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The Diagnostic Validity of the ^{13}C-Urea Breath Test in the Gastrectomized Patients: Single Tertiary Center Retrospective Cohort Study

ORIGINAL ARTICLE

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Background: This study was conducted to evaluate the diagnostic validity of the ^{13}C-urea breath test (^{13}C-UBT) in the remnant stomach after partial gastrectomy for gastric cancer.
Methods: The ^{13}C-UBT results after *Helicobacter pylori* eradication therapy was compared with the results of endoscopic biopsy-based methods in the patients who have received partial gastrectomy for the gastric cancer.
Results: Among the gastrectomized patients who showed the positive ^{13}C-UBT results (≥ 2.5‰, n = 47) and negative ^{13}C-UBT results (< 2.5‰, n = 114) after *H. pylori* eradication, 26 patients (16.1%) and 4 patients (2.5%) were found to show false positive and false negative results based on biopsy-based methods, respectively. The sensitivity, specificity, false positive rate, and false negative rate for the cut-off value of 2.5‰ were 84.0%, 80.9%, 19.1%, and 16.0%, respectively. The positive and negative predictive values were 44.7% and 96.5%, respectively. In the multivariate analysis, two or more *H. pylori* eradication therapies (odds ratio = 3.248, 95% confidence interval= 1.088-9.695, P = 0.035) was associated with a false positive result of the ^{13}C-UBT.
Conclusions: After partial gastrectomy, a discordant result was shown in the positive ^{13}C-UBT results compared to the endoscopic biopsy methods for confirming the *H. pylori* status after eradication. Additional endoscopic biopsy-based *H. pylori* tests would be helpful to avoid unnecessary treatment for *H. pylori* eradication in these cases.
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Key Words: *Helicobacter pylori*, ^{13}C-urea breath test, Eradication, Cut-off value, Subtotal gastrectomy

INTRODUCTION

Helicobacter pylori is known the primary cause leading to chronic atrophic gastritis and peptic ulcer disease.[1] This bacteria also associated with gastric cancers and mucosa- associated lymphoid tissue lymphoma.[2,3] World Health Organization has reported *H. pylori* as a group 1 carcinogen, and the eradication of *H. pylori* infection reduces the risk of gastric cancer development, and prevents recurrences of these diseases.[4-6] For the treatment of gastric cancer, curative attempts to resect gastric cancer leave a gastric stump, creating the possibility of metachronous tumors arising from the remnant stomach. Thus, many post-surgical management strategies have been attempted to lessen the risk of gastric cancer recurrence, such as adjuvant chemotherapy, regular endoscopic surveillance, and *H. pylori* eradication. After surgical resection, gastric surgery places patients in a different condition from those with a previous normal stomach. Subtotal gastrectomy can decrease the gastric emptying time,[7,8] and increased bile reflux cause the changes of the hydrophobic gastric mucosal barrier,[9] or enhanced blood flow in the remnant gastric body.[10] For these reasons, it is crucial to survive or to detect for *H. pylori* in the remnant stomach, and the reported rates of *H. pylori* infection in the remnant stomach after distal gastrectomy fall within a broad range (19%-70%).[11-13]

H. pylori infections can be diagnosed by a variety of invasive and non-invasive methods.[14] Endoscopic biopsy is the gold

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standard, but it is invasive and is prone to sampling error because *H. pylori* tends to be heterogeneously distributed in the stomach.[15] Serologic examinations are noninvasive and convenient, but do not accurately reflect infection status.[16] The urea breath test (UBT) using ^{13}C-labeled urea is a noninvasive test based on the potent urease activity of *H. pylori* in gastric mucosa and was developed to overcome the shortcomings of serologic testing. This test has been widely used because it has been reported to have a sensitivity and specificity greater than 90% for detecting *H. pylori* infection, and to be more convenient to use and safer for patients.[17,18] For these reasons, the ^{13}C-UBT is now routinely used for diagnosis of *H. pylori* infection. However, information on the diagnostic efficacy of the ^{13}C-UBT for the detection of *H. pylori* in the remnant stomach after partial gastrectomy is limited.[12,19-21] Furthermore, the diagnostic accuracy of this test is controversial for the reason that the changed gastric anatomy could affect the result of the ^{13}C-UBT. In this present study, we evaluated the diagnostic validity of the ^{13}C-UBT after *H. pylori* eradication by comparing ^{13}C-UBT result with that of endoscopic biopsy in the gastrectomized patients.

