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Functional ^{13}C-urea and glucose hydrogen/methane breath tests reveal significant association of small intestinal bacterial overgrowth in individuals with active *Helicobacter pylori* infection

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ABSTRACT

Objectives: *Helicobacter pylori* infection is considered to alter the bacterial flora in the upper gastrointestinal tract. This study aimed at investigating the presence of small intestinal bacterial overgrowth (SIBO) in patients with active *H. pylori* infection assessed by functional breath testing.

Design and methods: A total of 109 outpatients, who were referred for the *H. pylori* ^{13}C-urea breath test (^{13}C-UBT) by general practitioners and specialists, were also tested for the presence of SIBO by the glucose hydrogen (H_2)/methane (CH_4) breath test (HMBT). A detailed anamnesis was carried out about the history of *H. pylori* infection, eradication therapies, proton pump inhibitor intake, and comorbidities.

Results: In total, 36/109 (33.0%) patients had a positive *H. pylori* ^{13}C-UBT, and 35/109 (32.1%) patients had a positive glucose HMBT, the latter being indicative of SIBO. Interestingly, individuals with a positive *H. pylori* ^{13}C-UBT were significantly more often associated with a positive glucose HMBT ($p = 0.002$). Cohen's κ measuring agreement between the ^{13}C-UBT and the glucose HMBT was 0.31 (confidence intervals: 0.12–0.50) ($p = 0.001$). Altogether, 19 of 54 (35.2%) patients, who had completed up to four eradication therapies, were diagnosed with SIBO by HMBT.

Conclusions: *H. pylori* infection was found to be significantly associated with the presence of SIBO as determined by functional breath testing. In addition, SIBO rates appeared to have increased after completed eradication therapies. However, further longitudinal studies are warranted to fully elucidate the relationship and treatment modalities of coincident *H. pylori* infection and SIBO.

1. Introduction

Helicobacter pylori is still reported to be a common pathogen worldwide [1,2]. Since this Gram-negative bacterium was recognized as a risk factor of peptic ulcer disease, chronic atrophic gastritis, gastric cancer, and mucosa-associated lymphoid tissue lymphoma, numerous invasive and non-invasive diagnostic methods for accurate detection of *H. pylori* have been established. The functional ^{13}C-urea breath test (^{13}C-UBT) is considered as a valid laboratory method for the diagnosis of *H. pylori* infection [3].

H. pylori is well known as a bacterium that hydrolyses urea into ammonia and carbonic acid [4,5]. This chemical reaction is the basis for the detection of active infected individuals with the ^{13}C-UBT [6] and protects the bacterium against gastric acid [4,7], which plays an important protective role in the prevention of bacterial colonization in the stomach and small intestine [8]. The low intra-gastric pH value may increase due to *H. pylori* infection because of production of ammonia [9]. Therefore, this bacterium may be a causative agent of small intestinal bacterial overgrowth (SIBO).

SIBO is a complex condition of the upper gastrointestinal tract, which is defined as the presence of abnormal numbers of bacteria in the small intestine [10,11]. The etiology of SIBO is multifactorial comprising achlorhydria, previous surgery of the upper gastrointestinal tract, mechanically defective ileocecal valve, and/or impaired motility of the intestine [10]. The laboratory diagnostic glucose hydrogen (H_2)/methane (CH_4) breath test (HMBT) represents a non-invasive highly reproducible and inexpensive tool for SIBO diagnosis [12].

To date, SIBO rates in patients with active *H. pylori* infection have not been systematically evaluated by laboratory functional breath tests. Therefore, it is of great interest to investigate whether a close association exists between these two gastrointestinal conditions.

Abbreviations: ^{13}C-UBT, ^{13}C-urea breath test; SIBO, small intestinal bacterial overgrowth; H_2, hydrogen; CH_4, methane; HMBT, hydrogen methane breath test; PPI, proton pump inhibitor; δ, delta; κ, kappa; CI, confidence interval.

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As proton pump inhibitors (PPIs) are included in the treatment regime of *H. pylori* infections, the interference of this class of drugs with the gastric acidic barrier must also be considered [13]. PPIs can block the proton pump (H+/K+-ATPase) of parietal cells in the stomach. Nevertheless, the side effects of PPI use, such as altering the bacterial flora of the upper gastrointestinal tract through changes in gastric pH, may not be underestimated [14].

The aim of this study was to assess the presence of SIBO in patients with active *H. pylori* infection by the functional ^{13}C-UBT and glucose HMBT in a cohort of patients who were referred by general practitioners and specialists to our outpatient clinic. Additionally, a detailed anamnesis was carried out on gastrointestinal symptoms, the history of *H. pylori* infection, eradication therapies, PPI use, and comorbidities.

2. Materials and methods

2.1. Ethics

The ethical approval for this study was obtained from the Ethical Committee of Upper Austria, Linz, Austria. The study was carried out in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from all patients.

