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Effect of Citric Acid on Accuracy of ^{13}C-Urea Breath Test after *Helicobacter pylori* Eradication Therapy in a Region with a High Prevalence of Atrophic Gastritis

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Background/Aims: The validity of ^{13}C-urea breath test (^{13}C-UBT) for *Helicobacter pylori* detection is influenced by atrophic gastritis. The aim of this study was to evaluate the effect of citric acid on the accuracy of ^{13}C-Urea breath test after *H. pylori* eradication therapy in a region where atrophic gastritis is common. **Methods:** In this prospective study, *H. pylori*-positive patients received ^{13}C-UBT after *H. pylori* eradication regimen. They were classified into citric acid group and control group. To determine diagnostic accuracy of ^{13}C-UBT, patients were offered invasive methods. **Results:** A total of 1,207 who successfully took *H. pylori*-eradication regimen received UBT. They were assigned into the citric acid group (n=562) and the control group (n=645). The mean ^{13}C-UBT value of the citric acid group was 10.3±26.4‰, which was significantly (p<0.001) higher than that of that control group (5.1‰±12.6‰). Of these patients 122 patients were evaluated by endoscopic biopsy methods. Based on invasive tests, the accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of ^{13}C-UBT for the citric acid group were 83.3%, 91.7%, 81.3%, 55.0%, and 97.5%, respectively. Those of the control group were 87.7%, 90.9%, 88.2%, 62.5%, and 97.8%, respectively. They were not significantly different between the two groups. Although the presence of gastric atrophy and intestinal metaplasia (IM) decreased the accuracy, the decrease was not significant. **Conclusions:** In a country with high prevalence of atrophic gastritis or IM, false positivity remained common despite the use of citric acid in ^{13}C-UBT. **(Gut Liver 2019;13:506-514)**

Key Words: *Helicobacter pylori*; Gastritis, atrophic; Diagnosis

INTRODUCTION

The ^{13}C-urea breath test (^{13}C-UBT) is a noninvasive, simple, and widely available test for the initial diagnosis of *Helicobacter pylori* infection and confirmation of *H. pylori* eradication after treatment.[1,2] This test is considered an ideal test for those in whom endoscopy is not required because it offers the combination of simplicity, accuracy, absence of exposure to radioactivity, and reliability. However, the precise cutoff point to define whether UBT is positive or negative remains controversial.[3] One approach to solve this problem is to consider a range of ^{13}C-UBT values (2.5‰ to 5.0‰) as a "gray zone" for which results should be considered inconclusive. Calvet *et al.*[4,5] and our team have reported that most false-positive results are between 2.5‰ and 12‰, suggesting a larger "gray area" of delta values. Several studies have set up the cutoff point between 1.3‰ and 7.4‰, showing high sensitivity and specificity of ^{13}C-UBT after *H. pylori* eradication.[1,6] The selected cutoff value of ^{13}C-UBT could depend on several factors, including the dose of urea administered, the indication of ^{13}C-UBT, the measuring equipment for detecting δ $^{13}CO_2$, the presence and composition of a meal, and the time from the ingestion of urease to the expired gas sample of ^{13}C-UBT.[2,7,8] Thus, a unique and generally proposed cutoff level may not be possible. Our previous retrospective study has reported lower specificity (67.2%) and despite high sensitivity (96.4%) of ^{13}C-UBT for the range of 2.5‰ to 3.0‰ after *H. pylori* eradication.[4]

To decrease the rate of false positivity, the use of a citric acid

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meal has been proposed to increase the ^{13}C-UBT value in *H. pylori*-infected patients and reduce this value in uninfected patients.[3,9,10] Citric acid can increase urea hydrolysis by *H. pylori*[11] and decreases intragastric pH, which tends to inhibit non-*H. pylori* ureases. It may also retard gastric emptying, thus making gastric food contents remain longer in the stomach.[3] In the United States, coadministration of citric acid and urea is used to increase the diagnostic accuracy of UBT. Both the United States and European Standard Protocols include citric acid within the test kit.[12] In contrast, the use of a citric acid meal is not a standard part of the Asian Standard Protocol. In addition, severe atrophic gastritis (AG) or intestinal metaplasia (IM) was the cause of false positivity in this test.[4,13] Generally, the prevalences of endoscopic AG and IM were 40.7% and 12.5% in a previous Korean nationwide multicenter study.[14]

Based on this background, we hypothesized that a citric acid meal could increase the accuracy of ^{13}C-UBT. The aim of this study was to determine the effect of citric acid meal on the diagnostic validity of ^{13}C-UBT after *H. pylori* eradication and compare such ^{13}C-UBT with the use of citric acid and endoscopic biopsy-based methods for detecting *H. pylori*.