MATERIALS AND METHODS

1. Patient selection

Between January 2005 and March 2014, among 386 patients underwent the ^{13}C-UBT after partial gastrectomy (Billroth I,[22] Billroth II,[23] Roux-en-Y anastomosis,[24] wedge resection and proximal gastrectomy[25]) for gastric cancer in Seoul National University Bundang hospital. We retrospectively enrolled 161 patients with a ^{13}C-UBT result after *H. pylori* eradication and that subsequently underwent follow up endoscopic surveillance to confirm final *H. pylori* status. Three endoscopic biopsy methods (histology [the modified Giemsa test], the rapid urease test [CLOtest, Delta West, Bentley, Australia], and culture in anastomosis site and lesser curvature and grater curvature of body) were used, and the final *H. pylori* infection was determined by revealing *H. pylori* infection at least any two tests of three. Endoscopic surveillances were performed at least on two occasions during follow-up after ^{13}C-UBT without any further *H. pylori* treatment. The exclusion criteria applied were: (1) no follow up history of endoscopic surveillance after ^{13}C-UBT; (2) the administration of antibiotics or the consumption of bismuth salts within 4 weeks or the administration of a proton pump inhibitor (PPI) within 2 weeks prior to ^{13}C-UBT; (3) *H. pylori* eradication failure because of poor compliance; (4) a history of total gastrectomy; (5) *H. pylori* re-infection, where *H. pylori* status became positive after more 1 year after successful eradication.[26] The study protocol was approved by the Ethics Committee at Seoul National University Bundang Hospital (SNUBH B-1406).

2. *Helicobacter pylori* eradication

For the treatment of *H. pylori* infection, PPI-based triple therapy (standard dose of PPI b.i.d., clarithromycin 500 mg b.i.d., and amoxicillin 1 g b.i.d. for 1 week) was used as a first-line therapy in all study subjects.[27] When these first-line therapies failed, two types of rescue therapies were used, that is, 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 1-2 weeks,[28] or moxifloxacin-containing triple therapy (moxifloxacin 400 mg q.d., amoxicillin 1 g b.i.d., and PPI b.i.d.) for 1-2 weeks.[29] When second-line therapy failed then the other rescue therapy was used.

3. ^{13}C-urea breath test

Before the ^{13}C-UBT, patients were instructed to stop taking medications (such as bismuth salts or antibiotics for 4 weeks and PPI for 2 weeks), and fasted for a minimum of 4 hours. After washing the oral cavity by gargling, a predose breath sample was obtained. Then, 100 mg tablet of ^{13}C-urea (UBiTkit^{TM}, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) was administered free of 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, non-dispersive infrared spectrometer (UBiT-IR 300®, Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan). Despite the lack of validation, a ^{13}C-UBT cut-off value 2.5‰ was used as recommended by the manufacturer, and thus, a delta $^{13}CO_2$ of $\geq$ 2.5‰ was considered positive for *H. pylori* infection.

4. Endoscopic surveillance for the detection of *Helicobacter pylori* infection and histological evaluation

To improve the *H. pylori* detection rate, three gastric biopsies were obtained from the antral side of the anastomotic site, lesser curvature and greater curvature of body in the remnant stomach. The presence of *H. pylori* was assessed by modified Giemsa staining and degrees of inflammatory cell infiltration, atrophy, and intestinal metaplasia were assessed by hematoxylin and eosin staining. Histological features of gastric mucosae were graded using the updated Sydney scoring system, which has a

four-point scale (i.e., 0 = none, 1 = slight, 2 = moderate, and 3 = marked).[30]

5. Mucosa urease test

Two biopsy specimens, one from the lesser curvature of the anastomosis site and lesser curvature of body, were used for the rapid urease test (CLOtest, Delta West). Anastomosis site and body biopsy specimens in the remnant stomach were evaluated separately, and all urease tests were monitored for color change for up to 24 hours.

