The connection between irritable bowel syndrome and lactose intolerance, and the role of small intestinal bacterial overgrowth: from the diagnosis to the therapy.

Doctoral (Ph.D.) – thesis

2021

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INTRODUCTION

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a common chronic functional gastrointestinal disorder, which can be defined by the Rome IV criteria. It is characterized by abdominal pain related to defecation, and associated with a change in stool frequency or consistency (diarrhea, constipation or a combination of these), without any organic disease and routine histologic examination reveals no mucosal abnormality of the gut-wall. It can lead to significant impairment of quality of life (e.g. social isolation or stigmatization), decreased work productivity, and an increase of health care and societal costs. The incidence of the disease is high in Western countries, affecting 10–20% of the adult population, and it is twice more common among women. The exact pathomechanism remains unclear, but visceral hypersensitivity, altered gastrointestinal motility, changes in gut microbiota, altered brain-gut axis, low-grade digestive tract inflammation, and psychological factors may play a role. Because of the uncertain etiology and pathophysiology, only a few effective, non-specific, multimodal treatment options exist (laxatives, antidiarrheal agents, antispasmodics, antidepressants, dietary, and psychiatric interventions), improving only some key symptoms but not leading to the healing of IBS. Several studies have proven that certain foods worsen the symptoms in most IBS patients because they play an important role in the development of those symptoms. The most commonly reported foods are those containing lactose (milk, ice cream and yogurt) or fructose (honey, dates, oranges, cherries, apples and pears), gas-producing foods (beans, peas, broccoli, cabbage, and bran), wheat and wheat-containing products, and sweeteners (sorbitol, mannitol and xylitol). These findings suggest that dietary intervention that excludes symptom-triggering food components could be a promising treatment option for IBS. A novel treatment option is a diet low in FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols). FODMAPs can trigger symptoms in IBS patients, based on two major mechanisms. The ‘small bowel hypothesis’ states that FODMAPs are unabsorbed, osmotically active molecules (carbohydrates), so they increase the intraluminal water content in the small intestine. This leads to distension, which causes symptoms such as bloating and discomfort. The increased distension also leads to faster oro-cecal transit, which

impairs absorption in the small bowel. The second mechanism ('large bowel hypothesis') describes FODMAPs reaching the colon unabsorbed, where they are rapidly fermented by colonic bacteria. This causes flatulence, bloating, and discomfort through increased gas production and distension of the colonic wall. These findings suggested that the exclusion of FODMAPs from the diet could improve IBS symptoms. A growing number of studies have shown a positive effect of FODMAPs on IBS symptoms. The need has thus arisen for a meta-analysis with a focus on effectiveness in comparison with standard IBS diet to provide evidence and underpin recommendations for wider therapeutic use.

Lactose intolerance

Lactose intolerance (LI) is a clinical syndrome characterized by abdominal symptoms after the ingestion of lactose-containing products caused by lactose maldigestion (LM). The most common cause of primary LM is adult-type hypolactasia. Acquired organic disorders (e.g. small intestinal bacterial overgrowth [SIBO], celiac disease, inflammatory bowel disease [IBD], and infectious enteritis /e.g. giardiasis/), can lead to both downregulation of lactase expression and reduction of absorptive capacity and therefore to secondary lactose malabsorption. Approximately 47% of the Eastern European population is affected, males and females equally. The prevalence of LM increases with age, however, the LI symptoms decrease in the elderly. Because of insufficient lactase activity, lactose can reach the large intestine, where it is fermented by colonic bacteria; gases (H_2, CO_2, and CH_4), short-chain fatty acids, and other products that are formed there. Excessive gas production causes luminal distension and leads to different gastrointestinal symptoms. The most common complaints are abdominal pain and discomfort, bloating, flatulence, and diarrhea as with IBS or SIBO. The diagnostic methods available for LM or LI are based on the lactose breath test (LBT), lactose tolerance test (LTT), genetic test, and assessment of lactase activity in jejunal biopsy specimens, the LBT and LTT being the most popular methods. However, in most studies and at most centers, only one of the last two methods (LBT or LTT) is used, resulting in higher rates of incorrect diagnosis caused by SIBO, for example, which can lead to carbohydrate malabsorption and therefore to false-positive results during the LBT and LTT. Moreover, in some patients with methanogenic microbiota (e.g. Methanobrevibacter smithii), the bacteria convert hydrogen to methane,

leading to false negative LBT results. Due to the potential pathogenetic factors of IBS, food intolerances, such as LI, are more frequent in this disease, however, the prevalence of LM does not differ compared with the healthy population. More IBS patients have symptoms at lower lactose doses and their symptoms are more severe. Moreover, many IBS patients think that their abdominal symptoms are related to lactose intake, even though no objective tests of LM were carried out. Numerous clinical trials are investigating the connection between IBS, LM, and LI, but to our best knowledge, no meta-analyses have been performed up to this day.