MATERIALS AND METHODS

1. Study population

Patients with proven *H. pylori* infection (i.e., positive results for two of the following three endoscopic biopsy methods: histology [the modified Giemsa test], CLO test, and rapid urease test) were recruited prospectively between January 2015 and April 2018 at Seoul National University Bundang Hospital. They were classified into two groups depending on the test day regardless of *H. pylori* eradication regimen or other clinical setting. The testing day was chosen blindly by subjects. On Tuesdays, subjects received 4 g of citric acid dissolved in 200 mL of water immediately before receiving a ^{13}C-UBT tablet (citric acid group). On Mondays, subjects received ^{13}C-UBT without a citric acid meal (control group). Exclusion criteria were (1) after gastric operation; (2) administration of antibiotics or consumption of bismuth salts within 4 weeks or administration of a proton pump inhibitor (PPI) within 2 weeks prior to ^{13}C-UBT; (3) *H. pylori* eradication failure because of poor compliance; and (4) *H. pylori* reinfection where *H. pylori* status became positive for more than 1 year after successful eradication. The study protocol was approved by the Ethics Committee of Seoul National University Bundang Hospital (SNUBH B-1412/279-004). All participants gave written informed consent. This trial was registered with the UMIN Clinical Trials Registry (number: UMIN000001169).

2. *H. pylori* eradication

For treatment of *H. pylori* infection, standard Korean government-approved therapies were used, including PPI-based triple therapy (standard dose of PPI b.i.d. [twice a day], clarithromycin 500 mg b.i.d., and amoxicillin 1 g b.i.d. for 1 week) and sequential therapy (initial 5-day therapy with a combination of PPI b.i.d. and amoxicillin 1 g b.i.d., followed by 5 days of PPI b.i.d., clarithromycin 500 mg b.i.d., and metronidazole 500 mg t.i.d. [three times a day]) as first-line therapies in all study subjects.[15] When these first-line therapies failed, two types of rescue therapies were used, namely, bismuth-containing quadruple therapy (PPI b.i.d., tripotassium dicitrate bismuthate 300 mg q.i.d. [three tablets 30 minutes before meals and one tablet 2 hours after dinner], metronidazole 500 mg t.i.d., and tetracycline 500 mg q.i.d.) for 1 to 2 weeks, or moxifloxacin-containing triple therapy (moxifloxacin 400 mg q.i.d., amoxicillin 1 g b.i.d., and PPI b.i.d.) for 1 to 2 weeks. When second-line therapy failed, other rescue therapies were used.

3. Administration of a citric acid meal and ^{13}C-UBT

Before ^{13}C-UBT, patients were instructed to stop taking medications such as bismuth salts or antibiotics for 4 weeks and PPI for 2 weeks. They were asked to fast for a minimum of 4 hours. Patients were assigned to receive the test meal (citric acid group) for ^{13}C-UBT on Tuesdays or to the control group on Mondays. After washing the oral cavity by gargling, participants in the citric acid group received the citric acid solution (Dongwon, Seoul, Korea; 4 g in 200 mL of water containing 50 g [200 kcal] glucose polymer with artificial sweetener). After consumption of the meal, a predose breath sample was obtained, and then 100 mg tablet of ^{13}C-urea (UBiTkit™; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) was administered. In the control group, ^{13}C-UBT was performed with prior consumption of a glucose meal without citric acid. Breath samples were collected in the sitting position using special breath collection bags before ^{13}C-urea administration (baseline) and 20 minutes after administration. Collected breath samples were analyzed using an isotope-selective, nondispersive infrared spectrometer (UbiT-IR 300®; Otsuka Pharmaceutical Co. Ltd). Despite the lack of local validation, the ^{13}C-UBT cutoff value of 2.5‰ was used as recommended by the manufacturer and a delta $^{13}CO_2$ of ≥2.5‰ was considered positive.

4. Endoscopic surveillance for the detection of *H. pylori* infection

Two biopsy specimens were obtained from the antrum and gastric body for histology. The presence of *H. pylori* was assessed by modified Giemsa staining. Degrees of inflammatory cell infiltration, atrophy, and IM were assessed by hematoxylin and eosin staining. Histological features of gastric mucosae were graded using the updated Sydney scoring system with a 4-point scale (i.e., 0=none, 1=slight, 2=moderate, and 3=marked).[16] Another two biopsy specimens (from the lesser curvature of the antrum and gastric body) were used for the rapid urease test (CLOtest, Delta West, Bentley, Australia). Antral and gastric body biopsy specimens were evaluated separately. All urease

tests were monitored for color change for up to 24 hours. Further analysis was performed regarding the IM condition.

