

Validation of ^{13}C -Urea Breath Test for the Diagnosis of *Helicobacter pylori* Infection among Dyspeptic Patients at Dr. Soetomo Hospital, Surabaya

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ABSTRACT

Background: The urea breath test (UBT) has been published as the most sensitive and specific non-invasive test to detect *Helicobacter pylori* (*H. pylori*) infection. The limitation of UBT is the need of expensive equipment that is not always widely available. Recently, UBT has already been available in Surabaya. In the other hand, our experience using rapid urease test which detected urease enzyme produced by *H. pylori* as UBT showed low sensitivity.

Objective: To investigate the validation of UBT for the diagnosis of *H. pylori* infection in patient with dyspepsia.

Design: Cross-sectional study.

Method: Sixty patients who complained symptoms of dyspepsia were examined for *H. pylori* infection using UBT. Gastroscopy and biopsy was performed and the biopsy specimens were examined by Pathologist.

Results: Sixty patients consist of 28 male and 32 female were enrolled of this study. Eight patients had *H. pylori* positive by both UBT and histologic examination. One patient was *H. pylori* positive by UBT but negative by histologic examination. One patient was *H. pylori* negative by UBT but positive by histologic examination. The sensitivity of UBT was 88.9% and the specificity was 98 %. The negative predictive value was 98%.

Conclusion: In this study, UBT has lower sensitivity (88.9%) and comparable specificity (98%) for diagnosing *H. pylori* infection. Comprehensive studies to determine the doses of ^{13}C -urea, test meal and appropriate collection time, which is more suitable for local population was suggested.

Keywords: ^{13}C -Urea Breath Test, *Helicobacter pylori*, dyspepsia, diagnosis

INTRODUCTION

Helicobacter pylori (*H. pylori*), which is responsible for the most common infection worldwide, has been implicated in several gastrointestinal disease, such peptic ulcer disease, gastric adenocarcinoma, and MALT lymphoma.^{1,2}

A variety of highly sensitive and specific test are available to diagnose *H. pylori* infection, which divided into two types: noninvasive and invasive examination. Among those, many gastroenterologists still consider histology confirmation of the presence of *H. pylori* on

mucosal biopsy specimens or culture is the 'gold standard' of test.¹

Since have been proposed by Graham et al, the urea breath test (UBT) is generally regarded as the most sensitive and specific non-invasive test that indicates active *H. pylori* infection.³ The Maastricht 2-2000 Consensus,² recommended that UBT should be used as non-invasive diagnostic tool of *H. pylori* infection for 'test and treat' approach, because this is based on level-1 supporting evidence. The UBT may utilize either ^{14}C or ^{13}C and there are commercial test kits available for both. The main issue surrounding the use of the ^{14}C -urea breath test is the safety of ^{14}C -urea. Being a radioactive isotope, concerns exist as to its safe handling, administration and disposal as well as the appropriateness of administration to children,

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Table 1. Demographic and baseline characteristic of the patient

Characteristic	Total	(%)
Sex		
Male	28	46.67
Female	32	53.33
Age (years)		
20 – 29	16	26.67
30 – 39	18	30.00
40 – 49	17	28.33
50 – 59	8	13.33
≥ 60	1	1.67
Ethnics		
Javanese	50	83.33
Maduranese	3	5.00
Buginese	1	1.67
Bataknese	1	1.67
Sumbawane	1	1.67
Floresnese	1	1.67
Ambonese	1	1.67
Sundanese	1	1.67

Histologic Examination

Two senior pathologists at Soetomo hospital did the histology examinations. Among 60 specimens from 60 patients, the first pathologist found nine specimens were positive which came from five male and four female. The second pathologist also found that these nine specimens were positive and the other specimens were negative. The kappa index for inter-observers agreement among the pathologist was 1: nearly perfect.

After examined by the second pathologist, the histology specimens were renumbering randomly than reexamined by the first pathologist blindly. Among 60 specimens nine were *H. pylori* positive which came from the same patients as the first examination. The kappa index for intra-observers agreement of the first pathologist was 1: nearly perfect.

The Urea Breath Test (UBT)

Of sixty patients undergoing ¹³C-UBT, nine patients consisted of six men and three women were *H. pylori* positive. Compare to histology examinations, one patient with *H. pylori* infection positive by histology was negative by ¹³C-UBT and the other one who was positive by ¹³C-UBT was negative by histologic examination (table 2).

Table 2. The sensitivity and specificity of ¹³C-UBT

¹³ C-UBT	Histology		Total
	<i>H. pylori</i> positive	<i>H. pylori</i> negative	
<i>H. pylori</i> positive	8	1	9
<i>H. pylori</i> negative	1	50	51
Total	9	51	60

The sensitivity for ¹³C-UBT compared to histology was 88.9% and the specificity was 98%. The positive predictive value was 88.9% and the negative

predictive value was 98%.

