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Small intestinal bacterial overgrowth in patients with progressive familial intrahepatic cholestasis

Aleksandra Lisowska[1], Natalia Kobelska-Dubiel[1], Irena Jankowska[2], Joanna Pawłowska[2], Jerzy Moczko[3] and Jarosław Walkowiak[1]✉

[1]Department of Pediatric Gastroenterology and Metabolic Diseases, Poznań University of Medical Sciences, Poznań, Poland; [2]Department of Gastroenterology, Hepatology and Nutrition, Children's Memorial Health Institute, Warszawa, Poland; [3]Department of Computer Science and Statistics, Poznań University of Medical Sciences, Poznań, Poland

Background & Aims: To date, no studies concerning the presence of small intestinal bacterial overgrowth in patients with progressive familial intrahepatic cholestasis were published. Based upon characteristic of progressive familial intrahepatic cholestasis one can expect the coexistence of small intestinal bacterial overgrowth. The aim of the study was to assess the incidence of small intestinal bacterial overgrowth in patients with progressive familial intrahepatic cholestasis. Methods: 26 patients aged 8 to 25 years with progressive familial intrahepatic cholestasis were included in the study. Molecular analysis of *ABCB11* gene was performed in the vast majority of patients. In all patients Z-score for body weight and height, biochemical tests (bilirubin, bile acid concentration, fecal fat excretion) were assessed. In all patients hydrogen-methane breath test was performed. Results: On the basis of first hydrogen-methane breath test, diagnosis of small intestinal bacterial overgrowth was confirmed in 9 patients (35%), 5 patients (19%) had borderline results. The second breath test was performed in 10 patients: in 3 patients results were still positive and 2 patients had a borderline result. The third breath test was conducted in 2 patients and positive results were still observed. Statistical analysis did not reveal any significant correlations between clinical, biochemical and therapeutic parameters in patients with progressive familial intrahepatic cholestasis and coexistence of small intestinal bacterial overgrowth. Conclusions: Our results suggest that small intestinal bacterial overgrowth is frequent in patients with progressive familial intrahepatic cholestasis. Moreover, it seems that this condition has the tendency to persist or recur, despite the treatment.

Key words: small intestinal bacterial overgrowth, progressive familial intrahepatic cholestasis, hydrogen-methane breath test

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INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) is an autosomal recessive disorder, which results in disruption of bile formation and secretion leading to increased serum concentrations of bile acids. The most characteristic pathophysiological and clinical features of PFIC are cholestasis, jaundice, pruritus, hepatomegaly and growth failure (Jacquemin, 2012). Most children in the course of progressive cholestasis develop early (usually before adulthood) fibrosis and end-stage liver disease. On the basis of molecular studies, three types of PFIC have been identified: 2 with low serum γ-glutamyl transpeptidase activity (PFIC-1 and PFIC-2) and 1 with high enzyme activity (PFIC-3) (Jansen & Müller, 1998). Both PFIC-1 and PFIC-2 are associated with impaired bile salt secretion caused by defective genes: *ATP8B1* encoding FIC1 protein and *ABCB11* encoding the bile salt export pump protein (BSEP), respectively (Pawlikowska *et al.,* 2010). PFIC-3 is caused by defective *ABCB4* gene, encoding the multi-drug resistant 3 protein (MDR3), leading to impairment of biliary phospholipid secretion (Jacquemin *et al.,* 2001). Diagnosis of PFIC is based upon typical medical history and clinical manifestation, liver ultrasonography, as well as upon genetic tests. Ursodeoxycholic acid (UDCA) therapy is routinely used in PFIC patients as a first step to prevent liver damage. However, in many PFIC-1 or PFIC-2 patients surgical procedures, such as biliary diversion or ileal bypass, are required to relieve pruritus and to slow down progression of the disease. In most of PFIC patients with liver cirrhosis, liver transplantation is necessary as a life-saving procedure (Jansen & Müller, 1998).

Clinical studies conducted in patients with PFIC-1 and PFIC-2 revealed frequent presence of abnormal digestion and absorption, manifested by steatorrhoea (Walkowiak *et al.,* 2006). In some PFIC patients, in whom surgical procedures are required, complications like postsurgical adhesions are common findings, which — as the coexistent factor in the course of basic disease — can lead to impaired motility of the gastrointestinal tract. At the same time all clinical situations mentioned above can be considered as causative factors leading to development of small intestinal bacterial overgrowth (SIBO).

