Small intestinal bacterial overgrowth among patients with celiac disease unresponsive to a gluten free diet

Mohammad-Ayman A. Safi[1], Asif A. Jiman-Fatani[1], Omar I. Saadah[2]

[1]Department of Medical Microbiology and Parasitology, King Abdulaziz University Faculty of Medicine, Jeddah, Saudi Arabia
[2]Pediatric Gastroenterology Unit, Department of Pediatrics, King Abdulaziz University Faculty of Medicine, Jeddah, Saudi Arabia

Cite this article as: Safi MAA, Jiman-Fatani AA, Saadah OI. Small intestinal bacterial overgrowth among patients with celiac disease unresponsive to a gluten free diet. Turk J Gastroenterol 2020; 31(11): 767-74.

ABSTRACT

Background/Aims: Little is known about the relationship between small intestinal bacterial overgrowth (SIBO) and celiac disease (CeD) in patients who are unresponsive to a gluten-free diet (GFD). This study aimed to determine the SIBO prevalence in patients with CeD who are unresponsive to a GFD.

Materials and Methods: We conducted a case-control study from July 2012 to September 2014. We included 32 patients with CeD who were unresponsive to a GFD and 52 healthy age- and sex-matched controls. Demographic, clinical, and laboratory data were obtained from patients' medical records. Antitissue transglutaminase antibody determined by enzyme-linked immunosorbent assay was recorded, and lactulose hydrogen breath test (LHBT) was used to detect SIBO in all participants. Microbiological analysis, including jejunal aspirates obtained using upper endoscopy, was performed for only 20 patients with CeD.

Results: A total of 10 (31%) of 32 patients with CeD and 4 (7.7%) of 52 controls tested positive for LHBT, with a statistically significant difference (p=0.007). Of 20 cultures, 3 (15%) were positive with no statistically significant correlation between the cultures and LHBT (p=0.05). In a subgroup analysis of children who were 18 years old or younger, 7/24 (29.2%) patients with CeD had a positive LHBT compared with 3/32 (9.4%) controls, but this difference was not statistically significant (p=0.08).

Conclusion: The prevalence of SIBO was 31% in unresponsive patients with CeD according to LHBT and 15% in the quantitative culture of the jejunal aspirate, which is comparable with the published Western literature

Keywords: Bacteria, celiac disease, child, gluten-free diet, breath test, lactulose, Saudi Arabia

INTRODUCTION

Celiac disease (CeD) is an autoimmune enteropathy triggered by gluten ingestion in genetically susceptible individuals with a prevalence of about 1:100 in the Western countries (1, 2). In Saudi Arabia, seroprevalence rates of CeD are 2.8% and 2.2% in children and adolescents, respectively (3, 4). Small intestinal bacterial overgrowth (SIBO) is a condition characterized by excessive growth of bacteria in the small intestine (5). Presence of more than 10^5 colony-forming units (CFU)/mL of proximal jejunal aspiration is required to define this condition (5, 6). The main clinical symptoms related to SIBO are abdominal discomfort, bloating, flatus, chronic diarrhea, steatorrhea, nausea, and poor weight gain (7). Although the jejunal fluid aspiration remains the gold standard for diagnosis of SIBO, (5) other noninvasive diagnostic methods, such as lactulose hydrogen breath test (LHBT) and a glucose hydrogen breath test (GHBT), have been used (8, 9). SIBO has been reported in association with CeD in adult patients (10-12).

Several studies have investigated the association between SIBO and CeD in adult patients who are unresponsive to a gluten-free diet (GFD) (13-18). However, no studies have been conducted in a Saudi population where CeD is prevalent. Thus, this case-control study is the first and only study, thus far, to assess the prevalence of SIBO in patients with CeD, including children and adults who are unresponsive to a GFD in the Arab countries.

MATERIALS AND METHODS

Study Participants

We conducted a case-control study from July 2012 to September 2014. A total of 32 patients diagnosed with CeD, who were unresponsive to GFD, were included. The unresponsiveness to a GFD was defined as showing persistent symptoms despite strict adherence to a GFD for 12 months (14, 17). Adherence to a GFD was assessed by a celiac dietician who interviewed the patients using a dietary questionnaire and by detecting a significant decline

Corresponding Author: **Omar I. Saadah; osaadah@kau.edu.sa**
Received: **September 3, 2019** Accepted: **November 12, 2019**

DOI: **10.5152/tjg.2020.19627**

of the antitissue transglutaminase (anti-tTG) antibodies from the initial reading at diagnosis. The control group included 52 healthy individuals who were matched for age and sex. Data, including demographics, clinical symptoms, body mass index, and blood tests (albumin, hemoglobin, leukocyte counts, platelets count, and vitamin D level), were obtained from the patients' medical records. All participants were tested for bacterial overgrowth using LHBT and for CeD using anti-tTG antibodies. The jejunal aspirates for bacterial culture were obtained through endoscopy, which was performed only in a subset of patients with CeD.