5. Statistical analysis

Sensitivity, specificity, and likelihood ratios for a positive test result (LRp) for a negative test result (LRn) of ^{13}C-UBT were calculated for the citric acid and control groups. Statistical analysis was conducted using PASW Statistics version 18.0 (SPSS, Chicago, IL, USA). Positive predictive value (PPV) and negative predictive value (NPV) were included in LRs. Student t-test, Pearson chi-square test, and Fisher exact test were used, as appropriate, for univariate analysis of factors affecting the accuracy of ^{13}C-UBT. A logistic regression model was used for multivariate analysis. Statistical significance was considered at $p<0.05$.

RESULTS

1. Comparison of ^{13}C-UBT values

Fig. 1 shows the current study flow. Of 1,207 consecutive participants who underwent ^{13}C-UBT after *H. pylori* eradication therapy, 562 participants (46.6%) visited on Tuesdays for ^{13}C-UBT and received citric acid before ^{13}C-UBT (meal group), while 645 participants (53.4%) visited on Mondays without receiving citric acid (control group). Baseline characteristics of these participants are summarized in Table 1. Overall, 116 participants (20.6%) in the meal group and 139 participants (21.6%) in the control group showed positive results of ^{13}C-UBT after *H. pylori* eradication therapy. The mean δ of ^{13}C-UBT value showed a significant difference between the two groups. It was significantly ($p<0.001$) higher (10.3‰±26.4‰) in the meal group than in the control group (5.1‰±12.6‰) (Fig. 2). The mean δ of the ^{13}C-UBT value of positive ^{13}C-UBT results was also significantly ($p<0.001$) higher in the citric acid group (48.3‰±39.7‰ in the meal group vs 22.1‰±19.2‰ in the control group). The mean δ of the ^{13}C-UBT value of negative ^{13}C-UBT results did not show a significant difference (0.4‰±0.8‰ in meal group vs 0.5‰±0.4‰ in control group, $p=0.513$). Invasive testing via gastroscopy was recommended when the mean δ of the ^{13}C-UBT value was between 2.5‰ and 10‰. Endoscopy was limited to this group, as it was both invasive and expensive. Finally, 60 participants (10.7%) in the meal group and 62 participants (9.6%) in the control group accepted endoscopic biopsy to compare the existence of *H. pylori* with results of ^{13}C-UBT.

2. Effect of citric acid meal on diagnostic accuracy of ^{13}C-UBT

A total of 114 participants were evaluated by endoscopic biopsy for histological analysis (modified Giemsa test) of the antrum and gastric body, while 98 participants were evaluated by CLOtest of the antrum and gastric body for determination of *H. pylori* after ^{13}C-UBT. A total of 92 participants were evaluated by both histology and CLOtest. The mean δ of the ^{13}C-UBT value showed a significant difference between the two groups (10.9‰±25.6‰ in meal group vs 3.5‰±7.9‰ in the control