6. Microbiological examination

Two specimens from the antrum and body were sent for microbiological culture in brain heart infusion plates containing 7% horse blood. These plates were placed in a glass tank in a 5% O_2, 10% CO_2, and 85% N_2 atmosphere at 37°C for 3-5 days. Anastomosis site and body biopsy specimens in the remnant stomach were evaluated separately. Organisms were identified as *H. pylori* by Gram staining, colony morphology, and positive oxidase, catalase, and urease reactions.

7. Statistical analysis

Sensitivity, specificity, false positive rate, false negative rate, positive predictive value, negative predictive value for the ^{13}C-UBT in the partial gastrectomy state were calculated. Statistical analysis was conducted using PASW Statistics ver. 18.0 (IBM Co., Armonk, NY, USA). The Student's *t*-test, Pearson's chi-square test, and Fisher's exact test were used, as appropriate, for the univariate analysis of factors affecting the accuracy of the ^{13}C-UBT, and a logistic regression model was used for the multivariate analysis. *P*-values of < 0.05 were considered statistically significant.

RESULTS

1. Subject characteristics

Of the 161 patients with a ^{13}C-UBT result after partial gastrectomy state, forty seven patients (29.2%) had a ^{13}C-UBT result $\geq$ 2.5‰ and 114 patients (70.8%) had a value $<$ 2.5‰. Baseline patient characteristics are shown in Table 1. There were 97 males (60.2%) and 64 females (39.8%) with a mean patient age of 58.6 (30-78) years. Indications for operations were early gastric cancer in 125 patients (77.6%) and advanced gastric cancer in 36 patients (22.4%). Analyzing method of gastrectomy, Billroth I, II anastomosis, Roux-en-Y anastomosis, wedge resection and proximal gastrectomy were carried out for reconstruction of the gastrointestinal tract in 106 (65.8%), 6 (3.8%), 29 (18.0%), 11 (6.8%) and 9 (5.6%) patients. 17 (10.6%) and 27 (16.8%) patients had diabetes mellitus and hypertension, respectively. Before the ^{13}C-UBT, 110 patients (68.3%) underwent the first-line eradication therapy, 41 patients (25.5%) underwent second-line eradication therapy, and 10 patients (6.2%) underwent third-line eradication therapy (Table 1).

2. The diagnostic accuracy of ^{13}C-urea breath test in the gastrectomized patients

A flowchart of the study is shown in Figure 1. All 161 patients were evaluated for *H. pylori* status by histology and using the CLOtest. But *H. pylori* culture was performed in 8 patients during the endoscopic surveillance after the ^{13}C-UBT. The success rate of culture was 87.5%. 7 patients were proved *H. pylori* infection on the culture methods. When *H. pylori* statuses was analyzed based on biopsy-based methods, 25 patients (15.5%) had a positive result for *H. pylori* infection, and 136 patients (84.5%) were *H. pylori* negative (Fig. 1).

In patients with a negative ^{13}C-UBT result, histology and the CLOtest showed that 110 patients (96.5%) were *H. pylori* negative and 4 patient (3.5%) were *H. pylori*-positive. However, in the group with a positive ^{13}C-UBT result (n = 47), histology, the