Serology Testing (anti-tTG)

Anti-tTG results were obtained from the patients' medical records. Healthy controls underwent anti-tTG tests using enzyme-linked immunosorbent assay kits (Euroimmun Medical Laboratory Diagnostics, Seekamp, Luebeck, Germany). Results were calculated and interpreted according to the high positive cutoff, which was 20 unit/mL according to the manufacturer's instructions.

LHBT

All the participants were tested for SIBO using LHBT. We used a breath gas analyzer (Bedfont gastrolyzer; Bedfont Scientific Ltd., ME13QX, England) and lactulose syrup (65 g/100 mL) from the *Egyptian International Pharmaceutical Industries Company*. The patients were instructed not to take antibiotics for at least 2 weeks and discontinue probiotics 7–10 days before the test. They were also instructed not to eat fruits, grains, beans, and cereals for at least 12 hours before the test. An overnight fasting was required for the basal breath hydrogen level. Physically strenuous activities and cigarette smoking were not permitted at least 2 hours before and during the test to avoid hyperventilation. Just before the test, the patients had to brush their teeth and wash their mouths with an antiseptic mouthwash, followed by tap water to eliminate an early hydrogen peak that would occur because of the action of oral bacteria on the test sugar (19). An average of 3 values was taken as the basal breath hydrogen level. Oral lactulose syrup (10 mL) was administered containing 6.5 g of dissolved lactulose. The level of the breath hydrogen was measured using the Bedfont breath gas analyzer every 15 minutes for 3 hours, which was continued for up to 4–5 hours in case of absence of peaks. The rise of breath hydrogen by ≥20 parts per million (ppm) above the basal level within the first 100 minutes or 2 peaks ≥20 ppm over baseline was taken as evidence of SIBO (20). Although glucose has been reported to have better diagnostic accuracy than lactulose, both sugars are commonly used for the hydrogen breath test in clinical practice. We chose lactulose because it is the standard test in our hospital and has the advantage of measuring the orocecal transit time between its 2 recorded peaks.

Aspiration of Jejunal Fluids

Only a subset of patients with CeD underwent jejunal aspiration. It was performed using an aspiration catheter (Hobbs Medical Inc., Stafford Springs, CT, USA) of 240 cm in length and 2.3 mm in diameter that was passed through the biopsy channel of the gastroscope. A disinfected gastroscope and a sterile catheter were used to collect the aspirate. The catheter tip was positioned beyond the duodenum into the jejunum. To avoid any potential contamination, suction of secretions was not performed before positioning the aspiration catheter in the jejunum. The other end of the catheter was connected to a 10-mL sterile plastic syringe through which small bowel fluid was suctioned to obtain an amount of approximately 1 mL. The catheter (with the syringe) was returned to its sterile package and was then immediately taken to the microbiology laboratory where the aspirate was transported into a sterile micro vial and cultured for aerobic and anaerobic bacteria.

Aseptic Microtechnique for Delivering a Very Small Volume of the Jejunal Aspirate from a Catheter

An issue was encountered in the jejunal aspiration process. The aspirate mostly contained a very small volume that remained within the catheter. Thus, a novel aseptic microtechnique was developed for delivering small volumes of the jejunal aspirate from a catheter into a sterile micro vial. After aspiration, the catheter (with the attached plastic disposable syringe) was returned to its sterile bag and taken immediately to the microbiology laboratory within 10 minutes. Using a microbiological safety cabinet, the attached syringe was disconnected and the contents of the jejunal aspirate were emptied into a sterile micro vial. A 25-mL sterile plastic disposable syringe that was full of air was attached to the cath-

MAIN POINTS

- *SIBO should be always suspected in patients with CeD unresponsive to the gluten free diet.*
- *Breath hydrogen test using lactulose or glucose can be utilized as a noninvasive diagnostic test.*
- *Culture of the jejunal aspirate remains the gold standard for diagnosis.*
- *We found a high prevalence rate of SIBO in our patients with CeD that is comparable with the published Western literature.*

eter. The air inside the syringe was pushed to empty the jejunal aspirate inside the sterile micro vial. Thereafter, the syringe was disconnected from the catheter and air filled. Again, the inside air was pushed to empty the jejunal aspirate inside the sterile micro vial. The process was repeated until the entire jejunal aspirate was transferred into the sterile micro vial. This technique was found to be aseptic and harmless on anaerobic bacteria when tested 3 times for a suspension of *Clostridium sporogenes* in Robertson's cooked meat broth (RCMB). The culture of the suspensions showed heavy growth of only *Clostridium sporogenes* without any contamination.