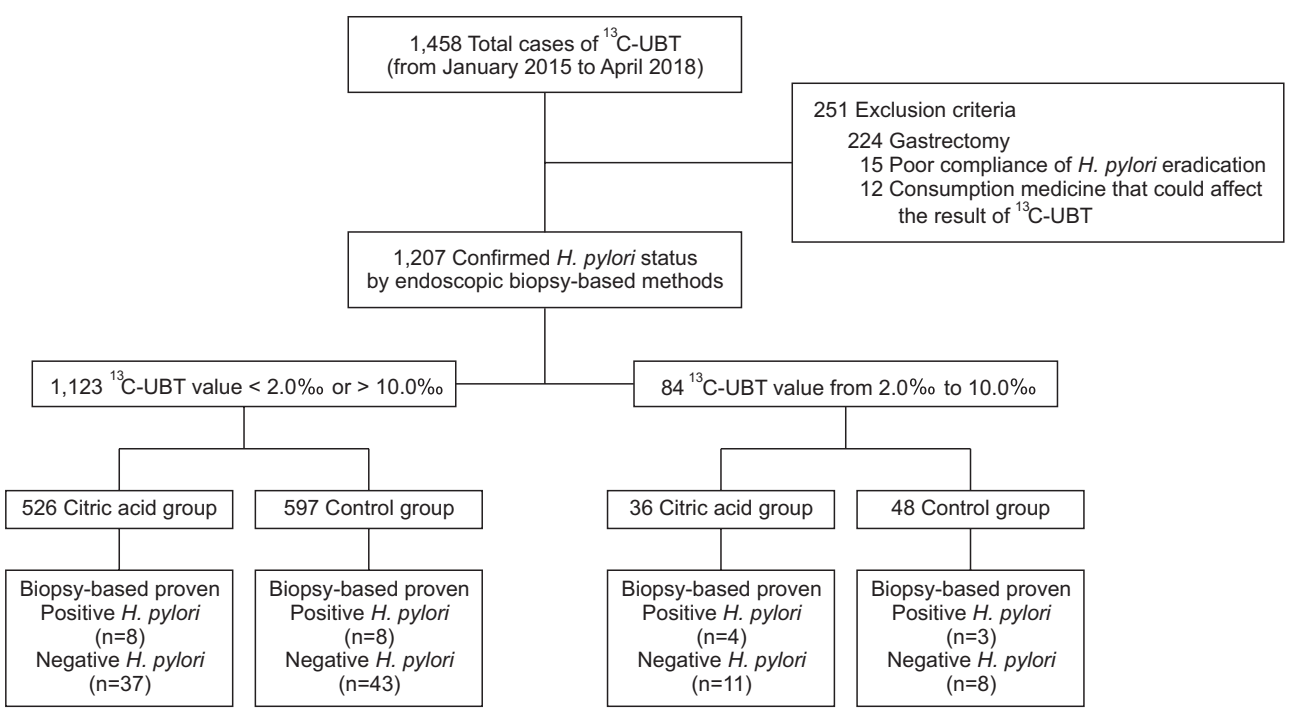

Fig. 1. Flowchart showing ^{13}C-UBT compared to endoscopic biopsy-based methods for evaluating *H. pylori* status after eradication.
^{13}C-UBT, ^{13}C-urea breath test; *H. pylori*, *Helicobacter pylori*.

Table 1. Baseline Characteristics of Participants in the Citric Acid Group and Control Group

Characteristic	Meal group (n=562)	Control group (n=645)	p-value*
Sex, male/female	280 (49.8)/282 (50.2)	343 (53.2)/302 (46.8)	0.134
Age, yr	56.3±12.3	55.3±11.8	0.783
Initial diagnosis			0.002
Functional dyspepsia	125 (22.2)	143 (22.2)	
Atrophic gastritis	302 (53.7)	339 (52.6)	
Benign peptic ulcer	75 (13.3)	104 (16.1)	
Gastric dysplasia	22 (3.9)	35 (5.4)	
Early gastric cancer	30 (5.3)	11 (1.7)	
Gastric MALT lymphoma	5 (0.9)	13 (2.0)	
ITP	3 (0.5)	0	
^{13}C-UBT value, ‰	10.3±26.4	5.1±12.6	<0.001
^{13}C-UBT positive, %	116 (20.6)	139 (21.6)	0.395
Mean ^{13}C-UBT value in positive results, ‰	48.3±39.7	22.1±19.2	<0.001
Mean ^{13}C-UBT value in negative results, ‰	0.4±0.8	0.5±0.4	0.513
No. of *H. pylori* eradications			0.994
First	471 (83.8)	541 (80.9)	
Second	61 (10.9)	69 (13.0)	
Third	30 (5.3)	35 (6.0)	
Mean time from *H. pylori* eradication to ^{13}C-UBT, wk	5.3±1.2	5.4±2.1	0.696

Data are presented as number (%) or mean±SD.
MALT, mucosa-associated lymphoid tissue; ITP, idiopathic thrombocytopenic purpura; ^{13}C-UBT, ^{13}C-urea breath test; *H. pylori*, *Helicobacter pylori*.
*Fisher exact test.

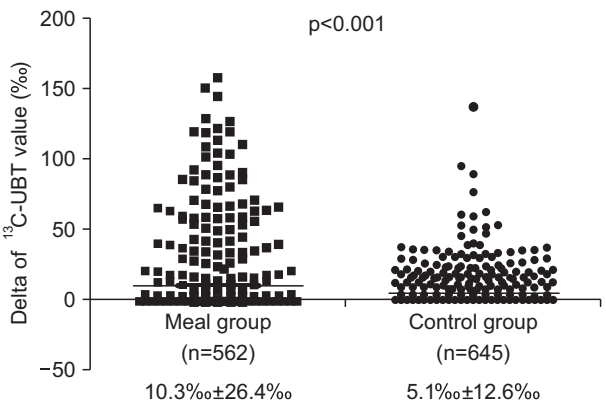

Fig. 2. Distribution of ^{13}C-UBT values after *H. pylori* eradication between the citric acid group and the control group. ^{13}C-UBT values showed a significant difference between the two groups. SD was greater in the citric acid group than in the control group (26.2‰ vs 6.7‰). Data are presented as the mean±SD.
^{13}C-UBT, ^{13}C-urea breath test; *H. pylori*, *Helicobacter pylori*.