Table 1. The baseline characteristics of patients with a ^{13}C-UBT value

Characteristic	Results
Age	58.6 ± 10.9 (30-78)
Gender (Male : Female)	97 (60.2%) : 64 (39.8%)
Underlying disease	
DM	17 (10.6%)
HTN	27 (16.8%)
Diagnosis for gastrectomy	
Early gastric cancer	125 (77.6%)
Advanced gastric cancer	36 (22.4%)
Type of gastrectomy	
Billroth I	106 (65.8%)
Billroth II	6 (3.8%)
Roux-en-Y anastomosis	29 (18.0%)
Wedge resection	11 (6.8%)
Proximal gastrectomy	9 (5.6%)
Total number of eradiation therapies for *H. pylori* infection	
First	110 (68.3%)
Second	41 (25.5%)
Third	10 (6.2%)
Final UBT result	
Positive	48 (29.8%)
Negative	113 (70.2%)

^{13}C-UBT, ^{13}C-urea breath test; DM, diabetes mellitus; HTN, hypertension; *H. pylori*, *Helicobacter pylori*.

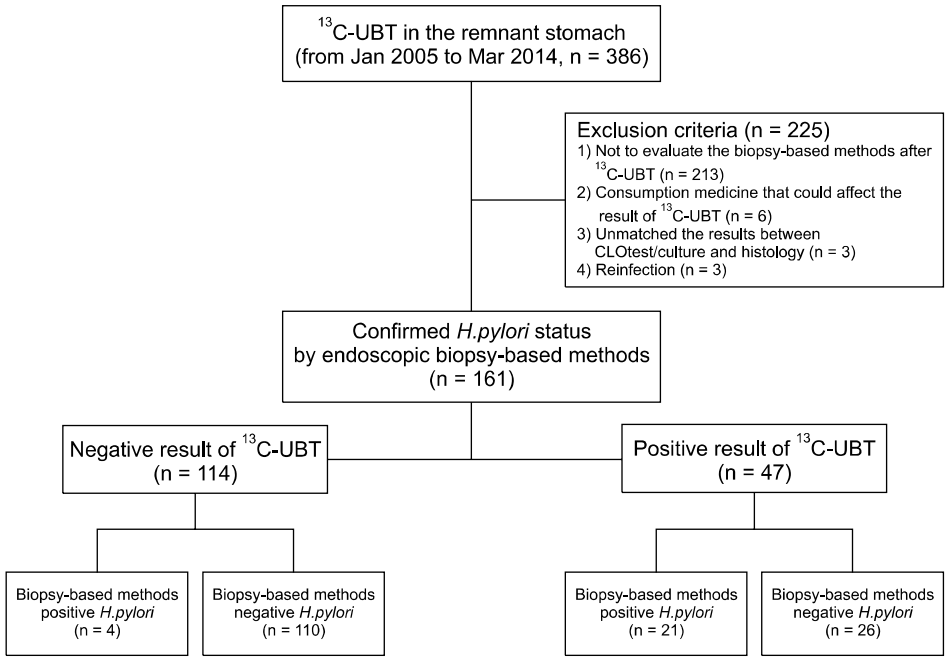

Figure 1. Flow chart of ^{13}C-urea breath test (^{13}C-UBT) and endoscopic biopsy based results of *Helicobacter pylori* (*H. pylori*) status after eradication in the partial gastrectomized patients. CLO, rapid urease test (Campylobacter-like organism).

Table 2. The sensitivity, specificity, and predictive values of the ^{13}C-UBT compared with endoscopic biopsy methods for detecting the *H. pylori* status

		Using the biopsy-based methods for *H. pylori* status		
		Positive	Negative	
^{13}C-UBT value	≥ 2.5‰	21 (13.1%)	26 (16.1%)	PPV 44.7%
	< 2.5‰	4 (2.5%)	110 (68.3%)	NPV 96.5%
		Sensitivity 84.0%	Specificity 80.9%	

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

CLOtest, and/or culture showed 21 patients (44.7%) were *H. pylori* positive and 26 patients (55.3%) were *H. pylori* negative (Fig. 1).

When the diagnostic accuracy of the ^{13}C-UBT was calculated versus endoscopic biopsy results, its sensitivity, specificity, false positive rate, and false negative rate were 84.0%, 80.9%, 19.1%, and 16.0%, respectively. Its positive and negative predictive values were 44.7% and 96.5%, respectively (Table 2).

