The prevalence of small intestinal bacterial overgrowth (SIBO) in adult patients with type 1 diabetes and relationship with metabolic control and the presence of chronic complications of the disease

Article ID: doi:10.20452/pamw.3501

ISSN: 1897-9483

Authors: Anna Adamska, Michalina Nowak, Stanisław Piłaciński, Aleksandra Araszkiewicz, Monika Litwinowicz, Małgorzata Tomaszewska, Bogna Wierusz-Wysocka, Marian Grzymisławski, Dorota Zozulińska-Ziółkiewicz

Article type: Original article

Received: June 1, 2016.

Revision accepted: July 29, 2016.

Published online: August 18, 2016.
ORIGINAL ARTICLE

The prevalence of small intestinal bacterial overgrowth (SIBO) in adult patients with type 1 diabetes and relationship with metabolic control and the presence of chronic complications of the disease

Short title: The prevalence of small intestinal bacterial overgrowth (SIBO) in adult patients with type 1 diabetes

Anna Adamska¹, Michalina Nowak², Stanisław Piłaciński¹, Aleksandra Araszkiewicz¹, Monika Litwinowicz², Małgorzata Tomaszewska¹, Bogna Wierusz-Wysocka¹, Marian Grzymisławski², Dorota Zozulińska-Ziółkiewicz¹

¹ Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poznań, Poland
² Department of Internal Medicine, Metabolic and Nutrition, Poznan University of Medical Sciences, Poznań, Poland

Correspondence to: Anna Adamska, MD, Katedra i Klinika Chorób Wewnętrznych i Diabetologii Uniwersytetu Medycznego im. Karola Marcinkowskiego w Poznaniu, Szpital Miejski im. Franciszka Raszei, ul. Adama Mickiewicza 2, 60-834 Poznań, Poland, phone: +48 61 2245 270, e-mail: ania@adamska.info

Received: June 1, 2016.

Revision accepted: July 29, 2016.

Published online:

Conflict of interest: none declared.
Abstract

Introduction  Gastrointestinal symptoms may occur in 50–70% of patients with diabetes. The aim of this study was to evaluate the prevalence of SIBO (small intestinal bacterial overgrowth) in patients with diabetes, as well as the prevalence of SIBO relationship with metabolic control of diabetes and the presence of chronic complications of the disease.

Patients and methods  The study included 148 patients with type 1 diabetes, they are under the care Clinics of Internal Medicine and Diabetology, Poznan University of Medical Sciences in the years 2013–2015. The control group consisted of 41 healthy volunteers. After drinking the test solution (20 g of lactulose suspended in 200 ml of water), hydrogen concentration was measured with a breath-hydrogen analyzer (Gastro+Gastrolyzer, Bedfont Scientific Ltd, UK). Measurements were performed at 15 min.-intervals in the first hour and at 30 min. intervals in the second hour. SIBO was defined as: an elevated fasting H$_2$ ≥ 20 ppm (parts per million) or by the presence of a peak H$_2$ >12 ppm occurring < 60 minutes of the test.

Results  We observed lower prevalence of SIBO in the test group in comparison with controls [56 patients (37.8%) vs. 30 healthy volunteers (73%); $P = 0.006$]. In the logistic regression model this association was independent of age, sex, BMI (body mass index), cigarette smoking, serum CRP (C-reactive protein) concentration and eGFR (estimated glomerular filtration rate) (odds ratio [OR], 0.26; 95% confidence interval [CI], 0.10–0.68; $P = 0.006$).
Conclusions  The prevalence of microbial proliferation in the small intestine in patients with diabetes is lower than in healthy subjects. One of the possible causes might be the beneficial effect of nutritional therapy in patients with diabetes.

Key words

diabetes type 1, hydrogen breath test, intestinal complications, small intestinal bacterial overgrowth (SIBO)
**Introduction** Despite advances in the treatment of diabetes prevention of its chronic complications remains an important clinical problem. It seems that the duration and metabolic control of diabetes has a major impact on the degree of organ involvement. It is believed that in the course of diabetes the gastrointestinal symptoms may occur in 50-70% of patients [1,2]. One of the possible causes is a syndrome of small intestinal bacterial overgrowth (SIBO). This is a heterogeneous syndrome, defined as amount of non-pathogenic bacteria at or exceeding $10^5$ colony forming units (CFUs) per ml of the contents of the small intestine. Value considered as upper normal range is $\leq 10^4$ CFU / ml [3,4]. The syndrome may occur with or without gastrointestinal symptoms, which may include nonspecific abdominal pain, bloating, excessive gas production, diarrhea and weight loss. [5,6] In severe cases the consequences of SIBO may be avitaminosis, fat malabsorption and malnutrition [5]. Stotzer et al proved that patients with SIBO had low bone mineral density, which may be the result of reduced concentration of vitamin D3 (serum 25-hydroxycholecalciferol) and impaired calcium metabolism [7].