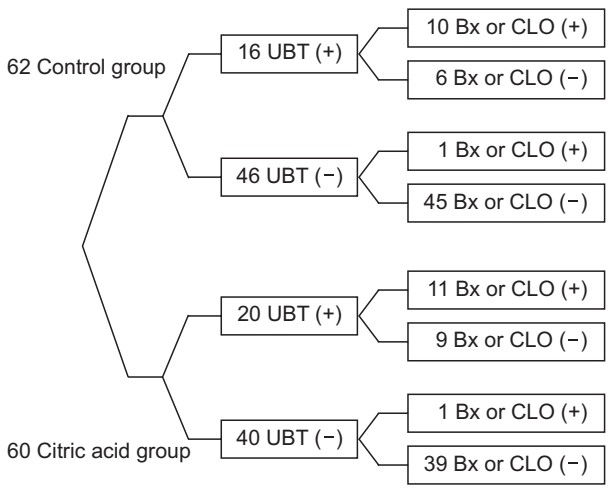

Fig. 3. Flowchart showing diagnostic accuracy of ^{13}C urea breath test (^{13}C-UBT) results compared with endoscopic biopsy (Bx) results. Values were calculated for a UBT cutoff value of 2.5‰.

group, p=0.032). Twenty participants (33.3%) in the meal group and 16 participants (25.8%) in the control group showed positive results of ^{13}C-UBT after *H. pylori* eradication (p=0.429) (Fig. 3). When the diagnostic accuracy of ^{13}C-UBT in the meal group was calculated based on endoscopic biopsy results (histology or rapid urease test), its accuracy, sensitivity, specificity, PPV, NPV, LRp, and LRn were 83.3% (95% confidence interval [CI], 71.5% to 91.7%), 91.7% (95% CI, 61.5% to 99.8%), 81.3% (95% CI, 67.4% to 91.1%), 55.0% (95% CI, 39.8% to 69.3%), 97.5% (95% CI, 85.6% to 99.6%), 4.89 (95% CI, 2.65 to 9.03), and 0.10 (95% CI, 0.02 to 0.67), respectively (Table 2). When the diagnostic accuracy of ^{13}C-UBT for the control group was calculated based on endoscopic biopsy results, its accuracy, sensitivity, specificity,

PPV, NPV, LRp, and LRn were 88.7% (95% CI, 78.1% to 95.3%), 90.9% (95% CI, 58.7% to 99.8%), 88.2% (95% CI, 76.1% to 95.6%), 62.5% (95% CI, 43.5% to 78.3%), 97.8% (95% CI, 87.4% to 99.7%), 7.73 (95% CI, 3.56 to 16.07), and 0.10 (95% CI, 0.02 to 0.67), respectively (Table 2).

3. Diagnostic accuracy of ^{13}C-UBT in the gray area based on invasive tests

Of 1,207 participants, 36 (3.0%) in the meal group and 48 (3.9%) in the control group had results in the gray area (2.0‰ to 10.0‰) for ^{13}C-UBT (Fig. 1). Fifteen participants in the meal group and 11 participants in the control group were analyzed by endoscopic biopsy methods (histology or rapid urease test). The diagnostic accuracy, sensitivity, specificity, PPV, NPV, LRp, and LRn of ^{13}C-UBT for the meal group in the "gray area" were 53.3% (95% CI, 26.6% to 78.7%), 75.0% (95% CI, 19.4% to 99.4%), 45.5% (95% CI, 16.8% to 76.6%), 33.3% (95% CI, 18.6% to 52.2%), 83.3% (95% CI, 44.8% to 96.9%), 1.38 (95% CI, 0.63 to 3.00), and 0.55 (95% CI, 0.09 to 3.88), respectively. They were 45.5% (95% CI, 16.8% to 76.6%), 100.0% (95% CI, 29.2% to 100.0%), 25.0% (95% CI, 3.2% to 65.1%), 33.3% (95% CI, 25.1% to 42.7%), 100.0%, 1.33 (95% CI, 0.89 to 1.99), and 0.00, respectively, for the control group in the "gray area" (Table 3).

4. Risk factors for a discrepancy between ^{13}C-UBT and invasive test after *H. pylori* eradication

Table 4 shows risk factors that caused mismatched results between ^{13}C-UBT and endoscopic biopsy-based tests. Except sex (p=0.039), there was no significant risk factor based on univariate analysis.