3. False positive results of the ^{13}C-urea breath test in the partial gastrectomy state

Figure 2 shows a discordant *H. pylori* status results for the ^{13}C-UBT and endoscopic biopsy in the ≥ 2.5‰ range (n = 26). The median ^{13}C-UBT value of this discordant group was 5.9‰ (2.6-9 8‰). 73.1% (19/26 patients) in the 2.5‰ to 10.0‰ range, 26.9% (7/26 patients) in the > 10.0‰ range had a discordant result between ^{13}C-UBT and endoscopic biopsy based results.

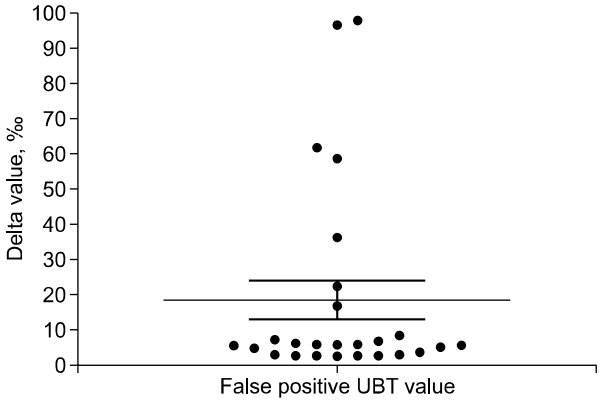

Figure 2. Individual values of the false positive ^{13}C-urea breath test (^{13}C-UBT) results after *Helicobacter pylori* (*H. pylori*) eradication in the partial gastrectomized patients (median ^{13}C-UBT value = 5.9‰).

Table 3. Risk factors for the mismatched ^{13}C-UBT positive result compared with biopsy based methods after *H. pylori* eradication in the partial gastrectomized patients

	^{13}C-UBT result compared with biopsy based methods		Univariate *P*-value	Multivariate *P*-value
	Matched group (n = 21)	Mismatched group (n = 26)		
Gender (Male : Female)	12 (57.1%) : 9 (42.9%)	17 (65.4%) : 9 (34.6%)	NA	
Age (years)	59.7 ± 8.9	60.5 ± 10.7	NA	
Underlying disease				
DM	5 (23.8%)	2 (7.7%)	NA	
HTN	5 (23.8%)	5 (19.2%)	NA	
Type of gastrectomy			NA	
Billroth I	10 (47.7%)	16 (61.5%)		
Billroth II	1 (4.8%)	1 (3.9%)		
Roux-en-Y	6 (28.6%)	4 (15.4%)		
Wedge resection	4 (19.0%)	4 (15.4%)		
Proximal gastrectomy	0 (0.0%)	1 (3.8%)		
Time to ^{13}C-UBT after eradication (weeks)	6.4	6.8	NA	
The mean delta value of the ^{13}C-UBT (‰)	12.3 ± 22.8	13.5 ± 15.1	NA	
The total number of *H. pylori* eradication therapies			< 0.005	0.035
Single	16 (76.2%)	7 (26.9%)		
Two	3 (14.3%)	17 (65.4%)		
Three	2 (9.5%)	2 (7.7%)		
The degree of gastric mucosal atrophy in the remnant stomach			NA	
None	20 (95.2%)	20 (76.9%)		
Mild	1 (4.8%)	4 (15.4%)		
Moderate	0 (0.0%)	0 (0.0%)		
Marked	0 (0.0%)	2 (7.7%)		
The degree of gastric mucosal intestinal metaplasia in the remnant stomach			NA	
None	19 (90.5%)	16 (61.5%)		
Mild	0 (0.0%)	4 (15.4%)		
Moderate	0 (0.0%)	3 (11.5%)		
Severe	2 (9.5%)	3 (11.5%)		

^{13}C-UBT, ^{13}C-urea breath test; NA, not available; DM, diabetes mellitus; HTN, hypertension; *H. pylori*, *Helicobacter pylori*.