Microbiology of the Jejunal Aspirates

After transferring the jejunal aspirates from the catheter into sterile micro vial, each jejunal aspirate was tested for SIBO. A smear was prepared, fixed, gram-stained, and then cultured aerobically and anaerobically using standard techniques (21). Each jejunal aspirate was homogenized by vortex and was serially diluted (1/20–1/20,000) with 0.5 mL of sterile distilled water for aerobic culture and with sterile RCMB for anaerobic culture. For aerobic culture, 10 µL of the undiluted sample and 100 µL of each dilution in distilled water were plated on the blood agar and MacConkey agar plates that were incubated at 37°C for 48 hours.

For anaerobic culture, 10 µL of the undiluted sample and 100 µL of each dilution in RCMB were cultured on plates of neomycin anaerobic blood agar and Wilkins-Chalgren-agar that were incubated in an anaerobic chamber at 37°C for 48 hours and 5 days, respectively. For lactobacilli, we used plates of Rogosa agar that were incubated in anaerobic conditions. Internal controls included two-third of each plate, with one-third being used for *Clostridium sporogenes* (anaerobic control) and the other third for *Pseudomonas aeruginosa* (aerobic control). At the end of the incubation, colonies were counted and bacterial species were identified using standard methodology and on Vitek 2 automation system (bioMerieux) (21, 22). Bacterial counts were expressed as a logarithm of CFU/mL of the jejunal fluid according to the following formula: number of colonies×dilution factor, which is the reciprocal of the dilution. A growth of more than 10^5 CFU/mL was considered significant to indicate SIBO and for further bacterial identification.

Histopathology

Biopsies of the small bowel (4-6 specimens) were evaluated by a single gastrointestinal pathologist to assess the severity of enteropathy. Biopsy specimens were classified according to Marsh-Oberhuber classification (23). All the patients with CeD at the initial diagnosis had severe enteropathy (Marsh 3c).

Statistical Analysis

All data analyses were performed using the Statistical Analysis System (SAS) v9.4 (SAS Institute, Cary, NC, USA). Descriptive statistics was performed using mean and standard deviation (SD), median and range, and frequencies and percentages. For comparisons, we used chi-squared test and Fisher's exact test for categorical variables and 2-sample t-test and Mann–Whitney U test for continuous variables per the data. To estimate the correlation between the different tests, we used tetrachoric correlation coefficients, which are used to test the correlation between binary variables. We also calculated the sensitivity and specificity of LHBT compared with the bacterial culture, which is the gold standard. A $p<0.05$ was considered statistically significant.

Ethical Oversight

Ethical considerations in accordance with the declaration of Helsinki were followed throughout the duration of this study. The study was approved by the research committee/biomedical ethics unit, King Abdulaziz University (Reference No 572-11). A written informed consent was obtained from each patient or the parent of the patient for participation, which included blood sampling, breath tests after sugar syrup intake, and endoscopy (for patients with CeD).

RESULTS

Baseline Characteristics

A total of 32 patients with CeD who were unresponsive to a GFD and 52 healthy controls matched for age and sex were included in the analysis.

Patients with CeD: The median age was 15.5 (range, 7-23) years. The mean age±SD for the patients was 14.9±4.5 years. There were 19 (59.4%) men and 13 (40.6%) women with a male/female (M/F) ratio of 1.5:1. There were 17 (53.0%) Saudi participants and 15 (47.0%) non-Saudi participants, with a Saudi/non-Saudi ratio of 1.3:1. There were 14 (43.8%) seronegative and 18 (56.3%) seropositive patients with a ratio of 1:1.3. Only 20 patients (62.5%) underwent endoscopy followed by culture of the jejunal aspirate.

Controls: The median age was 17 (7-23) years. The mean±SD age was 16.2±4.6 years. There were 41 (78.8%)

Table 1. Baseline characteristics of patients with celiac disease and controls.