SIBO is defined as an increase in the number and/or alteration in the type of bacterial flora in the upper gastrointestinal tract. Etiology of SIBO is complex, it includes impairment of protective antimicrobial mechanisms, anatomical anomalies and motility disorders. In the course of SIBO production of unabsorbable and toxic metabolites is present, which can cause mucosal damage and intensify maldigestion and malabsorption. Typical symptoms of SIBO include abdominal pain/discomfort, bloating, flatulence, di-

✉e-mail: jarwalk@am.poznan.pl

Abbreviations: BD, biliary diversion; BSEP, bile salt export pump protein; FIC, progressive familial intrahepatic cholestasis; HMBT, hydrogen-methane breath test; IB, ileal bypass. UDCA, ursodeoxycholic acid; SIBO, small intestinal bacterial overgrowth

Table 1. Clinical. biochemical and genetic data of the studied patients

Sex	ABCB11 gene defect	HMBT first/ second/ third	Age (y)	Body mass, Z–score	Height, Z–score	Treatment	Bilirubin (mg/dl)	Bile acids (μmol/l)	Fecal fat excretion (g/day)
M	–	B/–/–	7	–0.67	–0.77	BD	0.9	1.4	10
M	–	B/B/N	7.11	–0.88	–1.21	BD	0.9	1.5	6.7
F	–	B/P/–	15	–0.41	–1.36	BD	0.6	3.6	9.9
F	–	B/N/–	8.11	–0.25	–1.11	IB/BD	0.9	4.1	0.5
M	c.1445 A>G; p.Asp482Gly. c.1544 A>C; p.Asn515Thr	B/–/–	11.4	–1.99	–3.42	BD	0.9	2.6	2.1
M	c.890 A>G; p.Glu297Gly (HOM)	P/N/–	19.7	0.41	0.56	IB	1.1	405	2.2
F	–	P/N/–	12.11	–1.31	–0.73	UDCA	1.5	21.7	1.1
F	c.1445 A>G; p.Asp482Gly. c.1550 G>A; p.Arg517His	P/P/–	10.9	0.02	–0.79	BD	0.9	4.8	2.4
F	–	P/B/P	18.2	–0.25	–1.06	BD	0.9	8.7	9.7
M	c.1445 A>G; p.Asp482Gly. c.1685 G>A; p.Gly562Asp	P/N/–	16.6	–1.34	–1.75	BD	1.2	73.6	4.1
M	No parental consent for genetic tests	P/N–	11.1	–1.50	–4.07	BD	4.3	364.4	4.3
F	c.2576 C>G; p.Thr859Arg. c.2178+1 G>C; Splice Defect	P/–/–	14.5	–1.09	–1.38	UDCA	1.3	0.5	0.3
M	c.1445 A>G; p.Asp482Gly. c.1544 A>C; p.Asn515Thr	P/–/–	11.4	–0.81	–0.60	BD	0.9	4	1.5
F	c.1445 A>G; p.Asp482Gly. c.890 A>G; p.Glu297Gly	P/P/P	9.8	–1.01	–1.48	BD	0.9	4	4.5
M	c.890 A>G; p.Glu297Gly (HET)	N/–/–	12.5	0.83	–1.14	BD	1.1	2.1	2
M	c.1445 A>G; p.Asp482Gly. c.2494 C>T; p.Arg832Cys	N/–/–	11.9	–1.73	–3.27	BD	0.9	5.6	13
M	c.890 A>G; p.Glu297Gly. c.1643 T>A; p.Phe548Tyr	N/–/–	13.1	–1.13	–1.49	BD	1.4	3.5	70
M	c.1445 A>G; p.Asp482Gly*	N/–/–	8.9	–0.72	0.07	UDCA	0.9	3.1	1.2
M	–	N/–/–	18.5	–1.51	–2.26	IB/BD	0.9	67.8	4.3
M	c.1445 A>G; p.Asp482Gly (HOM)	N/–/–	20.7	–0.90	–0.38	UDCA	0.9	2.4	8.5
M	c.1445 A>G; p.Asp482Gly (HOM)	N/–/–	13.5	–1.15	–1.15	UDCA	0.9	2.4	6.2
F	c.1445 A>G; p.Asp482Gly (HOM)	N/–/–	24.2	–1.68	–2.21	BD	0.9	2.4	2.7
F	c.1445 A>G; p.Asp482Gly. c.3086 C>A; p.Thr1029Lys	N/–/–	12.11	–1.31	–1.71	UDCA	0.9	8.8	8.9
M	–	N/–/–	12.7	0.34	–1.63	UDCA	0.9	7.7	0.3
F	–	N/–/–	15.6	–1.05	0.70	BD	1.7	4.3	3.7
M	c.1445 A>G; p.Asp482Gly. c.1763 C>T; p.Ala588Val	N/–/–	20.5	–2.01	–2.42	BD	2.2	2	8.2

F — female, M — mal, P — positive, N — negative, B — borderline, BD — biliary diversion, UDCA — ursodeoxycholic acid, IB — ileal bypass, HMBT — hydrogen–methane breath test.

arrhoea. Symptoms usually last from few minutes to hours after meal. To assess SIBO a non-invasive hydrogen-methane breath test (HMBT) is used (Singh & Toskes, 2004).