DISCUSSION

Among 60 patients who enrolled this study, 10 (16.67%) patients consisted of six men and four women were detected to be infected by *H. pylori* by Histology and or UBT. Although the frequency of *H. pylori* infection in male was more frequently than female patients as reported by several authors, but the different between both sexes in this study did not reached statistically significant. This result might be due to small number of sample.

The sensitivity of UBT is calculated as all subjects who detected have *H. pylori* infection by both histologic examination and UBT divided by all subjects who have *H. pylori* positive by histologic examination. In this study, the sensitivity of UBT was 88.9 %. It was not as high as had been published, which reported the sensitivity of ¹³C-UBT were 94 -100%, even though using low doses of ¹³C-urea^{5,6,7}. In this study, the collection time was before ingestion of ¹³C-urea and 20 minutes after ingestion of ¹³C-urea.¹³ Some studies reported that the doses of ¹³C-urea, collection time and test meal influenced the sensitivity and specificity of ¹³C-UBT.^{7,9,14} Perhaps we should determine the appropriate doses, test meal and collection time for our clinics.

The specificity was calculated as the negative results from both test divided by the negative results from histology test. In this study, the specificity of ¹³C-UBT was 98%. This specificity was comparable with the other study.^{4,5,6} The negative predictive value of UBT in this study was 98%. It was similar with other study and confirmed that the UBT as the most accurate methods to documented eradication of *H. pylori*.¹

CONCLUSION

In this study, the sensitivity of ¹³C-UBT was 88.9%. It was less sensitive than have been reported. In the other hand, the specificity and the negative predictive value were 98% and 98% respectively. There were comparable with the previously study.

Other comprehensive studies to determine the doses of ¹³C-urea, test meal and appropriate collection time, which is more suitable for local population are needed.

REFERENCES

- Knigge KL. The role of *Helicobacter pylori* in gastrointestinal disease: A guide to identification and eradication. Postgrad Med [serial online] 2001[cited 2006 Aug 17];110(3):71-82. Available from: URL:http://www.postgradmed.com/issues/2001/09_01/knigge.htm.
- Malferteiner P, Megraud F, O'morain C, Hungin APS, Jones R, Axon A, et al & The European *Helicobacter pylori* Study Group (EHPG). Current concepts in the management of

pregnant women or women of childbearing age. Regulations governing the use of radioactive material also make its use restricted to selected sites.⁴ The ^{13}C -urea breath test (^{13}C -UBT) is also safe and accurate test for the detection of *H. pylori* infection in adult and children.^{5,6} Recently, a study using low dose ^{13}C shows the similar sensitivity and specificity to diagnose *H. pylori* infection.⁷ Although, the ^{13}C -UBT proved to be a safe, non invasive, and accurate test for the detection of *H. pylori* infection, it need a special equipment for measuring ^{13}C which is not widely available especially in Indonesia. Recently, one of private laboratory offers ^{13}C -urea breath test to use and we did not have sufficient experience regarding the test.

In the other hand, Rapid urea test is the most commonly used and less expensive invasive test to document *H. pylori* infection.¹ Rapid urea test has the similar principle with UBT which detect the urease enzyme activity of *H. pylori*. Our experience using rapid urea test showed lower sensitivity compared with histologic examination. Maybe there was false-negative results which might occur because of sampling error or low inoculums, especially in patient with prior proton pump inhibitors (PPI) use.⁸

METHODS

Study Design

This study was a cross-sectional study. Patients were recruited from Gastroenterology Clinic - Dr. Soetomo Hospital, Surabaya. The study was conducted in accordance with the ethic principles of the Declaration of Helsinki and was consistent with Good Clinical Practice guidelines and applicable to local regulatory requirement. Written informed consent was obtained from all randomly assigned patients.

Selection of the Patients

Sixty patients among 157 new patients who arrived at the outpatient Gastroenterology Clinic of Dr. Soetomo hospital, with uninvestigated dyspepsia symptom for at least 1 month were selected randomly. The inclusion criteria were: (1) Male or female, 21 - 60 years old; (2) Suffering of at least two of the following symptoms: upper abdominal pain or discomfort, bloating, nausea, vomiting for 4 weeks or more; (3) Agreed to participate in the study and sign the informed consent; (4) Have no previous abdominal surgery; (5) Not pregnant nor breast feeding mother; (6) Have no alarm sign symptoms; (7) Have no serious underlying disease.^{4,7,9}

Determination of *H. pylori* Infection

• Histology

A 'gold standard' for diagnosing *H. pylori* infections, so far, is difficult.^{4,10} The sensitivity and specificity of histological detection of *H. pylori* depend on the experience of pathologist.^{11,12} Careful histological examination of at least two biopsies appears to come closest to 'gold standard'.^{1,10}

Sixty selected patient were prohibited to taking antibiotic, proton pump inhibitor, H_2 receptor antagonist, bismuth compound at least 4 weeks prior of the study. The patients were undergoing upper endoscopy by Gastroenterologist and two biopsy specimens were taken from the antrum and two from the corpus for histologic examination. The biopsy specimens were processed routinely, embedded in paraffin wax and stained with giemsa. The specimens were examined by two different pathologist who were blinded to the UBT and other pathologist results, *H. pylori* infection was described as positive if the curved bacilli was found, than the results were analyzed for inter observer agreement, after that the pathology specimens were renum bering randomly and than re-evaluated by one of them blindly, and intra-observer agreement wascalculated.