	CeD patients (n=32)	Controls (n=52)	p
Age, median (range) Yrs.	15.5 (7-23)	17 (7-23)	0.22*
Age, mean±SD	14.9±4.5	16.2±4.6	0.24**
Age groups			
0-18 years	24	32	0.20***
>18 years	8	20	
Male: female ratio	1.6:1	3.7:1	0.06***
Saudi nationality (%)	17 (53%)	29 (56%)	0.80***
Clinical manifestations, n (%)			
Abdominal pain	17 (20.2%)		
Abdominal distention	16 (19%)		
Diarrhea	15 (17.9%)		
Poor weight gain	14 (16.7%)		
Loss of appetite	10 (11.9%)		
Pallor	6 (7.1%)		
Positive serology, n (%)	18 (56%)	0.0 (0.0%)	<0.001****
Marsh Classification, n (%) (Histopathology)			
Type 0	12 (37.5%)		
Type 1	9 (28.1%)		
Type 3a	5 (15.6%)		
Type 3b	3 (9.4%)		
Type 3c	3 (9.4%)		
Hemoglobin (g/dl), mean (SD)	12 (2.1%)		
Leukocyte counts (k/ul), mean (SD)	5.8 (1.8%)		
Platelets counts (k/ul), mean (SD)	301 (99%)		
Albumin (g/L), mean (SD)	38 (3.2%)		
25-hydroxyvitamin D level (ng/mL) mean (SD)	40.8 (19.7%)		

*Mann-Whitney U test
** Two-sample t-test
***chi-square test ****Fisher's exact test

Table 2. Relationship between the jejunal aspirate culture and lactulose hydrogen breath test results.

		Culture of jejunal aspirates			
		Negative N (%)	Positive N (%)	Total N (%)	p
LHBT	Negative	16 (94.1)	1 (33.3)	17 (85)	0.05*
	Positive	1 (5.9)	2 (66.7)	3 (15)	
	Total	17 (100)	3 (100)	20 (100)	

*Fisher's exact test
LHBT: lactulose hydrogen breath test

men and 11 (21.2%) women with M/F ratio of 3.7:1. There were 17 (53.0%) Saudi participants and 15 (47.0%) non-Saudi participants, with a Saudi/non-Saudi ratio of 1.3:1. All controls were seronegative, and none had an endoscopy performed for the jejunal fluid aspiration. Demographic and clinical characteristics are shown in Table 1.

Prevalence of SIBO Using LHBT and Bacterial Culture

A total of 10 (31%) of 32 patients with CeD and 4 (7.7%) of 52 controls were positive for LHBT, with a significant difference between both the groups (p=0.004). A total of 3 (15%) of 20 cultures were positive, but 1 had a negative LHBT. The culture of the jejunal fluid revealed significant growth of gram-positive *Streptococcus viridans* in 2 patients and *Lactobacillus* in 1 patient. Of the 17 negative cultures, 1 had a positive LHBT (Table 2). Compared with the culture, which is the gold standard for SIBO diagnosis, the sensitivity and specificity of LHBT were 66.7% and 94.1%, respectively. There was no statistically significant association between LHBT and culture tests (p=0.05) with no statistically significant correlation (tetrachoric rho=0.85; p=0.05). Furthermore, there was no significant correlation (p>0.05) among anti-tTG, culture, and Marsh score (anti-tTG and culture, tetrachoric rho=1.00; anti-tTG and Marsh score, tetrachoric rho=0.61; culture and Marsh score, tetrachoric rho=1.00).

There was no significant difference in the clinical and laboratory variables between the patients with positive and negative LHBT among those with CeD (Table 3). There was also no significant difference in the demographic and clinical variables between LHBT-positive and -negative patients among all the participants, except for the anti-tTG serology test (p=0.004) (Table 4).

In children who were 18 years or younger, positive LHBT was detected in 7/24 (29.2%) patients and in 3/32

Table 3. Comparison of patients with celiac disease with positive and negative lactulose hydrogen breath test.