Table 4. Logistic regression model for risk factors of the mismatching result between ^{13}C-UBT and endoscopic biopsy based methods in the diagnosis of *H. pylori* infection

Risk factors	β	S.E	*P*-value	OR	95% CI
Multiple eradication therapies for *H. pylori* infection	1.178	0.558	0.035	3.248	1.088-9.695

^{13}C-UBT, ^{13}C-urea breath test; *H. pylori*, *Helicobacter pylori*; β, coefficient; S.E, standard error; OR, odds ratio; CI, confidence interval.

4. The risk factors for a false positive result of the ^{13}C-urea breath test after *H. pylori* eradication

The mean delta value of the ^{13}C-UBT (≥ 2.5‰) after *H. pylori* eradication was not shown a statistical difference between matched (12.3‰) and unmatched group (13.5‰). Among those patients with a positive ^{13}C-UBT result, 23 patients (48.9%), 20 patients (42.6%), and 4 patients (8.5%) underwent first, second, or third eradication therapies for *H. pylori* infection before enrollment in this study. Mean time from eradication to ^{13}C-UBT was not significantly different for those with matching ^{13}C-UBT and biopsy results. However, univariate analysis of the risk factor that caused mismatched results showed that total number of *H. pylori* eradication therapies ($P < 0.005$) significantly contributed to mismatching (Table 3). Logistic regression analysis confirmed that multiple prior eradication therapies (odds ratio = 3.248, 95% confidence interval = 1.088-9.695, $P = 0.035$) was associated with the mismatching of ^{13}C-UBT and biopsy results after *H. pylori* eradication (Table 4).

DISCUSSION

The results of the present study suggest that there was a poor diagnostic concordance between the ^{13}C-UBT and endoscopic biopsy-based results in the positive ^{13}C-UBT values ($\geq$ 2.5‰) after *H. pylori* eradication in remnant stomach after partial gastrectomy for gastric cancer. Generally, the ^{13}C-UBT has been reported to be one of the most accurate diagnostic tools for assessing *H. pylori* status,[31,32] and due to its speed, cost- effectiveness, and convenience, this test has been widely adopted in clinical practice.[33] After partial gastrectomy, the gastric anatomy is altered and the test urea might be expected to pass through the stomach faster, giving different reactant percentages in reaction time, or the ^{13}C-UBT results could be influenced by bile acid reflux. Several reports have shown that ^{13}C-UBT provides lower diagnostic accuracy when using histology as a reference in the remnant stomach after partial gastrectomy.[34-36] Previously, Lotterer et al.[20] reported that the ^{13}C-UBT had shown a 100% sensitivity and 80% specificity in patients with partial stomach resection. In a Japanese study, the sensitivity, specificity, and accuracy of the ^{13}C-UBT were shown to be 96.3%, 100%, and 97.1% in the remnant stomach.[21] However, Schilling et al.[12] reported that the sensitivity of the ^{13}C-UBT was 52%, the specificity 93%. The positive predictive value was 81.25%, the negative predictive value 76.9% and the accuracy was 77.9%. In our study, the sensitivity, specificity, false positive rate, and false negative rate of the ^{13}C-UBT were 84.0%, 80.9%, 19.1%, and 16.0%, respectively. Its positive and negative predictive values were 44.7% and 96.5%. Overall, the diagnostic validity of the ^{13}C-UBT was disappointing especially in the positive range of the ^{13}C-UBT results.

Histological examination of the gastric mucosal biopsy specimen is generally considered to be the current gold standard for the diagnosis of *H. pylori* infection. In this study, the observed false positive ^{13}C-UBT results raise the question as to whether endoscopic biopsy-based methods are reliable for determining the final status of *H. pylori*. A previous study showed that the ^{13}C-UBT occasionally has poor diagnostic ability as compared with endoscopic biopsy-based methods.[18] However, endoscopic biopsy-based methods are susceptible to sampling errors because of discontinuous *H. pylori* colonization of the stomach.[37] In our study, gastric biopsies were performed and the samples were obtained from the anastomosis site and the body of the remnant stomach for histological analysis (modified Giemsa staining), and a gastric sample was obtained from both anastomosis site and body for rapid urease testing. *H. pylori* culture was performed using samples from the gastric biopsies, that is, 2 samples from the anastomosis site and from the body. Furthermore, all patients received at least two or more follow-up endoscopic surveillances, which re-confirmed final *H. pylori* statuses. Thus, we believe the possibility of a gastric biopsy sampling error was slight.