2.2. Patients

Overall, 109 outpatients, who were referred for laboratory *H. pylori* ^{13}C-UBT by general practitioners and specialists, were also tested for SIBO by functional glucose HMBT during a second consultation. Thirty-six (33.0%) individuals were males and 73 (67.0%) were female. The mean age was 44 ± 16 years. The basic characteristics of the study participants are illustrated in Table 1. Taken together, 97/109 (89.0%) patients presented one or more gastrointestinal symptoms (i.e., upper abdominal pain, bloating, nausea, diarrhea, or obstipation), 21/109 (19.2%) individuals had a preexisting gastrointestinal or hepatic disorder (i.e., reflux esophagitis and hepatitis) in their case histories, and 12/109 (11.0%) individuals were diagnosed with a psychiatric disorder. All patients had a negative case history of previous surgery of the upper gastrointestinal tract (i.e., esophagus, stomach, and small intestine).

The inclusion criteria for this study were a minimum age of 15 years, an overnight fasting state, and a non-smoking period >12 h before breath testing. Patients who underwent antibiotic-based therapy at least 4 weeks before and/or PPI therapy at least 2 weeks before the breath test performance were excluded from the study. A detailed anamnesis was carried out on the history of *H. pylori* infection, the intake of PPI, completed eradication therapies, and comorbidities.

Table 1
Basic characteristics of the study population.

	Study population (n = 109)
Gender	
Female	73 (67.0%)
Male	36 (33.0%)
Age (years, mean ± SD)	44 ± 16
Presence of gastrointestinal symptoms (anamnesis)	
Upper abdominal pain	54 (49.5%)
Bloating	27 (24.7%)
Nausea	15 (13.7%)
Diarrhea	14 (12.8%)
Obstipation	1 (0.9%)
Gastrointestinal and hepatic disorders (case histories)	
Hiatus hernia	7 (6.4%)
Reflux esophagitis	4 (3.7%)
Barrett syndrome	1 (0.9%)
Fatty liver	7 (6.4%)
Autoimmune hepatitis	1 (0.9%)
Hepatitis C	1 (0.9%)
Psychiatric disorders (case histories)	
Depression	10 (9.2%)
Anxiety disorder	1 (0.9%)
Eating disorder	1 (0.9%)
Chronic comorbidities (case histories)	
Arterial hypertension	13 (11.9%)
Obesity	5 (4.5%)
Diabetes type 2	4 (3.6%)
Coronary heart disease	3 (2.7%)
Bronchial asthma	4 (3.7%)
Obstructive sleep apnea syndrome	2 (1.8%)
Hashimoto thyroiditis	1 (0.9%)
Multiple sclerosis	1 (0.9%)
Bechterew's disease	1 (0.9%)
Hereditary angioedema	1 (0.9%)

2.3. H. pylori ^{13}C-UBT

The ^{13}C-UBT was performed by isotope ratio mass spectrometry using an IRIS®-^{13}C-Infrared Isotope Analyzer System (Wagner Analysen Technik GmbH, Bremen, Germany). The test protocol was implemented according to the manufacturer's instructions. In brief, after a 12-h fasting period, breath samples were obtained before (baseline) and 30 min after the test drink intake (75 mg ^{13}C-urea from the capsule dissolved in 200-mL fruit juice) early in the morning (8:00–10:00 a.m.). After giving the patient the ^{13}C-urea dose in liquid form, an immediate mouth rinsing was carried out to prevent false-positive results by oral bacteria with urease activity [15,16]. The $^{13}C/^{12}C$-isotope ratio on CO_2 in the breath samples was determined as the delta (δ) value (‰) versus PDB (Pee Dee Belemnite, international limestone standard). The increase in the δ value 30 min after ingestion of the tracer was expressed in delta over baseline ((DOB) ‰) (formula: DOB, ‰ = δ value of baseline breath sample − δ value 30 min after ingestion of the tracer) [15]. A sample was considered positive if the 30 min value was above a 4‰ cut-off level [6,17]. Eating, drinking, and/or smoking were not allowed until the ^{13}C-UBT had been completed.

2.4. Glucose HMBT

A glucose HMBT protocol was established with the QuinTron Model DP Plus MicroLyzer™ (QuinTron, Milwaukee, Wisconsin, United States of America) to detect patients with SIBO. After an overnight fasting state of 12 h, 50 g glucose dissolved in 200 mL of water was orally administered [12]. The H_2 and CH_4 breath concentrations were measured at 0 (baseline before sugar ingestion), 15, 30, 45, 60, 90, and 120 min by gas chromatography. According to the literature [12,18], patients were classified to have SIBO if a H_2 and/or CH_4 increase of ≥10 ppm above the baseline was observed. During the test procedure, patients were instructed to avoid physical effort, smoking, and/or eating and to report clinical symptoms.

2.5. Statistical analysis

Descriptive statistics were performed to analyse and compare the functional ^{13}C-UBT and glucose HMBT results. Fisher's exact test (2 × 2 tables) was calculated for subgroup comparisons of categorical parameters. The agreement between the ^{13}C-UBT and the HMBT results was calculated using Cohen's kappa (κ) with 95% confidence intervals (CIs). All statistical tests were used in an explorative way; therefore, no correction of the type I error (two-sided, 5%) was made. This means that the results were only descriptive. For all calculations, the Analyse-it® software version 2.30 (Analyse-it Software, Ltd., Leeds, United Kingdom) was used.

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