	Positive LHBT		Negative LHBT		
Variable	N	Mean (SD)	N	Mean (SD)	p
Age (years)	10	13.5 (5.5)	22	15.6 (3.9)	0.30*
BMI	9	18.1 (5.6)	20	17.9 (3.4)	0.92
tTG (u/ml)	10	114.4 (104)	19	99.5 (69)	0.79
Albumin (g/L)	10	37.7 (2.9)	21	38.2 (3.4)	1.0
Hemoglobin (g/dl)	10	12.2 (2)	20	11.9 (2.2)	0.59
Leukocyte count (k/ul)	10	5.8 (1.7)	21	5.9 (1.9)	0.96
Platelets counts (k/ul)	10	332 (102)	21	286 (96)	0.24
25-hydroxyvitamin D level (ng/mL)	10	34.3 (18.3)	21	43.9 (20)	0.35

* Two sample t-test
LHBT: lactulose hydrogen breath test; BMI: body mass index

(9.4%) controls (p=0.08). In adults, 3/8 (37.5%) patients tested positive for LHBT and only 1 control tested positive for LHBT (p=0.06). There was a significant difference in the prevalence of SIBO detected by LHBT between patients and controls (p=0.007). However, there was no significant difference in the prevalence of SIBO between children and adults (p=0.765) (Table 5).

Among patients who had a jejunal culture, 3 (15%) had a positive culture; 2/14 (14.3%) children and 1/6 (16.7%) adults were found positive of the 20 patients tested (Table 6).

DISCUSSION

CeD is an autoimmune enteropathy triggered by gluten ingestion in genetically susceptible individuals, which usually manifests itself as symptoms of malabsorption, including diarrhea, abdominal pain, abdominal distention, and poor weight gain (1). Patients with CeD usually improve on GFD; however, 7%–30% of the patients continue to have symptoms of malabsorption despite adherence to the GFD and require further evaluation (24). The lack of response to a prescribed GFD or recurrence of gastrointestinal symptoms despite GFD maintenance in patients who responded initially to GFD is usually termed "unresponsive CeD" (13). Unresponsiveness suggests gluten contamination or coexistence of other conditions, such as SIBO, pancreatic insufficiency, giardiasis, lymphocytic colitis, ulcerative jejunitis, and refractory CeD (13).

SIBO occurs usually because of an increased number and/or abnormal types of bacteria in the small intestine (25). Symptoms, such as diarrhea, bloating, and malabsorption, can manifest in SIBO. The gold standard for the diagnosis of SIBO is the bacterial culture of the jejunal fluid aspirate, obtained through upper gastrointestinal endoscopy and diagnosed when bacteria $\geq 10^5$ CFU/mL of the proximal jejunal fluids is found (5). However, this method is invasive and not practical. Despite low sensitivity and specificity, hydrogen breath test is considered more practical by most authors. The test can achieve a sensitivity of 62.5% and a specificity of 82%, with a diagnostic accuracy of 72% after glucose use and a sensitivity of 52%, specificity of 86%, with a diagnostic accuracy of 55% after lactulose use (26). Mattson et al. (27) reported that LHBT is more often positive than GHBT. However, a recent systematic review (28) showed no difference in the prevalence of SIBO reported by studies using either GHBT or LHBT.

SIBO has been reported in relation to CeD by several studies (10, 11, 14, 18). SIBO plays a role in the pathogenesis of CeD by changing the expression of the tight junction protein, zonula occludens-1, leading to gliadin translocation through impaired tight junction function and increase in the luminal gastrointestinal response to gliadin, resulting in inflammation and dysmotlity (29).

The mean reported prevalence of SIBO among adults with CeD using LHBT was 29.7%, with a range of 5%-66.6% (10, 14, 15, 18, 30), 16.9%, with a range 8.3%-21.57% using GHBT (11, 12, 31), and 24.6%, with a range 9.39%-50% using cultures of the jejunal fluids (13, 16, 17).

Table 4. Bivariate analysis of the clinical and demographic variables in relation to lactulose hydrogen breath test.

Variable	Positive LHBT N (%)	Negative LHBT N (%)	Total N (%)	p*
Age category				
Children	10 (71.4)	46 (65.7)	56 (66.7)	0.76
Adult	4 (28.6)	24 (34.3)	28 (33.3)	
Gender				
Male	8 (57.1)	52 (74.3)	60 (71.4)	0.19
Female	6 (42.9)	18 (25.7)	24 (28.5)	
Nationality				
Saudi	7 (53.8)	39 (54.9)	46 (54.7)	0.94
Non-Saudi	6 (46.2)	32 (45.1)	38 (45.2)	
Poor weight gain				
Yes	4 (50)	10 (55.5)	14 (53.8)	0.79
no	4 (50)	8 (44.4)	12 (46.1)	
Loss of appetite				
Yes	5 (27.7)	5 (62.5)	10 (38.4)	0.19
no	13 (72.2)	3 (37.5)	16 (61.5)	
Abdominal pain				
Yes	4 (50)	13 (72.2)	17 (65.3)	0.38
no	4 (50)	5 (27.7)	9 (34.6)	
Diarrhea				
Yes	5 (62.5)	10 (55.5)	15 (57.6)	1.0
no	3 (37.5)	8 (44.4)	11(42.3)	
Abdominal distension				
Yes	4 (50)	12 (66.6)	16 (61.5)	0.66
no	4 (50)	6 (11.3)	10 (38.4)	
Serology				
Positive	7 (50.0)	11 (15.7)	18 (21.4)	0.004**
Negative	7 (50.0)	59 (84.3)	66 (78.6)	
Histopathology grading				
Marsh 0	3 (30)	9 (40.9)	12(37.5)	0.87
Marsh 1	4 (40)	5 (22.7)	9 (28.1)	
Marsh 3a	1 (10)	4 (18.1)	5 (15.6)	
Marsh 3b	1 (10)	2 (9.1)	3 (9.4)	
Marsh 3c	1 (10)	2 (9.1)	3 (9.4)	