Considering a change of anatomical status of the stomach, postgastrectomy-induced hypochlorhydria often results in bacterial colonization of the remnant stomach. Urease-producing bacteria (*Streptococcus, Staphylococcus, Gardnerella, Lactococcus*, and *Enterococcus*) could cause false positive results.[38-41] In a hypochlorhydric state of the remnant stomach, it accelerates the colonization and the overgrowth of non-*H. pylori* urease-positive bacteria. Furthermore, elevation of intragastric pH removes the neutralizing action of hydrochloric acid on local ammonia production by *H. pylori* urease, leading to the ultimate death of the bacterium as a result of overalkalization.[42] Previous study showed that the persistence of *H. pylori* in the residual stomach decreased from 68.8% to 36% as time elapsed after surgery went from less than 1 year to more than 3 years without any eradication.[19] According to a Taiwan study, spontaneous clearance of *H. pylori* develops in a certain number of patients who underwent distal gastrectomy.[43] In this study, they reported decreased prevalence of *H. pylori* colonization was found after partial gastrectomy without additional eradication therapy: 1-15 years, 29.5%; 16-30 years, 13.6%; and $>$ 31 years, 10%. Final spontaneous clearance rate of *H. pylori* after partial gastrectomy was 43%. For these backgrounds, the possibility of the spontaneous clearance of *H. pylori* (duration from ^{13}C-UBT to endoscopic biopsies: 6 month to 1 year) would be caused the mismatch results.

For another important factor, regarding the role of bile reflux on *H. pylori* colonization in the remnant stomach after partial gastrectomy, many conflict results were published. According to a study by Onoda et al.,[44] the prevalence of *H. pylori* infection was lower in Billroth-II reconstruction patients with severe bile reflux and subsequent stomal gastritis, suggesting a spontaneous eradication of *H. pylori* by the reflux of bile contents. Thus, subtotal gastrectomy allows bile reflux; they therefore cause more severe gastritis with decreased *H. pylori* infection. Although we could not evaluate the degree of bile reflux in the patients who had received subtotal gastrectomy, the reflux of bile acid might influence on *H. pylori* survival after ^{13}C-UBT, and it might cause the mismatch result between the ^{13}C-UBT and endoscopic biopsy methods.

However, a previous study reported that Roux-en-Y reconstruction after distal gastrectomy produces smaller amounts of bile reflux and as a result had a lower rate of *H. pylori* infection.[45] Nakagawara et al.[46] reported that bile refluxate facilitated the survival of *H. pylori*, speculating that *H. pylori* was perhaps

inhibited by other bacteria in the gut. Pylorus-preserving gastrectomy for gastric cancer also resulted in significantly lower *H. pylori* prevalence after surgery.[43] Thus, the precise mechanism between bile acid reflux and *H. pylori* survival would be required to further investigation.