The microflora of the human digestive tract is a part of the complex ecosystem, regulated by the defense mechanisms of host, environmental factors and interactions between bacteria. Many factors may interfere with this balance, for instance: anatomical anomalies, motoric disorders (eg. scleroderma and other inflammatory diseases of the connective tissue involving the digestive tract, diabetic autonomic neuropathy, radiation enteropathy, the syndrome of alleged obstruction intestines) and the conditions conducive to bacterial overgrowth: achlorhydia, long-term treatment with proton pump inhibitors or H2-receptor blockers (drugs that inhibit acid secretion are often overused in clinical practice [8]), insufficiency of exocrine pancreas, congenital and acquired immunodeficiency syndromes. The majority of cases involve more than one causative factor [1,3,5]. The diagnosis of SIBO is made on the basis of invasive and non-invasive tests. Invasive tests require aspiration of contents present in the
light of the small intestine. Non-invasive tests, including hydrogen-tests (BT-H2, H2-breath tests) require administration of glucose or lactulose [3,4]. The aim of the study was to investigate the prevalence of SIBO in patients with type 1 diabetes, as well as its association with the metabolic control and the prevalence of diabetic neuropathy.

**Patients and methods** The study included 148 people with type 1 diabetes (DM1) (94 male, 54 female), they are under the care Clinics of Internal Medicine and Diabetology, Poznan University of Medical Sciences in the years 2013–2015. Exclusion criteria were: age <18 years, duration of diabetes <5 years, systemic treatment with antibiotics, proton pump inhibitor or H2-receptor blockers within 30 days before the test, history of comorbidities (inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), celiac disease, endocrinopathies, malignant neoplasms, immune deficits).

The control group consisted of 41 healthy volunteers with normal carbohydrate metabolism (based on medical history). Almost half of them (43.9%) were recruited from the personnel of the hospital and their relatives. The clinical characteristics of the study group and control group are presented in TABLE 1. All participants gave written informed consent to participate in the study, which received approval of local Bioethics Committee.

The study was conducted in the Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences.

**Procedure of data collection** Patients with diabetes and healthy volunteers participating in the study completed a questionnaire containing: demographic data (age, sex), smoking-related data, family record of diabetes and information about other concomitant diseases and taken medication. All subjects underwent a physical examination including anthropometric measurements (weight, height, waist circumference, hip circumference) and blood pressure (twice using a sphygmomanometer in sitting position after 10 min of rest). Participants completed a questionnaire on gastrointestinal symptoms: incidence of flatulence,
excessive amounts of gas, the occurrence of abdominal pain, number of bowel movements per day, the presence of loose stools, fatty stools, as well as information about the loss of body weight, incidence of skin lesions and the duration of the symptoms. Questionnaire for DM1 patients also included data on duration of the disease and method of treatment.

**Laboratory tests** Blood samples were taken on after 10 hours of fasting, after a period of rest, with minimum occlusion of the vein in a Monovette system. Total concentration of cholesterol in serum, the fraction of high density lipoproteins (HDL), low density lipoproteins (LDL), triglyceride (TG), creatinine were measured by standard methods. The estimated glomerular filtration rate (eGFR) was calculated in accordance with the Modification of Diet in Renal Disease Study Group (MDRD). Serum concentrations of C-reactive protein (hsCRP) was determined by a highly sensitivity method. In the DM1 group glycated hemoglobin was also determined (HbA1c) by high performance liquid chromatography, the values of which were calibrated with respect to Diabetes Control and Complications Trial/National Glycohemoglobin Standardisation Program (DCCT/NGSP) and albumin/creatinine ratio taken for analysis from the first sample of urine in the morning. Laboratory tests were performed in a certified laboratory.