* chi-square/ Fisher's exact test
**p<0.05
LHBT: lactulose hydrogen breath test

Although the most common cause of CeD that is unresponsiveness to GFD is the inadvertent consumption of gluten, other causes have been described including SIBO (13, 18). Our study focused on CeD unresponsive to GFD in children and adults. Few studies were conducted in patients with unresponsive CeD (13-18). However, those studies differ in their definition of unresponsiveness to the GFD; 2 studies used "persistent symptoms after 12 months of GFD" to define unresponsiveness (14, 17), 1 used "persistent symptoms for 24 months" (15), 1 used "persistence of symptoms for 6–8 months" (18), 1 used "persistence of symptoms and histological atrophy after 12 months" (13), and 1 used "persistent histological atrophy" (16). In our study, we relied on the "persistence of symptoms after 12 months of GFD" as a criterion to define the unresponsiveness in the population studied. All the reported studies were conducted in adults only, and our study cohort included pediatric patients and controls.

A total of 3 studies using LHBT showed prevalence of SIBO of 9%, 20%, and 48% (10, 14, 15). Our study showed an overall prevalence of 31% in patients with CeD and 7.7% in the controls that showed a statistically significant difference (p=0.007). The prevalence of SIBO in our patients with CeD is close to that reported by Chang et al (14), and 1 of the 3 studies had a case-control design, similar to our study (10). However, unlike our study, no significant difference in the prevalence of SIBO between the patients and controls was detected (20% vs. 13.33%).

In our study, we also performed bacterial culture of the jejunal fluid aspirate that was positive in 16.7% adults and 14.3% children. Comparing our results with 2 other similar studies using culture and sharing the same criteria for defining unresponsiveness, our prevalence using the culture method was low and comparable with the prevalence of 11.4% and 14.3% reported in these studies (13, 17). The discrepancy between the results of the LHBT and bacterial culture may be explained partly by the relatively high frequency of false-positive results of LHBT, especially in patients with rapid orocecal transit time (6). In addition, culture methods of the jejunal aspirate may not be perfect either because they may miss patchy or more distal SIBO and because they are poorly reproducible (25). Therefore, from a practical viewpoint, LHBT is more widely used than culture methods in clinical settings.

In our subgroup analysis, patients older than 18 years had SIBO prevalence of 37.5% vs. 5% in the controls, and patients who were 18 years or younger had SIBO prev-

Table 5. Results of lactulose hydrogen breath test in patients and controls stratified by age group (children vs. adults).

	Children (0-18) n=56 N (%)			Adults >18 n=28 N (%)				
LHBT	cases	controls	P value	cases	controls	P value	Total N (%)	Overall p*
Positive	7 (29.2)	3 (9.4)	0.08	3 (37.5)	1 (5)	0.06	14 (16.7)	0.765
Negative	17 (70.8)	29 (90.6)		5 (62.5)	19 (95)		70 (83.3)	
Total	24	32		8	20		84	

*Fisher's exact test
LHBT: lactulose hydrogen breath test

Table 6. Jejunal aspirate culture in patients with celiac disease stratified by age group (children vs. adults).

