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Helicobacter pylori 감염에서 새로운 저용량 ^{13}C-요소캡슐과 기존의 정제형 ^{13}C-요소제제를 이용한 요소호기검사간 비교

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Comparison Between a New Low Dose Urea Capsule Test and the Conventional UBiT® Tablet Test for the Detection of *Helicobacter pylori* Infection

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Background : The urea breath test (UBT) is regarded as a highly reliable, noninvasive tool for diagnosing *Helicobacter pylori* infection. We compared a recently developed low-dose 38 mg ^{13}C-urea capsule, which is able to eliminate oral urease effects and does not require positional changes during the test, with the conventionally used 100 mg ^{13}C-urea tablet method.

Methods : Thirty-nine volunteers were tested under informed consent with both ^{13}C-UBT methods, Helifinder® and UBiT-IR300®, with a minimum 2-week washout period. The pre-ingestion and 20-minute post-ingestion breath samples were analyzed with an isotope ratio mass spectrometer for Helifinder, and a nondispersive isotope-selective infrared spectrophotometer for UBiT samples.

Results : Helifinder method showed excellent agreement with UBiT among 19 positive and 20 negative cases (weighted kappa value, 1.0). Helifinder results (y) showed good agreement but with a proportional bias compared to UBiT results (x) by Passing and Bablok method (y=0.551x-0.255, r=0.74, P<0.0001).

Conclusions : Since the low-dose 38 mg ^{13}C-urea capsule (Helifinder) test, which is more convenient and economic, showed comparable results with the conventional UBiT method, it can be used as an alternative for the diagnosis of *H. pylori* infection. ***(Korean J Lab Med 2006;26:81-5)***

Key Words : *Urea breath test, Helicobacter pylori, Low-dose urea*

INTRODUCTION

The role of *Helicobacter pylori* in various gastroduodenal pathologies, including gastritis, duodenal ulcer, gastric ulcer, stomach cancer, and stomach mucosa-associated lymphoid tissue (MALT)oma, has led to the development of many diagnostic methods for detecting *H. pylori* in the stomach, both invasive and non-invasive[1-3]. Among the non-invasive tests, the ^{13}C-urea breath test (^{13}C-UBT), first introduced by Graham et al. in 1987, is one of the most important, accurate, and commonly used methods for detecting *H. pylori* infection[4]. Due to its excellent diagnostic accuracy, the ^{13}C-UBT has been promoted as the preferable method for confirming eradication as well

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as epidemiological investigations[5-7]. The ^{13}C-UBT is based on the capacity of the *H. pylori* urease to hydrolyze urea to ammonia and carbon dioxide, which diffuses into the blood, and is excreted by the lungs. Thus, by labeling urea with ^{13}C, the presence of *H. pylori* in the stomach can be identified by detecting ^{13}C in the expired breath sample with measuring equipments such as an isotope ratio mass spectrometer (IRMS), a nondispersive isotope-selective infrared spectroscopy (NDIRS), or a laser-assisted ratio analysis (LARA). During recent years, the ^{13}C-UBT has made several modifications to provide a more innocuous, simplified, and applicable procedure to be used in the clinical setting. Although the ^{14}C-labelled urea can also be utilized for UBTs, this radioactive isotope brought up problems dealing with hospital department licensing for storage and disposal of radioactive substrates and, more importantly, radiation exposure for the patients[8, 9]. In contrast, the non-radioactive stable isotope ^{13}C is innocuous enough that the ^{13}C-UBT can be repeated as often as required in the same patient and can also be safely performed in children and women of child-bearing age. Moreover, lowering the dose of ^{13}C is an advantageous attempt to provide a more risk-free UBT technique for *H. pylori* detection.

In this study, we compared a recently developed low-dose urea capsule that uses an IRMS system with the conventional ^{13}C-UBT for detection of *H. pylori* infection. The currently used UBiT method was employed as the reference standard since a growing number of studies with favorable results for this tablet-based ^{13}C-UBT implementing the NDIRS have been reported[10, 11].

MATERIALS AND METHODS

1. Study population

Apparently healthy volunteers were recruited for the comparative ^{13}C-UBT study. On receiving approval from the Institutional Review Board (IRB), the study was conducted from November 2004 until January 2005. Participants who had a history of treatment with antibacterial agents, proton pump inhibitors, histamine receptor antagonists, bismuth preparations, and ecabet sodium preparations within 4 weeks prior to the start of the study were excluded. Other exclusion criteria included a history of gastric surgery, possible or current pregnancy, participation in a clinical study within 4 weeks prior to the start of this study, alcoholism or drug abuse, and pulmonary abnormalities such as asthma or chronic pneumonia. All thirty-nine subjects submitted their written informed consent prior to testing.