5. Diagnostic accuracy depending on the degree of gastric atrophy

To exclude the influence of gastric mucosal atrophy on the diagnostic accuracy of ^{13}C-UBT, subgroup analysis was performed for the diagnostic validity using 69 patients who were revealed to have no or mild gastric atrophy based on gastric mucosal biopsy (31 subjects in the meal group and 38 subjects in the control group). The diagnostic accuracy was 86.8% (95% CI, 71.9% to 95.6%) for the control group and 80.7% (95% CI, 62.5% to 92.3%) for the meal group (p=0.637). Diagnostic sensitivity, specificity, PPV, NPV, LRp, and LRn were 100.0% (95% CI, 47.8% to 100.0%), 76.9% (95% CI, 56.4% to 91.0%), 45.5% (95% CI, 29.2% to 62.7%), 100.0%, 4.33 (95% CI, 2.15 to 8.74) and 0.0 for the meal group and 100.0% (95% CI, 47.8% to 100.0%), 84.9% (95% CI, 68.1% to 94.9%), 50.0% (95% CI, 30.8% to 69.2%), 100.0%, 6.6 (95% CI, 2.94 to 14.80), and 0.00 for the control group, respectively (Supplementary Table 1). Among those in the gray zone, the diagnostic accuracy was

Table 2. Diagnostic Validity of ^{13}C-UBT Compared to Those of Endoscopic Biopsy Methods

^{13}C-UBT value		Using both endoscopic biopsy-based methods for *H. pylori* status		
		Positive	Negative	
Meal group (n=60)	≥2.5‰	11	9	PPV 55.0%
	<2.5‰	1	39	NPV 97.5%
		Sensitivity 91.7%	Specificity 81.3%	
Control group (n=62)	≥2.5‰	10	6	PPV 62.5%
	<2.5‰	1	45	NPV 97.8%
		Sensitivity 90.9%	Specificity 88.2%	

^{13}C-UBT, ^{13}C-urea breath test; *H. pylori*, *Helicobacter pylori*; PPV, positive predictive value; NPV, negative predictive value.

Table 3. Diagnostic Validity of ^{13}C-UBT Compared to Those of Endoscopic Biopsy Methods in the Gray Zone (Value of ^{13}C-UBT: 2.0‰–10.0‰)

^{13}C-UBT value		Using both endoscopic biopsy-based methods for *H. pylori* status		
		Positive	Negative	
Citric acid group (n=15)	≥2.5‰	3	6	PPV 33.3%
	<2.5‰	1	5	NPV 83.3%
		Sensitivity 75.0%	Specificity 45.5%	
Control group (n=11)	≥2.5‰	3	6	PPV 33.3%
	<2.5‰	0	2	NPV 100.0%
		Sensitivity 100.0%	Specificity 25.0%	

^{13}C-UBT, ^{13}C-urea breath test; *H. pylori*, *Helicobacter pylori*; PPV, positive predictive value; NPV, negative predictive value.

Table 4. Risk Factors for Mismatched Results between ^{13}C-UBT and Endoscopic Biopsy-Based Methods after *Helicobacter pylori* Eradication

Variable	^{13}C-UBT result compared with both endoscopic biopsy-based methods		p-value*
	Matched group (n=105)	Mismatched group (n=17)	
Sex, male/female	60 (57.1)/45 (42.9)	5 (29.4)/12 (70.6)	0.039
Use of citric acid	50 (47.6)	10 (58.8)	0.441
Age, yr	56.2±11.3	57.0±12.0	1.000
Diagnosis			0.623
Functional dyspepsia	19 (18.1)	3 (17.6)	
Atrophic gastritis	23 (21.9)	6 (35.3)	
Benign peptic ulcer	22 (21.0)	1 (5.9)	
Gastric dysplasia	13 (12.4)	1 (5.9)	
Early gastric cancer	20 (19.0)	5 (29.4)	
Gastric MALT lymphoma	8 (7.6)	1 (5.9)	
Mean time from *H. pylori* eradication to ^{13}C-UBT, wk	5.6±0.6	5.5±0.8	0.831
Mean time from ^{13}C-UBT to endoscopic biopsy, wk	31.8±27.6	25.2±21.0	0.285
Gastric mucosal status			
Gastric atrophy			0.961
Not investigated	7 (6.7)	1 (5.9)	
Not applicable	23 (21.9)	3 (17.6)	
None	45 (42.9)	8 (47.1)	
Mild	13 (12.4)	3 (17.6)	
Moderate	14 (13.3)	2 (11.8)	
Marked	3 (2.9)	0	
Gastric intestinal metaplasia			0.862
Not investigated	7 (6.7)	1 (5.9)	
None	54 (51.4)	10 (58.8)	
Mild	20 (19.0)	2 (11.8)	
Moderate	17 (16.2)	2 (11.8)	
Marked	7 (6.7)	2 (11.8)	

Data are presented number (%) or mean±SD.
^{13}C-UBT, ^{13}C-urea breath test; MALT, mucosa-associated lymphoid tissue.
*Fisher exact test.