Culture results	Children, n=14 N (%)	Adults, n=6 N (%)	Total N (%)	p*
Positive	2 (14.3)	1 (16.7)	3 (15)	1.00
Negative	12 (85.7)	5 (83.3)	17 (85)	
Total	14 (100)	6 (100)	20 (100)	

*Fisher's exact test
LHBT: lactulose hydrogen breath test

alence of 29.2% vs. 9.4% in the controls. However, for both the age groups, the differences in the SIBO prevalence were not significant. No studies reported SIBO prevalence in the younger age group for unresponsive CeD. The overall prevalence of SIBO and prevalence in both adults and children in our study are consistent with the prevalence reported by other studies using similar diagnostic methodology. Other factors, such as obesity, may be a risk factor for SIBO. Recent reports have suggested an increasing incidence of obesity in patients with CeD (32, 33); however, none of the patients in this study were obese (Table 2).

Our study has several strengths. We included a sample of both children and adults using a healthy control group for comparison. We used 2 techniques, LHBT and quantitative bacterial culture, to diagnose SIBO.

In conclusion, the prevalence of SIBO was high using LHBT in unresponsive Saudi and Arab children and adults with CeD compared with healthy matched controls. Although SIBO is possible cause for unresponsiveness to GFD in a significant proportion of patients, other potential causes must be excluded. Further studies are needed to evaluate patients' symptoms and responsiveness to a GFD after the treatment of SIBO.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of King Abdulaziz University (Reference No 572-11)

Informed Consent: Informed consent was obtained from all the participants or their guardians.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A.S.; Design - M.A.S., A.J.F., O.I.S.; Supervision - M.A.S., O.I.S.; Resource - M.A.S., O.I.S.; Materials - M.A.S., A.J.F., O.I.S.; Data Collection and/or Processing - M.A.S., A.J.F., O.I.S.; Analysis and/or Interpretation - M.A.S., O.I.S.; Literature Search - M.A.S., O.I.S.; Writing - M.A.S., O.I.S.; Critical Reviews - M.A.S., A.J.F., O.I.S.

Acknowledgements: The authors acknowledge with thanks DSR for technical and financial support.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The project was funded by the Deanship of Scientific Research (DSR) a King Abdulaziz University, Jeddah, under grant no. 411/140/1432.

REFERENCES

1. Fasano A, Catassi C. Celiac Disease. N Eng J Med 2012; 367: 2419-26. [Crossref]
2. Singh P, Arora A, Strand TA, et al. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2018; 16: 823-36. [Crossref]
3. Al-Hussaini A, Troncone R, Khormi M, et al. Mass Screening for Celiac Disease Among School-aged Children: Toward Exploring Celiac Iceberg in Saudi Arabia. J Pediatr Gastroenterol Nutr 2017; 65: 646-51. [Crossref]
4. Aljebreen AM, Almadi MA, Alhammad A, Al Faleh FZ. Seroprevalence of celiac disease among healthy adolescents in Saudi Arabia. World J Gastroenterol 2013; 19: 2374-8. [Crossref]
5. Bures J, Cyrany J, Kohoutova D, et al. Small intestinal bacterial overgrowth syndrome. World J Gastroenterol 2010; 16: 2978-90. [Crossref]
6. Corazza GR, Menozzi MG, Strocchi A, et al. The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing. Gastroenterology 1990; 98: 302-9. [Crossref]
7. Kirsch M. Bacterial overgrowth. Am J Gastroenterol 1990; 85: 231-7.

