptly to avoid leakage.

Each subject also underwent a Bio M *pylori* Serologic test using a nitrocellulose membrane attached to a test card. The test card is placed in a room temperature for 20 minutes and taken out from the aluminium pack. A 20 μL serum from blood vein sample was dropped to the blue pad on the test card, and one drop of buffer is added in the same pad. Another two drops of buffer is added in the red pad. After serum reached limit line, the test card was closed and the result is read after 5-20 minutes. The test result is positive if two lines appeared and negative if only one line appeared. The test is considered invalid if no line appeared.

The diagnostic value (sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio) of the Bio M *pylori* Serologic test were calculated using the UBT as the gold standard.

Results

Samples were collected in May – June 2009. Subject's characteristic are shown in Table 4.1. The prevalence of H. pylori infection in children with recurrent abdominal pain based on urea breath test and Bio M pylori serologic test were 8% and 52%, respectively (**Table 4.2.**).

Results of Bio M *pylori* serologic test compared to UBT are shown in **Table 4.3.**

In this study, the Bio M *pylori* serologic test had a 100% sensitivity (95% CI 47 to 100%) and 53%

Table 4.1. Subject's characteristic

Variable	Total
Sex, n	
Male	13
Female	47
Age, years	
12 year-old	9
13 year-old	26
14 year-old	22
15 year-old	3
Nutritional status, n	
Undernourished	29
Well-nourished	21
Overweight	4
Obesity	6
Other complaint besides abdominal pain, n	
Nausea	47
Vomiting	13
Bloated abdomen	48
Awaken at night	44
Recurrent diarrhea	28

Table 4.2. Prevalence of *H. pylori* infection in children with recurrent abdominal pain

Diagnostic test	N	%
UBT	5/60	8%
Bio M pylori serologic test	31/60	52%

Tabel 4.3. Results of *H. pylori* infection in children with recurrent abdominal pain

	UBT		
	Abnormal	Normal	Total
Serologic positive	5	26	31
Serologic negative	0	29	29
Total	5	55	60

specificity (95%CI 39 to 66%). The area under the receiver operating characteristic (ROC) curve (AUC) was 0.764 (95%CI 0.697 to 0.83). The positive and negative predictive value were 16% (95% CI 5 to 34%) and 100% (95% CI 88 to 100%), respectively. The positive and negative likelihood ratio were 2.12 (95% CI 1.6 to 2.8) and 0, respectively.

Discussion

This study was conducted in children aged 12-15 years with recurrent abdominal pain. This is a good diagnostic study to reveal the sensitivity and specificity of a test (Bio M *pylori* serologic test) compared to diagnostic case-control studies which take samples from subjects with a positive *H. pylori* infection and control from normal subjects.

The prevalence of *H. pylori* infection in this study was 8% based on the urea breath test (UBT). This finding is lower than the study by Susanto et al[6] that found a prevalence of 28%. This may be due to the difference of subjects used. Our study used school children aged 12-15 years with recurrent abdominal pain, while Susanto used samples from patients in referral hospitals, where other disease has been ruled out before.

The sensitivity of the serologic M pylori bio kit was 100%. This value is higher than the study by Muttaqin et al[16] that found a 95% sensitivity in adult population, but the 95% confidence interval is wide (48% to 100%). Specificity was much lower (53%) compared to study by Muttaqin et al[16] (92%) in adult population. This low specificity value may be due to: a) transient *H. pylori* infection that is common in children (spontaneous infection clearance), false positive result occur due to a decrease antibody after clearance;[17] b) non-direct *H. pylori* eradication due to antibiotic given for other indication.[18]

Antigen feces test had been reported to have good sensitivity and specificity compared to urea breath test (>90%) in adult and children population.[19] This test had been studied in Indonesia but the validity has not been reported. Syam et al[20] conducted a study in adult population and reported good sensitivity (100%) but low specificity (37%) compared to this study (with a 0.16 cut-off according to the manufacture). If cut-off is made higher (0.274) the sensitivity will be lower (67%) and the specificity higher (79%).

The area under the receiver operating characteristic (ROC) curve (AUC) of this serologic Bio M *pylori* test was 0.764 (95% CI 0.697 to 0.83). It means that this kit is considered as a good diagnostic tool. Study by Syam et al[20] using antigen faeces test in adult population revealed a slightly lower AUC compared to serologic M *pylori* bio kit with a value of 0.722 (95% CI 0.518 to 0.927).

The low prevalence of *H. pylori* in this study (8%) will give a low positive predictive value (16%). If this test is performed in a population with low prevalence of *H. pylori* infection and the serologic result is positive, then the possibility of having *H. pylori* infection is low. Further examination, such as UBT, antigen faeces test or endoscopy should be perform to establish diagnosis in this case. But in high prevalence population, a positive result is more possible to be truly positive and is useful in pre-endoscopic screening in children with recurrent abdominal pain. On the other hand, high negative predictive value (100%) means that a negative result in a low prevalence population will assure us that *H. pylori* infection is not present.

The serologic M *pylori* bio kit have a positive likelihood ratio of 2.12 (95% CI 1.6 to 2.8). This value is higher than the study of Syam et al[20] that used antigen faeces test with a value of 1.6 (cut-off according to manufacture), but higher value is found with higher cut-off (3.16).

The serologic M *pylori* bio kit has several benefits compared other tools. A screening test,using this kit, will avoid spending too much cost in further advance test, is easy to perform even in children (no

need to collect breath samples), can be done in a simple laboratory and needs no expensive tool. But the result of this test cannot decide whether a child with recurrent abdominal pain needs treatment of *H. pylori* eradication or not, because a positive result has to be confirmed by other advanced test for *H. pylori* infection. If used as a pre-screening endoscopy, this test can reduce the number of patient that should be examined by endoscopy. The limitation of this study is the small sample size, due to limitation of budget.

In conclusion, this kit is potential in screening children with recurrent abdominal pain suspected to have *H. pylori* infection before further invasive test is performed. Further study with larger sample is needed to answer this question.

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