To date, no studies concerning the presence of SIBO in PFIC patients were published. Based upon characteristic of PFIC one can expect the coexistence of SIBO. Therefore, studies assessing the incidence of SIBO and the potential efficacy of the treatment seem to have a reasonable base in patients with PFIC.

The aim of the study was to assess the incidence of SIBO in patients with PFIC-1 and PFIC-2.

PATIENTS AND METHODS

Twenty six children and adolescents (10 females and 16 males) aged 8 to 25 years with the diagnosis of PFIC were included in the study. The patients were non-selected. All PFIC subjects attending outpatient clinic within the period of study were asked for the participation and agreed to take part in it. The study was conducted in the years 2006 to 2007. Investigation protocols were approved by the Bioethical Committees of the Children's Memorial Health Institute in Warsaw and also by Poznan University of Medical Sciences in Poznan. Informed

consent was obtained from all patients included in the study and their parents. The diagnosis of PFIC was established on medical history, clinical manifestation, findings in biochemical tests and genetic studies (Table 1).

Inclusion criteria were: the willingness to participate in the study, confirmed diagnosis of PFIC. Exclusion criteria comprised: use of antibiotic, H2-blocker or proton pump inhibitor (i.v. or per os) six weeks prior to the test, use of systemic steroids, diabetes mellitus.

Molecular analysis of *ABCB11* gene was performed in 16 patients. In 1 child parents didn't consent to the test. The genotypes of the studied patients are presented in table 1.

Among studied patients, 19 were treated surgically: most of them (n=18) underwent biliary diversion (BD), in 2 patients of this group ileal bypass (IB) was performed prior to biliary diversion. Ileal bypass as a sole surgical procedure was performed in 1 patient. In the remaining subjects (n=7) treatment with ursodeoxycholic acid was conducted.

In studied subjects Z-score for body weight and height, biochemical tests including bilirubin and bile acid concentration as well as fecal fat excretion were assessed by standard methods.

SIBO was diagnosed on the basis of glucose HMBT. The test was performed in all patients after overnight fasting. To ensure accurate and reliable results, eating or drinking was not allowed in patients within at least 12 hours before the test. Patients were instructed to exclude from the diet slowly digested foods like beans and similar vegetables, brans or high-fiber cereals on the day before the test. Patients were also asked to avoid vigorous exercises, sleeping and smoking for at least 1 hour before or at any time during the test. Each patient was given water solution of glucose in a dose of 1.5 g/kg up to maximum dose of 75 g. Breath samples were collected at baseline (fasting) and at 15, 30, 45, 60, 90 and 120 minutes after glucose intake. Collected samples were consecutively analyzed with QuinTron MicroLyser DP Plus (Quintron, USA). A pathological HMBT result was defined as a high baseline value (>20 ppm for hydrogen or >10 ppm for methane) and early increase of gas excretion (Δ>12 ppm for hydrogen or >6 ppm for methane).

In all patients with abnormal results of first HMBT standard first-line treatment (metronidazole and a four-week probiotics therapy) was introduced with subsequent HMBT. In those patients, in whom the second HMBT was positive, a second-line treatment with ciprofloxacine and a four-week probiotics therapy was conducted and again HMBT was performed.

In patients with borderline results of both first and second HMBT a monthly therapy with probiotics was recommended.

All calculations were performed using STATISTICA (data analysis software system), version 10.0 StatSoft, Inc. (2010). If not stated otherwise, measures of location are reported as medians [1st-3rd quartiles]. The level of significance was set at $p<0.05$. The difference in distribution of SIBO between groups of patients with different types of treatment (surgical — BD, IB or UDCA) and sex was analyzed by Fisher-Freeman-Halton test. The logistic regression analysis was used to assess the correlation of different therapeutic options, clinical status and the results of biochemical tests with the presence of SIBO in PFIC patients.