55.6% (95% CI, 21.2% to 86.3%) for the meal group and 37.5% (95% CI, 8.5% to 75.5%) for the control group (p=0.620). Supplementary Table 2 shows diagnostic validity for the gray area except severe to moderate gastric mucosal atrophy.

6. Diagnostic accuracy depending on gastric intestinal metaplasia

To exclude any influence of gastric mucosal IM on the diagnostic accuracy of ^{13}C-UBT, subgroup analysis was performed for the diagnostic validity using 86 patients who were revealed to have no or mild IM based on gastric mucosal biopsy (48 subjects in the meal group and 38 subjects in the control group). The diagnostic accuracy was 86.8% (95% CI, 71.9% to 95.6%) for the control group and 85.4% (95% CI, 72.2% to 93.9%) for the meal group (p=1.000). Diagnostic sensitivity, specificity, PPV, NPV, LRp, and LRn were 90.0% (95% CI, 55.5% to 99.8%), 84.2% (95% CI, 68.8% to 93.9%), 60.0% (95% CI, 41.2% to 76.3%), 97.0% (95% CI, 83.2% to 99.5%), 5.70 (95% CI, 2.66 to 12.22), and 0.12 (95% CI, 0.02 to 0.77) for the meal group and 80.0% (95% CI, 28.4% to 99.5%), 87.9% (95% CI, 71.8% to 96.6%), 50.0% (95% CI, 26.5% to 73.5%), 97.7% (95% CI, 83.3% to 99.4%), 6.60 (95% CI, 2.38 to 18.26), and 0.23 (95% CI, 0.04 to 1.32) for the control group, respectively (Supplementary Table 3). Among those in the gray zone, the diagnostic accuracy was 58.3% (95% CI, 27.6% to 84.8%) for the meal group and 33.3% (95% CI, 4.3% to 77.7%) for the control group (p=0.620). Supplementary Table 4 shows diagnostic validity for the gray area except severe to moderate IM.

DISCUSSION

The use of 4 g of citric acid as a test meal failed to increase the diagnostic validity of ^{13}C-UBT after *H. pylori* eradication therapy. Although the mean ^{13}C-UBT value of the citric acid group (10.3‰±26.4‰) was significantly higher than that of the control group (5.1‰±12.6‰, p<0.001), the sensitivity was only slightly higher in the meal group (91.7% vs 90.9%) while the specificity was higher in the control group (88.2% vs 81.3%). The overall accuracy was also higher in the control group (87.7% vs 83.3%), although the sensitivity of ^{13}C-UBT for the meal group was higher than for the control group (90.0% vs 80.0%) in subgroup analysis for those without or with mild gastric atrophy or IM. Gastric atrophy and IM might have blunted the effect of citric acid on the accuracy of ^{13}C-UBT.

Generally, moderate to severe gastric atrophy and IM were associated with increased risk of a mismatched result of ^{13}C-UBT value after *H. pylori* eradication, suggesting that the presence of hypochlorhydria or achlorhydria and resulting changes in gastric microbiome might affect the result.[4,13,17] A previous Japanese study demonstrated that five bacterial species with urease activity (*Proteus mirabilis, Citrobacter freundii, Klebsiella pneumoniae, Enterobacter cloacae*, and *Staphylococcus aureus*) were subsequently isolated from the oral cavity and/or stomach, and all patients with a false positivity result were suffering from AG.[18] Furthermore, there are marked geographic differences in the frequency of non-*H. pylori* contamination in duodenal ulcer disease (high in Colombia and low in Korea or United States).[19]

Taken together, these results suggest that in regions of the world where AG is common, the diagnostic accuracy of the test needs to be increased, such as by using citric acid meals and different doses or formulations of urea. The original ^{13}C-UBT employed a test meal designed to slow gastric emptying and maximize the distribution of the substrate within the stomach to increase the area and time of contact between bacteria and the substrate.[20] Different doses (1, 2, or 4 g) of citric acid can produce significant increases in breath $^{13}CO_2$ activity compared to other meals, such as ascorbic acid, subcutaneous pentagastrin, and glucose polymer.[9] Citric acid could slow gastric emptying and enhance the intragastric distribution of urea.[3] Most studies evaluating the role of citric acid in UBT have shown higher delta values with citric acid in comparison with other pretest meals or no test meals.[12,21,22] Similarly, the standard deviation of δ ^{13}C-UBT value in the citric acid group of the present study was significantly higher than that of the control group, while the mean δ ^{13}C-UBT value for positive ^{13}C-UBT results in the citric acid group was significantly higher. Furthermore, the sensitivity was higher in the citric acid meal group. These results reflect the increase in urease activity by citric acid (i.e., increase of δ ^{13}C-UBT value in *H. pylori* infected patients without a consistent change in the delta value in uninfected patients). Thus, the use of citric acid as a meal theoretically could increase the diagnostic accuracy of ^{13}C-UBT and could especially increase the discriminative capacity in the gray zone. However, prior fasting and test meals may not be essential.[23-26] Ng *et al*.[27] reported that sensitivities and specificities of ^{13}C-UBT were 97.4% and 95.8%, respectively, in the group with fasting and use of citric acid as a test meal. They were 96.5% and 93.9%, respectively, in the group without fasting and absence of a test meal in a Chinese population. Gisbert *et al*.[22] reported that using citric acid as a test meal might result in higher ^{13}C-UBT results at different sampling times (15, 30, and 45 minutes) in *H. pylori*-positive patients, suggesting that these results did not imply a better discrimination between infected and noninfected patients.