• ^{13}C -Urea Breath Test

One week after upper endoscopy examination the ^{13}C -urea breath test was done, the baseline expired breath samples were collected before the patients taking ^{13}C -urea. Patients were asked to drink a solution containing ^{13}C -urea and then asked to lie on their left side. After 20 minutes, expired breath samples were collected.¹³ The collected breath samples were analyzed using UBiT®-IR300 infrared spectrophotometer.

Statistical Analysis

Two by two tables were performed to determine the sensitivity and specificity of the UBT compare to Histology test. The kappa index was used to assess the proportion of intra-observer and inter-observer agreement.

RESULTS

Study Population

Sixty patients consisted of 28 male and 32 female were eligible for this study. Most of them were Javanese ethnic and the ages were between 20-60 years old (mostly under 50 years) as showed in table 1.

- Helicobacter pylori* infection - The Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002;16:167-80.
3. Graham DY, Evans DJ, Alpert LC, Klein PD. *Campylobacter pylori* detected non invasively by the ¹³C urea breath test. Lancet 1987;1:1174.
 4. Chua TS, Fock KM, Teo EK, Ng TM. Validation of ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection in the Singapore population. Singapore Med J [serial online] 2002 [cited 2006 May 25];43(8):408-11. Available from: URL: <http://www.sma.org.sg/smj/4308/4308a5.pdf>.
 5. Bazzoli F, Cecchini L, Corvaglia L, Dall'Antonia M, De Giacomo C, Fossi S, et al. Validation of the ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection in children: A multicenter study. Am J Gastroenterol 2000;95(3):646-50.
 6. Ralf Herold, Michael Becker. ¹³C-Urea breath test threshold calculation and evaluation for the detection of *Helicobacter pylori* infection in children. BMC Gastroenterology [serial online] 2002 [cited 2006 May 25];2:12. Available from: URL: <http://www.biomedcentral.com/1471-230X/2/12>.
 7. Gatta L, Ricci C, Tampieri A, Osborn J, Pema F, Bemabucci V, et al. Accuracy of breath tests using low doses of ¹³C-urea to diagnose *Helicobacter pylori* infection: A randomized controlled trial. Gut 2006;55:457-62.
 8. Javed Y, Wasmin J, Shahab A, Nadim J, et al. Role of rapid urease test and histopathology in the diagnosis of *Helicobacter pylori* infection in developing country. BMC Gastroenterology [serial online] 2005 Jan-Mar [cited 2006 May 25];5(38):[2 screens]. Available from: URL: <http://www.biomedcentral.com/1471-230X/5/38>.
 9. Peng NJ, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, et al. Capsule ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection. World J Gastroenterol [serial online] 2005 [cited 2006 Aug 17];11(9):1361-4. Available from: URL: <http://www.wjgnet.com/1007-9327/11/1361.pdf>.
 10. Wyatt JI, Gray SF. Detection of *Helicobacter pylori* by histology. In: Rathbone BJ, Heatley RV editors. *Helicobacter pylori* and gastroduodenal disease. Oxford: Blackwell Sci Publ 1992.p.51-7.
 11. Maconi G, Vago L, Galletta G, Imbesi V, Sangaletti O, Parente F, et al. Is routine histological evaluation an accurate test for *Helicobacter pylori* infection? Aliment Pharmacol Ther 1999;13(3):327-31.
 12. Deltenre M, Glupczynski Y, De Prez C, Nyst JF, Burette A, Labbé M, Jonas C, DeKoster E. The reliability of urease tests, histology and culture in the diagnosis of *Campylobacter pylori* infection. Scand J Gastroenterol Suppl 1989;160: 19-24.
 13. Abdullah M, Rositawati W. Pemeriksaan ¹³C-urea breath test untuk diagnosis infeksi *Helicobacter pylori* aktif. Informasi Laboratorium 2003;1:3-6.
 14. Peng NJ, Hsu PI, Lee SC, Tseng HH, Huang WK, Tsay DG, et al. A 15-minute [¹³C]-urea breath test for the diagnosis of *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. J Gastroenterol Hepatol 2000;15(3): 284-9.