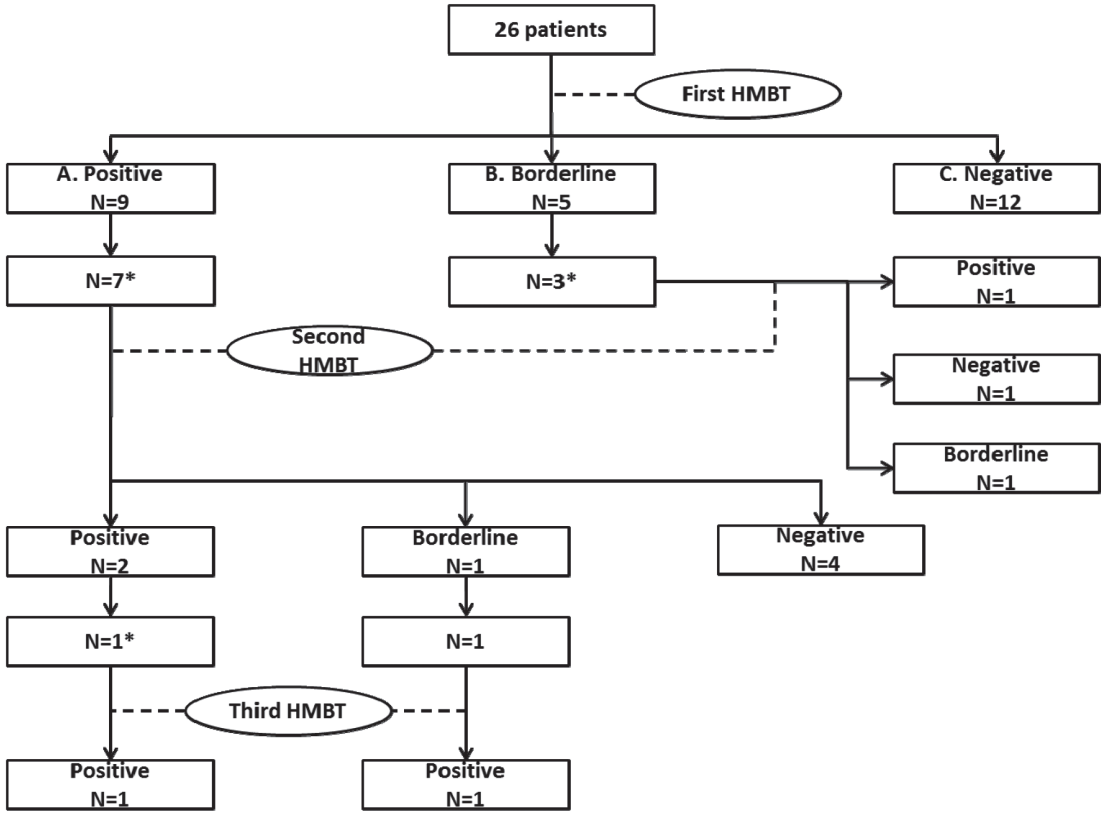

Figure 1. Results of the first, second and third hydrogen-methane breath test (HMBT).
*Five patients studied dropped out of the study (4 for the second and 1 for the third HMBT).

Table 2. Hydrogen-methane breath test (HMBT) results and type of treatment in PFIC patients

HMBT test result	Biliary diversion	Ileal bypass	Ileal bypass/ biliary diversion	Ursodeoxycholic acid
Positive	6	1	–	2
Borderline	4	–	1	–
Negative	6	–	1	5

RESULTS

On the basis of the first HMBT, studied patients were divided into 3 subgroups: A — 9 patients (35%; 5 females and 4 males) with SIBO, B — 5 patients (19%; 2 females and 3 males) with borderline results of HMBT and C — 12 patients (46%; 3 females and 9 males) with normal results of HMBT (Fig. 1).

Among 7 patients from group A (4 females and 3 males), in whom a second HMBT measurement was performed, in 2 patients (females) results typical for SIBO were present and in 1 patient (female) a borderline result was confirmed. In the remaining 4 patients of group A a negative HMBT result was observed. Among 3 patients from group B (2 females and 1 male), who underwent a second HMBT procedure, in 1 patient (female) the diagnosis of SIBO was confirmed, 1 patient (female) had a negative result and in 1 patient (male) a borderline result was still observed. The third HMBT procedure was conducted in 2 patients from group A (2 females). In 1 patient, in whom on the basis of second HMBT procedure SIBO was confirmed, positive results indicating SIBO were still observed. In 1 patient with previously observed borderline results of the second HMBT procedure, third HMBT confirmed the diagnosis of SIBO (Fig. 1). Five patients studied dropped out of the study (4 for the second and 1 for the third HMBT).