2. Study phases

The low-dose 38 mg ^{13}C-urea capsule (Helifinder®; InBioNet Pharmaceutical, Ichon, Korea) method was tested followed by a 2-week washout period before performing the ^{13}C-UBT using the 100 mg ^{13}C-urea tablets (UBiT®; Otsuka Pharmaceutical, Tokyo, Japan). Both urea breath tests required a minimum of 4-hour overnight fasting prior to testing for all participants. ^{13}C was measured as the $^{13}CO_2$:$^{12}CO_2$ isotope ratio and expressed as delta over baseline (DOB) per mil (‰).

The first phase of the study protocol was done by urea breath testing using the Helifinder capsules, which contained polyethylene glycol (PEG) 4000 to enhance the dissolution of urea. The baseline breath sample was collected in a white-labeled test tube through a straw, and 20 minutes after ingestion of the capsule with 50 mL of water, a second breath sample was collected in a blue-labeled test tube. The $^{13}CO_2$ levels in the before and after ^{13}C administration breath samples were analyzed using an isotope ratio mass spectrometer (IRMS, Medichems, Seoul, Korea). If the change (Δ^{13}C: ‰) from the baseline (pre-ingestion) level at 20 minutes after ingestion was 2.0‰ or higher, the patient was determined as *H. pylori*-infected, and non-infected if lower than 2.0‰[12].

The second phase required collection of a pre-administration breath sample in a special breath-sampling bag, before ingestion of one film-coated ^{13}C-urea tablet (UBiT) with 100 mL of water. Subsequently, participants remained in the left lateral decubitus position for 5 minutes upon which they sat up to a sitting position for further 15 minutes, before the 20-minute breath sample was taken. The $^{13}CO_2$ levels in these breath samples were analyzed by a nondispersive isotope-selective infrared spectrophotometer (NDIRS; UBiT-IR300; Otsuka Electronics, Osaka Japan) and likewise, *H. pylori* infection was determined using Δ^{13}C(‰), the calculated difference between pre-administration and 20-minute breath sample. A *H. pylori*-positive result was given when the Δ^{13}C was more than 2.5‰,

Table 1. Patient distribution of urea breath test-positive versus -negative results

Gender (No)	No. of patients with urea breat test	
	Positive	Negative
Male (12)	6	6
Female (27)	13	14
Total (39)	19	20

Table 2. Agreement of ^{13}C-urea breath test results between Helifinder® and UBiT®

UBT Method	HeliFinder®		Total
	Negative	Positive	
UBiT®			
Negative	20	0	20
Positive	0	19	19
Total	20	19	39

and *H. pylori*-negative when the Δ^{13}C was less than 2.5‰. We repeated the Helifinder test following the UBiT test for 8 available cases after an additional 2-week washout period. A rapid urease test (CKD Bio Hp, Seoul, Korea) was performed during the endoscopic gastrointestinal biopsy in some of the patients.

3. Statistical analysis

The comparison of the ^{13}C-UBTs (Helifinder® versus UBiT®) were tested by linear regression analysis and Pearson's coefficient for correlation, Passing-Bablok comparison methods, kappa testing for agreement, and chi-square test using Analyse-it for Microsoft Excel (Analyse-It Software, Ltd. Leeds, UK).

RESULTS

Of the 39 apparently healthy volunteers between the age of 22 to 60 (mean±standard deviation, 37.6±9.75) with a male to female ratio of 12:27 (Table 1), 19 were positive by both Helifinder and UBiT methods with excellent agreement between the two methods (weighted kappa value, 1.0, Table 2). The DOB value of Helifinder (y, ‰) showed good agreement but with a proportional bias compared to UBiT (x, ‰) by the Passing and Bablok method (y=0.551x-0.255, r= 0.74, P<0.0001, Fig. 1). Initially, a discrepant case with Helifinder-positive (13.97‰) and UBiT®- negative (0.5‰) results was detected but, on follow-up, both UBiT and Helifinder tests were positive (42.3‰ and 13.6‰, respectively). The rapid urease test was performed during the endoscopic gastrointestinal biopsy in this patient and a positive rapid urease test result supported the positive results from both urea breath tests. The replicate study of Helifinder for the 8 available cases also showed excellent agreement (y=0.972x+0.0141, r=0.9, P=0.0023; kappa statistics, 1.0) with no significant difference (P>0.05 by paired t-test).

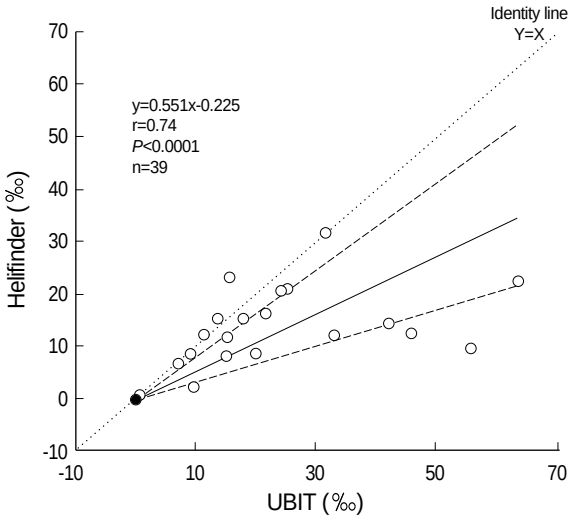

Fig. 1. Passing and Bablok comparison of Helifinder® and UBiT® ^{13}C values (‰). Dashed lines show the 95% confidence limits of the regression line.