**Assessment of neuropathy in DM1 patients** Symptoms of peripheral neuropathy were evaluated on the basis of the medical history. During the standard examination touch sensation was evaluated with the use of 10g monofilament, feeling of vibration of the 128 Hz tuning fork, temperature sensation with TipTherm, pain sensation with neurotips and Achilles tendon reflexes. Diabetic neuropathy was diagnosed in patients with 2 or more of the following 5 elements: occurrence of symptoms, abnormal sensation of touch, vibration and/or temperature, the absence of ankle reflexes. Assessment of cardiac autonomic neuropathy with the use of ProsciCard III programme. Heart rate variability at rest in supine position and under standardized stimuli (deep breathing test, Valsalva maneuver, orthostatic test) was studied.
During the examination the ECG of the subject was monitored on the computer screen. On the basis of the calculated R-R interval parameters of autonomic neuropathy were calculated and subsequently compared with age- and sex-specific standard values. Autonomic neuropathy was diagnosed if the results 2 of 4 tests were abnormal.

**Assessment of SIBO (Small intestinal bacterial overgrowth)**  All subjects were studied after an overnight fast (for 8 hours). They were requested not to smoke 12 hours before and during the test. Patients drank the test solution (20 g of lactulose suspended in 200 ml of water). Hydrogen concentrations were measured with a breath-hydrogen analyzer (Gastro+Gastrolyzer, Bedfont Scientific Ltd, UK). Measurements were taken at 15 min-intervals in the first hour and at 30 min-intervals in the second hour. SIBO was defined as: an elevated fasting H2≥ 20 ppm (parts per million) or by the presence of a peak H2 >12 ppm occurring < 60 minutes of the test.

Figure 1 outlines an example of the test result.

**Statistical analysis**  The results were evaluated statistically with the use of a computer program Statistica 10 (Stat-Soft, Tulsa, Oklahoma, United States). Values were given as medians and interquartile ranges [IQR] or as numbers and percentages [n (%)]. Normality of distribution of quantitative data was evaluated with Shapiro-Wilk test. Since the distribution of the vast majority of variables was skewed, Mann-Whitney test was used in comparative analyses. Comparisons of categorical variables were conducted using an exact Fisher test. In order to simultaneously assess the potential factors associated with the occurrence of SIBO multivariate logistic regression model was used.

**Results**  We found the presence of SIBO in 56 patients with type 1 diabetes (37.8%) and in 30 healthy volunteers (73%), \( P = 0.006 \). In the logistic regression model, presence of diabetes was associated with a lower incidence of SIBO regardless of age, sex, body mass index (BMI), cigarette smoking, hsCRP and eGFR (OR, 0.26; 95% CI, 0.10–0.68; \( P = 0.006 \)).
TABLE 2 displays the comparison of individuals without evidence of SIBO and those with positive test in subjects with diabetes. There was no association between SIBO symptoms and positive hydrogen test, but absence of symptoms does not exclude the presence of SIBO. The intestinal bacterial overgrowth may be asymptomatic.\textsuperscript{[1]}

**Discussion**

Intestinal microflora is a complex and dynamic ecosystem. Qualitative analysis of this ecosystem is challenging, mainly because of the difficulty of the in-vitro studies. Sequencing of the 16S rRNA gene (from amplification of bacterial genetic material from stool samples) allowed for more accurate assessment of the intestinal flora\textsuperscript{[9,10]}. Eckburg et al conducted a study of intestinal microflora and found that 90% are: Bacterioides and Firmicutes\textsuperscript{[11]}. According to research (using molecular biology methods), we know that the same types of bacteria are present in the parts of the intestine, but on the location of other subtypes predominate\textsuperscript{[12]}. SIBO is defined as overgrowth bacterial (increase in the number and changes in the type of bacteria) in the small intestine.\textsuperscript{[1]} The issue of characteristics of microflora not only gastrointestinal, but also skin and the genitourinary, has been raised by international Human Microbiome Project (HMP). One the most important factors influencing the composition of intestinal microflora is diet\textsuperscript{[13]}. Other important factors influencing intestinal microflora include age, sex, genetic and environmental factor \textsuperscript{[14]}. Significant differences in the flora composition were observed between people living in different regions of the world. It is currently believed that the modification intestinal microflora can have a positive flow in the metabolism host. In the Pol Arch Med Wewn, Kasinska and Drzewoski showed in meta-analysis, that supplementation with probiotics was significant on the reduction in HbA1c levels and insulin resistance in patients with type 2 diabetes. To this time, we don’t have study in group patients with type 1 diabetes.\textsuperscript{[15]}

Possible limitation of the work is not including detailed nutritional data e.g using dietary questionnaires. However, this method is difficult and time-consuming for the patients (which
can limit the size of the study group) and subjective. The incidence of SIBO in the general population is unknown. Many authors recognize the direct aspiration duodenal fluid as a “gold standard”[3]. However, the method is invasive and expensive, which limits its wide application. In contrast, the H2-breath test is a non-invasive, easily accessible and acceptable by the subjects.