Considering the accuracy of the ^{13}C-UBT, Sheu et al.[19] reported that the sensitivity and specificity of the UBT in the gastrectomy group were lower than those in the normal group. Applying a cut-off value as 2.5‰, the sensitivity and specificity were only 82.2% and 87.8%, respectively. They explained that such a poor UBT diagnostic efficacy in the gastrectomized patients can be attributed in part to the lower bacterial loads, inadequate coating of the stomach by urea, and disuse of test meal. Kubota et al.[21] established a standardized protocol and cut-off value for the ^{13}C-UBT in gastrectomized patients. Using receiver operating characteristic analysis, they selected 40 minutes and a cut-off of 2.0‰. As the delta over baseline (DOB) $^{13}CO_2$ of *H. pylori* non-infected patients mainly reflect urease activity in the mouth, surgery should have no effect and the cut-off should be set appreciably higher.[47] Graham et al.[48] originally reported that a test meal was required before urea ingestion to similarly extend the period of contact between urea and *H. pylori*. Most studies evaluating the need for citric acid in UBT showed higher delta values with citric acid when compared with other test meals or no test meals.[49-51] Citric acid is expected to increase delta values in infected patients and not change delta values in uninfected ones. Adding citric acid may, therefore, well increase the discriminative capacity of the test.[49] In previous our reports,[52] we also reported a high false positive results of the ^{13}C-UBT after eradication of *H. pylori* in the normal patients. In line with previous reports, no application of the citric acid test meal for the test would be cause such a high rate of false positive results in this study.

In the present study, we also sought to identify clinical factors that caused false positive ^{13}C-UBT results after *H. pylori* eradication in the remnant stomach. A previous history of multiple *H. pylori* eradication therapies was found to be correlated with false positive ^{13}C-UBT results (odds ratio = 3.248, 95% confidence interval = 1.088-9.695, $P = 0.035$). Total 19 patients underwent second- or third-line eradication therapy for *H. pylori* infection in the ^{13}C-UBT range over 2.5‰ in mismatched group. Of 2 patients who underwent third line eradication the ^{13}C-UBT value were 6‰ and 22.4‰. In these cases, DOBs of the $^{13}CO_2$ were decreased compared to first eradication therapy for *H. pylori*, but the change DOB $^{13}CO_2$ range was variable. This consequence might imply that the diagnostic accuracy of the ^{13}C-UBT is imprecise in positive value, thus additional second or third eradication would be needed according the UBT results. This is important point that if the clinicians should perform additional eradication of *H. pylori* infection more cautiously when the results fall into the positive results of the ^{13}C-UBT after *H. pylori* eradication. Considering the altered stomach environment and the diagnostic reliability of the ^{13}C-UBT, it would be better to postpone additional eradication and to perform endoscopic biopsy methods to detect for *H. pylori* infection in the remnant stomach.

However, this present study also has some limitations. First, we could not compare ^{13}C-UBT and endoscopic biopsy results at the same time. According to the Korean Health Insurance service, these two tests should not be performed simultaneously after *H. pylori* eradication. Therefore, in this study, we evaluated *H. pylori* status endoscopically at least 6 months after the ^{13}C-UBT. Thus, the high false positive results of the ^{13}C-UBT might be influenced by the altered stomach environment, such as, increasing pH, bile reflux acid, and unused of test meal. Second, this study is intrinsically limited by its retrospective design. In particular, 42.3% of patients who performed ^{13}C-UBT after receiving subtotal gastrectomy were enrolled, which introduce the possibility of sampling bias. Especially, the cases of the positive ^{13}C-UBT result were lesser than half of negative results of the ^{13}C-UBT. Third, the results of our study could be applied to post-*H. pylori* eradication status rather than initial diagnosis of *H. pylori* infection. Under the Korean National Medical Insurance system, it is difficult to obtain ^{13}C-UBT values before *H. pylori* eradication. Furthermore, a well-designed, randomized, controlled study is needed to confirm the diagnostic validity of the ^{13}C-UBT before *H. pylori* eradication in partial gastrectomized patients.

Summarizing, this study shows that there were too many mismatched results between the ^{13}C-UBT and endoscopic biopsy methods after eradication of *H. pylori* infection in the patients who received partial gastrectomy for gastric cancer. Especially, in the range 2.5‰ to 10‰ of the ^{13}C-UBT after *H. pylori* eradication in the gastrecomized patients, the clinicians should be consider the possibility of false positive result of ^{13}C-UBT, and additional endoscopic surveillance with biopsy-based methods would be helpful to avoid unnecessary additional treatment for *H. pylori* infection.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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