In group A, 6 patients underwent biliary diversion (3 females and 3 males), 1 patient (male) underwent ileal bypass and 2 patients (females) were treated with UDCA. In group B, 4 patients (1 female and 3 males) underwent biliary diversion and 1 patient (female) underwent ileal bypass prior to biliary diversion. In group C, 6 patients underwent biliary diversion (2 females and 4 males), 1 patient (male) underwent ileal bypass prior to biliary diversion and 5 patients (1 female and 4 males) were treated with UDCA (Table 2).

Statistical analysis did not reveal important differences in distribution of SIBO between patients with different types of treatment (biliary diversion, ileal bypass, UDCA) and sex. Similarly, statistical analysis did not reveal any relation between biochemical tests results (bilirubin concentration, bile acid concentration and fecal fat excretion) and the coexistence of SIBO in PFIC patients.

DISCUSSION

It is the first study documenting coexistence of PFIC and SIBO. In SIBO positive patients bacterial overgrowth seemed to be persistent. The small size group of studied patients is a major limiting factor. We failed to detect any risk factors predisposing to development of SIBO in PFIC patients. Statistical analysis did not reveal any significant correlations between clinical, biochemical and therapeutic parameters of PFIC patients and coexistence of SIBO. However, its frequent occurrence is an important finding requiring further studies.

In some clinical entities coexisting with SIBO (e.g. cystic fibrosis), methanogenic bacterial flora was proven to be more common (Lisowska et al., 2009). One could presume similar situation in PFIC patients. Therefore, we performed mixed hydrogen – methane breath test in our patients. This test is certainly more advocated than standard hydrogen breath test in PFIC patients.

SIBO is caused by proliferation of enteric flora in the proximal small bowel, thus resembling a healthy colon. There are several medical and surgical risk factors predisposing to SIBO; it often results from conditions that predispose to delayed motility in the small intestine (e.g. scleroderma, autonomic enteropathy in diabetes mellitus, post-radiation enteropathy, small intestinal pseudo-obstruction), conditions that alter the intestinal environment and are associated with disorders of protective antibacterial mechanisms (e.g. pancreatic exocrine insufficiency, immunodeficiency syndromes), functional disorders of the gastrointestinal tract (e.g. irritable bowel syndrome), some endocrionpathies and other causes leading to altered intestinal peristalsis and anatomy e.g. abdominal surgeries (e.g. surgical blind loops, previous ileo-cecal resections, diverticula, fistulae, small intestinal obstruction) (Singh&Toskes, 2004; Bures *et al.,* 2010). Gastric acid suppression caused by prevalent use of proton pump inhibitors and histamine-2-blockers can also contribute to the development of SIBO (Gutierrez *et al.,* 2012).

Moreover, there are some data suggesting a strong interaction between gut and liver named "gut-liver axis". It was proved that beneficial substances produced by the liver are absorbed by the gut. The liver receives approximately 70% of its blood supply from the intestine and thus is exposed to a large number of gut-derived antigens and toxic factors, particularly when gut barrier is impaired. On the other hand, liver pathology may contribute to gut dysfunction. Thus, an important role in the maintenance of gut-liver axis health has been attributed to gut microbiota. When appropriate immune cell regulation and gut barrier function is impaired, gut bacteria may contribute to development of various acute and chronic liver diseases by activating the innate and adaptive immune responses and wound healing processes. It was proved, that the levels of bacterial lipopolysaccharide are increased in the portal and/or systemic circulation in chronic liver diseases. SIBO occurs in a large percentage (20-75%) of patients with chronic liver disease. There are suggestions, that modulation of the gut microbiota may even represent a new way to treat or prevent a variety of liver diseases (Compare et al., 2012).

To date, there are no available data concerning coexistence of SIBO in patients with PFIC. However, based on previous studies, in patients suffering from other types of chronic GI disorders (as mentioned above) one can expect, that also in PFIC patients symptoms of excessive bacterial proliferation and inflammation typical for SIBO may occur. Patients with PFIC are commonly treated using surgical procedures like biliary diversion or ileal bypass, which can alter the intestinal motility and thus interfere with natural

bacterial milieu in the gut, leading to development of SIBO. Similarly, liver function in PFIC patients is at least not fully normal.

Our results suggest that SIBO is frequent in patients with PFIC. Moreover, it seems that this condition has the tendency to persist or recur, despite the treatment.

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