8. Ghoshal UC. How to interpret hydrogen breath tests. J Neurogastroenterol Motil 2011; 17: 312-7. [Crossref]
9. Triantafyllou K, Pimentel M. Understanding Breath Tests for Small Intestinal Bacterial Overgrowth. Clin Gastroenterol Hepatol 2016; 14: 1362-3. [Crossref]
10. Lasa JS, Zubiaurre I, Fanjul I, Olivera P, Soifer L. Small intestinal bacterial overgrowth prevalence in celiac disease patients is similar in healthy subjects and lower in irritable bowel syndrome patients. Rev Gastroenterol Mex 2015; 80: 171-4. [Crossref]
11. Mooney PD, Evans KE, Sanders DS. Letter: coeliac disease and small intestinal bacterial overgrowth--is dysmotility the missing link? Aliment Pharmacol Ther 2014; 39: 902-3. [Crossref]
12. Rana SV, Sinha SK, Lal S, Sikander A, Singh K. Small intestinal bacterial overgrowth in North Indian patients with celiac disease. Trop Gastroenterol 2007; 28: 159-61.
13. Abdulkarim AS, Burgart LJ, See J, Murray JA. Etiology of nonresponsive celiac disease: results of a systematic approach. Am J Gastroenterol 2002; 97: 2016-21. [Crossref]
14. Chang MS, Minaya MT, Cheng J, Connor BA, Lewis SK, Green PH. Double-blind randomized controlled trial of rifaximin for persistent symptoms in patients with celiac disease. Dig Dis Sci 2011; 56: 2939-46. [Crossref]
15. Dewar DH, Donnelly SC, McLaughlin SD, Johnson MW, Ellis HJ, Ciclitira PJ. Celiac disease: management of persistent symptoms in patients on a gluten-free diet. World J Gastroenterol 2012; 18: 1348-56. [Crossref]
16. Prizont R, Hersh T, Floch MH. Jejunal bacterial flora in chronic small bowel disease. I. Celiac disease. II. Regional enteritis. Am J Clin Nutr 1970; 23: 1602-7. [Crossref]
17. Rubio-Tapia A, Barton SH, Rosenblatt JE, Murray JA. Prevalence of small intestine bacterial overgrowth diagnosed by quantitative culture of intestinal aspirate in celiac disease. J Clin Gastroenterol 2009; 43: 157-61. [Crossref]
18. Tursi A, Brandimarte G, Giorgetti G. High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms after gluten withdrawal. Am J Gastroenterol 2003; 98: 839-43. [Crossref]
19. Thompson DG, O'Brien JD, Hardie JM. Influence of the oropharyngeal microflora on the measurement of exhaled breath hydrogen. Gastroenterology 1986; 91: 853-60. [Crossref]
20. Sharara AI, Aoun E, Abdul-Baki H, Mounzer R, Sidani S, Elhajj I. A randomized double-blind placebo-controlled trial of rifaximin in patients with abdominal bloating and flatulence. Am J Gastroenterol 2006; 101: 326-33. [Crossref]
21. Collee JG MR, Walt B. Tests for the identification of bacteria. In: Mackie and Mccartney Practical Medical Microbiology. (eds. Collee, JG, Fraser, AG, Marmion, BP, Simmons, A.) New York: Churchill Livingstone 1996: 131-49.
22. Anonymous. Processing clinical specimens for anaerobic bacteria: Isolation and identification procedures. In Bailey and Scott's Diagnostic Microbiology. (eds. Baron, EJ, Peterson, LR, Finegold, SM) Philadelphia: Mosby. 1994: 474-503.
23. Marsh MN, Johnson MW, Rostami K. Rebutting Oberhuber- Again. Gastroenterol Hepatol Bed Bench 2015; 8: 303-5.
24. Abdallah H, Leffler D, Dennis M, Kelly CP. Refractory celiac disease. Curr Gastroenterol Rep 2007; 9: 401-5. [Crossref]
25. Gasbarrini A, Lauritano EC, Gabrielli M, et al. Small intestinal bacterial overgrowth: diagnosis and treatment. Dig Dis 2007; 25: 237-40. [Crossref]
26. Parodi A CG, Perri F, Cuoco L, Lauritano EC. H2-breath testing for small-intestinal bacterial overgrowth. Aliment Pharmacol Ther 2009; 29: 18-22.
27. Mattsson J, Minaya MT, Monegro M, et al. Outcome of breath tests in adult patients with suspected small intestinal bacterial overgrowth. Gastroenterol Hepatol Bed Bench 2017; 10: 168-72.
28. Losurdo G, Marra A, Shahini E, et al. Small intestinal bacterial overgrowth and celiac disease: A systematic review with pooled-data analysis. Neurogastroenterol Motil 2017; 29(6). [Crossref]
29. Cinova J, De Palma G, Stepankova R, t al. Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: study in germ-free rats. PLoS One 2011; 6: e16169. [Crossref]
30. Rana SV, Sinha SK, Sikander A, Bhasin DK, Singh K. Study of small intestinal bacterial overgrowth in North Indian patients with irritable bowel syndrome: a case control study. Trop Gastroenterol 2008; 29: 23-5.
31. Ghoshal UC, Ghoshal U, Misra A, Choudhuri G. Partially responsive celiac disease resulting from small intestinal bacterial overgrowth and lactose intolerance. BMC Gastroenterol 2004; 4: 10. [Crossref]
32. Stein AC, Liao C, Paski S, Polonsky T, Semrad CE, Kupfer SS. Obesity and Cardiovascular Risk in Adults With Celiac Disease. J Clin Gastroenterol 2016; 50: 545-50. [Crossref]
33. Ierardi E, Losurdo G, Sorrentino C, et al. Macronutrient intakes in obese subjects with or without small intestinal bacterial overgrowth: an alimentary survey. Scand J Gastroenterol 2016; 51: 277-80. [Crossref]