In our study, the use of citric acid as a test meal improved the diagnostic sensitivity after *H. pylori* eradication therapy in comparison with that of the control group (Table 2). However, there was no benefit for the diagnostic validity in the gray zone (Table 3). Although false-negative tests were rare, false positivity was common, and sensitivity was low even with the use of citric acid. To determine whether such disappointing results could be due to severe gastric mucosal atrophy or IM, we further analyzed those with or without mild atrophy or IM. However, the increase did not reach significant difference (p=0.637 in atrophy and p=1.000 in IM subgroup analysis). Although the sensitivity was equal or higher in the meal group (100.0% both group in atrophy and 90.0% vs 80.0% in IM subgroup analysis), the specificity was higher in the control group (84.9% vs 76.9% in atrophy and 87.9% vs 84.2% in IM group subgroup analysis). In the meal group, the sensitivity was rather low (75.0%) for the gray zone in IM subgroup analysis. There was no significant difference between the two groups, possibly due to the small number of subjects. However, this study was conducted over three years. The accuracy of UBT should apply to all post-eradication subjects regardless of atrophy or IM.

To obtain a statistically significant improvement of diagnostic accuracy from 90% to 95% would likely require an extremely large sample size. However, previous studies could not meet the needed sample size, either, due to limitations in the clinical situation, such as high cost of endoscopy and the invasiveness of endoscopic biopsy-based *H. pylori* tests.[4,5,22,23] Furthermore, the National Cancer Screening Program in Korea offers either ^{13}C-UBT or the rapid urease test for diagnosis after *H. pylori* eradication with the exception of the endoscopic ^{13}C-urea test. These two tests cannot be performed together after *H. pylori* eradication. Thus, we could only compare ^{13}C-UBT with biopsy-based *H. pylori* tests in 138 subjects (9.9%) after *H. pylori* eradication therapy over a 3-year period. We were unable to prove whether endoscopic biopsy results were more accurate than ^{13}C-UBT results.

Our results are consistent with our previous suggestion that moderate to severe IM is an independent risk factor for a false positivity.[4,28] As the degree of gastric atrophy becomes severe, the environment in the stomach changes to a hypochlorhydric

state and causes overgrowth of non-*H. pylori* urease-positive bacteria.[29] CLOtest also has a low detection rate for *H. pylori* in the presence of mucosal atrophy. IM as this mucosa is not conducive to the growth or attachment of *H. pylori*. This becomes more prominent in the presence of higher levels of IM and AG.[30] A recent Japanese study suggested a possibility that patients with autoimmune gastritis were sometimes misdiagnosed as refractory to eradication therapy for *H. pylori* because of the presence of urease-positive bacteria other than *H. pylori* that colonized the stomach.[13] In addition, we did not give citric acid and urea together, as in Western countries. Confirmatory tests did not follow right away in some cases due to the invasiveness of endoscopy. A previous study reported that, in case of "false-positive ^{13}C-UBT results," re-endoscopic biopsy-based methods showed that all of them had positive histology when multiple antral biopsy specimens were taken.[30]

In conclusion, our results show that citric acid did not increase the diagnostic accuracy or specificity of ^{13}C-UBT after *H. pylori* eradication therapy. Thus, those with multiple treatment failures as confirmed by ^{13}C-UBT should not automatically be considered infected. The diagnosis should be confirmed by another test, such as histology or stool antigen, before starting retreatment.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Guarantor of the article: N.K. Study design, data analysis, statistical analysis, data interpretation and manuscript drafting: Y.H.K. Study design, enrolled the subjects, data interpretation, and critical revision: N.K. Enrolled subjects and edited the manuscript: H.Y., C.M.S., Y.S.P., D.H.L. All authors approved the final version of the manuscript.

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