H2-breath test is based on the concept that part of the gas produced by bacteria fermentation diffuse into the blood and is rapidly excreted by breath, where it can be measured. In patients with diabetes lactulose is more appropriate, as it does not increase glycaemia. The lactulose H2-BT was used by Mendoza et al, who validated this test by comparison with the bacterial cultures from the duodenal aspirate [16]. Reported sensitivity of H2-BT test 85.7% and specificity of 90.9% show a high concordance of these methods. These results indicate the applicability of H2-BT giving lactulose as a screening tool for SIBO[16].

There is little data on SIBO in patients with type 1 diabetes. Faria et al. in group of 28 patients with long-standing (>10 years) type 1 diabetes assessed the orocecal transit time (OCTT) and presence SIBO, in which lactulose H2-breath test was used. Analysis showed that was no statistically association between prolonged OCTT and SIBO[17].

Virally-Monod et al found that SIBO was present in 43% of people with diabetes and therefore should be suspected in the case of chronic diarrhea in patients with diabetes[18]. In our study among patients with type 1 diabetes the prevalence of SIBO was 37.8%.

In the current study, the incidence of SIBO was independent of BMI, which was contrary to the results of survey of obese asymptomatic patients, where SIBO was found in 17% of obese vs 2.5% of non-obese patients[19]. This discrepancy may result from low prevalence of obesity in our study group.

Zieth et al found that SIBO in one-third of patients with diabetes was associated with cardiovascular autonomic neuropathy. Contrarily to this finding, our study did not confirm the
association between diabetic autonomic neuropathy and SIBO (by means of the ProsciCard III program) [20].

Appropriate therapy in patients with SIBO allows permanent or temporary elimination of causes of intestinal complaints. Recommendation on treatment of SIBO are inconclusive. Shah et al. performed a systematic review and meta-analysis comparing the effectiveness of antibiotic therapies in the treatment of patients with SIBO. The most studied antibiotic was rifaximin (8 of 10 studies)[21]. According to Lauritano et al. rifaximin showed higher SIBO decontamination rate than metronidazole at the tested doses (the glucose breath test was reassessed 1 month after) and the prevalence of adverse events was significantly lower in rifaximin with respect to metronidazole[22]. Furnari et al showed that 87% of patients treated with oral antibiotic achieved normalization during hydrogen tract tests [23]. In a pilot study conducted by Khalighi et al. the objective of which was to evaluate the efficiency of probiotic with lactic acid bacteria for the treatment of SIBO demonstrated better response in patients receiving probiotic compared with the control group (negative H2-BT in 93.3% of those receiving probiotic compared with 66.7% in the control group). In all cases, patients receiving the probiotic abdominal pain completely subsided[24].

In available literature, to this time there is not study about treatment recommendations for the group of patients with type 1 diabetes and SIBO than standard recommendations for patients with SIBO. In the future, when we obtain prospective data, we may draw conclusions with clinical implications.

Hydrogen respiratory tests after treatment in a patient population with diabetes and SIBO have not been conducted to date.

**Conclusions**  In diabetic patients, the incidence of SIBO is lower than in healthy subjects. The results necessitate further research to explain observed association.
Acknowledgments  Funding: Poznan University of Medical Sciences
References