DISCUSSION

There are many diagnostic tests available to date for the detection of *H. pylori* infection, including endoscopic gastrointestinal biopsy, campylobacter-like organism (CLO) test, *H. pylori* culture, ELISA and the urea breath test. Stringent conditions are required for *H. pylori* culture, so this culture technique is not regarded as an appropriate main diagnostic method. Moreover, most patients tend to avoid the biopsies or CLO tests since a gastrointestinal endoscopic procedure is necessary for the tests. Furthermore, given that *H. pylori* isn't evenly dispersed among the gastric mucosa, there is always the likelihood of false negative results for the biopsy and CLO test. Hence, ^{13}C-UBTs proved to have a higher sensitivity and specificity compared with biopsy and CLO test[12].

With the advent of this promising ^{13}C-utilizing UBT, a test that provides diagnosis for infection as well as deter-

mining infective status after antibiotic treatment[6], various alterations have been made to lower the costs, decrease the amount of ^{13}C-urea intake, reduce the number of breath samples needed, shorten the duration between the two breath samples and overall produce an easily applicable, convenient, and accurate test. The Helifinder capsules have accommodated many of these needs by not increasing the costs despite using an expensive isotope ratio mass spectrometer by lowering the ^{13}C-urea dosage to 38 mg, another benefit in itself. Using capsules with PEG4000 enabled ^{13}C-urea dose to be reduced and enhanced the dissolution of urea, which subsequently provided protection from the oral urease effect and contributed to the maintenance of satisfactory diagnostic quality. Moreover, these capsules offered convenience for the examinee by omitting the lateral decubitus position step required in the previous tests, including the UBiT method, and collecting only 10 mL of expired gas as opposed to 300 mL needed for the conventional method. The reduction in sample volume is an advantage when testing for the elderly, children, and patients with pulmonary disease. Nevertheless, education of the patients is imperative because this method uses test tubes and straws instead of balloons.

The limitation of this study was that the confirmatory tests were not performed for all cases and the relatively small number of recruited patients. Nevertheless, we propose the Helifinder capsule test for clinical use based on this head-to-head comparison study in the same individuals, with excellent agreement with the validated urea breath test (e.g. 0.76‰ with Helifinder and 1.4‰ with UBiT; 6.27‰ with Helifinder and 7.6‰ with UBiT, etc). In addition, the one inconsistent case in our study group may suggest that UBiT may show false negative results, which may be due to inappropriate positional changing or inadequately exhaled breath samples.

We could conclude that in comparison to the conventional UBiT (100 mg ^{13}C-urea tablet) method, the new low-dose capsule was appealing to a wide range of patients including children and the elderly as well as being comparable in quality and cost. Therefore, the Helifinder® ^{13}C-UBT is considered to be a convenient and valid alternative to the conventional UBiT for the diagnosis of *H. pylori* infection.

요 약

배경 : 요소호기검사법은 *Helicobacter pylori* 감염을 진단하는데 있어서 정확하며 비침습적인 방법이다. 저자들은 최근 개발된 38 mg 저용량 ^{13}C-요소캡슐(Helifinder®)을 이용한 요소호기검사의 임상적인 유용성을 평가하였다.

대상 및 방법 : 임상시험 참여에 동의하고 서면 동의서에 서명한 39명의 지원자를 대상으로 2004년 11월부터 2005년 1월까지 검사를 시행하였다. 대상자들은 38 mg ^{13}C-요소캡슐을 사용한 요소호기검사를 시행한 후 최소 2주간의 간격을 두고 기존에 사용되던 UBiT-IR300®를 사용한 방법과 비교검사를 하였다. 저용량 요소캡슐은 질량분석기를 통해 20분 후 호기 표본을 분석하였고 기존 방법에서는 적외선 분광광도계을 이용하여 분석하였다.

결과 : Helifinder 방법은 요소용량을 줄였지만 UBiT-IR300 방법과 비교하여 모두 *H. pylori* 양성 19명, 음성 20명으로 두 검사간 우수한 일치도를 보였다(weighted kappa value 1.0). Δ^{13}C 값은 비례오차를 보였으나 좋은 일치도를 보였다(Passing and Bablok method; y=0.551x-0.255, r=0.74, P<0.0001).

결론 : 새롭게 개발된 38 mg 저용량 ^{13}C-요소캡슐을 이용한 요소호기검사는 기존의 검사와 동등한 결과를 보였으며 더 편리하고 경제적인 검사로서 Helicobacter 감염증 진단에 사용할 수 있는 유용한 검사라고 판단되었다.

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