<table>
<thead>
<tr>
<th>Rated variables</th>
<th>study group (n = 148)</th>
<th>control group (n = 41)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, years</td>
<td>45 (35–54)</td>
<td>31 (27–39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sex f/m, n</td>
<td>54/94</td>
<td>24/17</td>
<td>0.001</td>
</tr>
<tr>
<td>duration of diabetes, years</td>
<td>20 (13.5–28)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>smoking, n %</td>
<td>36 (24.3)</td>
<td>1 (2.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>hypertension, n %</td>
<td>71 (47.9)</td>
<td>2 (4.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>clinical symptoms, n %</td>
<td>58 (53.7)</td>
<td>28 (68.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>24.8 (22.7–28.4)</td>
<td>22.6 (20.8–24.7)</td>
<td>0.0002</td>
</tr>
<tr>
<td>waist circumference, m</td>
<td>0.9 (0.8–1.0)</td>
<td>0.77 (0.72–0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.7 (7.1–8.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>1.16 (0.56–2.47)</td>
<td>0.65 (0.46–1.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>1.03 (0.76–1.4)</td>
<td>0.91 (0.68–1.17)</td>
<td>0.17</td>
</tr>
<tr>
<td>LDL, ch, mmol/l</td>
<td>2.71 (2.2–3.4)</td>
<td>2.87 (2.38–3.57)</td>
<td>0.28</td>
</tr>
<tr>
<td>HDL, ch, mmol/l</td>
<td>1.8 (1.45–2.1)</td>
<td>1.74 (1.45–1.97)</td>
<td>0.5</td>
</tr>
<tr>
<td>creatinine, mg/dl</td>
<td>0.89 (0.79–1.01)</td>
<td>0.87 (0.81–0.92)</td>
<td>0.23</td>
</tr>
<tr>
<td>eGFR (MDRD), ml/min./m(^2)</td>
<td>115.3 (100.6–139.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>peripheral neuropathy, n %</td>
<td>50 (33.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>autonomic neuropathy, n %</td>
<td>14 (9.5)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR) or number (percentage)

Fisher’s test, \(p\) Mann-Whitney U test

BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycated
hemoglobin; HDL-ch, high-density lipoprotein; hsCRP, C-reactive protein with a highly determined method; LDL-ch, low-density lipoprotein; TG, triglycerides

Table 2 Clinical characteristics of patients with type 1 diabetes with positive and negative SIBO result

<table>
<thead>
<tr>
<th>Rated variables</th>
<th>Negative results (n = 92)</th>
<th>Positive results (n = 56)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, years</td>
<td>45 (35.5–54.5)</td>
<td>44 (34–53)</td>
<td>0.8</td>
</tr>
<tr>
<td>sex f/m, n</td>
<td>33/59</td>
<td>21/35</td>
<td>0.86</td>
</tr>
<tr>
<td>duration of diabetes, years</td>
<td>20 (13.5–29)</td>
<td>20.5 (13.5–27.5)</td>
<td>0.74</td>
</tr>
<tr>
<td>smoking, n %</td>
<td>20 (21.7)</td>
<td>16 (28.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>hypertension, n %</td>
<td>43 (46.7)</td>
<td>28 (50)</td>
<td>0.74</td>
</tr>
<tr>
<td>clinical symptoms, n %</td>
<td>38 (41.3)</td>
<td>20 (35.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9 (22.9–28.6)</td>
<td>24.2 (22.7–27.8)</td>
<td>0.43</td>
</tr>
<tr>
<td>waist circumference, m</td>
<td>0.91 (0.8–1.0)</td>
<td>0.90 (0.76–1.00)</td>
<td>0.61</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.7 (7.1–8.7)</td>
<td>7.7 (7.05–8.8)</td>
<td>0.86</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>1.13 (0.52–2.28)</td>
<td>1.16 (0.66–2.48)</td>
<td>0.45</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>1.03 (0.77–1.4)</td>
<td>1.01 (0.73–1.49)</td>
<td>0.91</td>
</tr>
<tr>
<td>LDL – ch, mmol/l</td>
<td>2.73 (2.3–3.4)</td>
<td>2.69 (2.07–3.26)</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL – ch, mmol/l</td>
<td>1.8 (1.51–2.1)</td>
<td>1.72 (1.36–2.11)</td>
<td>0.39</td>
</tr>
<tr>
<td>creatinine, mg/dl</td>
<td>0.87 (0.79–1.01)</td>
<td>0.91 (0.8–1.02)</td>
<td>0.43</td>
</tr>
<tr>
<td>eGFR (MDRD), ml/min./m²</td>
<td>117.3 (100.8–139.2)</td>
<td>113.5 (100.5–139.7)</td>
<td>0.7</td>
</tr>
<tr>
<td>peripheral neuropathy, n %</td>
<td>32 (34.8)</td>
<td>18 (32.1)</td>
<td>0.74</td>
</tr>
</tbody>
</table>
autonomic neuropathy, n % | 8 (8.7) | 6 (10.7) | 0.66

Data are presented as median (IQR) or number (percentage)

Fisher’s test, p Mann-Whitney U test

Abbreviations: see TABLE 1

FIGURE 1 Example of positive test result (SIBO is present)