Small intestinal bacterial overgrowth

SIBO is a condition in which the small intestine is excessively colonized by aerobic and anaerobic bacteria. Normally, there are fewer than 10^5 bacteria per milliliter (ml) in the duodenal and jejunal part of the small intestine, with ileal counts reaching 10^8 per ml. The prevalence of SIBO is unclear, depending on the population and the diagnostic test used. It is more frequent among the elderly due to reduced gastric acid secretion and medications causing hypomotility. Disorders disturbing mucosal defense mechanisms can predispose one to SIBO, intestinal motility disorders, and chronic pancreatitis being the most common causes. Other etiological factors are motility disorders (diabetes mellitus, IBS, use of narcotics, intestinal pseudo-obstruction, etc.), anatomic disorders (adhesions, strictures, diverticulosis, etc.), immunological disorders (e.g. human immunodeficiency virus [HIV]), metabolic and systemic diseases (e.g. cirrhosis). SIBO causes mucosal damage and altered motility and therefore leads to complex malabsorption (of carbohydrate, fatty acids, proteins, and vitamins), diarrhea, bloating, flatulence, and abdominal discomfort. A diagnosis of this disease can be based on carbohydrate breath tests or an assessment of bacterial concentration from the jejunal aspirate. Although jejunal aspirate culture is the gold standard method, it is not widely used due to its invasiveness, poor reproducibility, possible contamination, and patchy disease localization. Carbohydrate breath tests are simple, non-invasive, inexpensive, and therefore widely used. The treatment comprises the correction of the underlying cause, antibiotic therapy (rifaximine), and nutritional support (e.g. lactose-free diet, vitamin replacement, and correction of nutrient deficiencies). The effectiveness of probiotics is inconclusive, and generally, they are not recommended in SIBO.

AIMS

1. Given the uncertain connection between IBS and lactose consumption-related disorders, we performed a systematic literature search and meta-analysis in this important topic intending to assess the prevalence of:
   a) LM
   b) objective LI, and
   c) subjective LI

   in IBS patients compared to healthy controls (HC).
2. We aimed to:
   a) assess the prevalence of LM and LI in South-West Hungary (Baranya County, except for the Mohács district, with a population of 317,000 people),
   b) show that parallel testing for SIBO could reduce false positive cases determined by LBT and/or LTT, and
   c) investigate the effect of a combined diagnostic method (parallel use of LBT and LTT) compared to standard LBT method in improving diagnostic accuracy.

   A retrospective observational study was performed to answer these questions.
3. Our third goal was to carry out a meta-analysis to prove whether a low-FODMAP diet improves the symptoms of adult IBS patients more effectively than other (standard) dietary interventions (i.e. without the restriction of FODMAP content) recommended by the latest guidelines.

METHODS

Methods for AIM 1

Our work was planned and conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 statement.

Searching strategy

Our systematic literature search was based on the PICO format: Participants: subjects who underwent any form of LM or LI assessment; Intervention: IBS patients; Comparison: HCs; Outcomes: prevalence of LM, subjective/objective LI. The search covered three major

databases (PubMed, Embase, and the Cochrane Library) with the terms '('irritable bowel syndrome' OR 'IBS') AND ('lactose intolerance' OR 'lactose maldigestion' OR 'lactose malabsorption')'.

Eligibility criteria

In our meta-analysis, we included all studies investigating the connection between IBS, lactose consumption-related symptoms, and maldigestion in comparison with the HC group. Short conference abstracts or papers not available in full-text format were excluded. By definition, adult IBS patients had to be diagnosed according to the Rome or, in articles that were not recently published, according to any other well-defined criteria system.

Quality assessment of the individual studies

The quality and the biases of the included studies were analyzed with the Newcastle-Ottawa Scale (NOS) for case-control studies.

Data extraction

At the end of the screening process, relevant data were independently extracted from studies by two independent reviewers (JC and PV). These included: prevalence of LM and LI (subjective or objective) as the outcome parameters, first author, year of publication and country of origin, study design, basic characteristics of the study population (age, percentage of females and IBS subtypes, size of the study groups), diagnostic criteria for IBS, diagnostic methods, thresholds and lactose dose used to diagnose maldigestion.

Outcome measure

The prevalence of LM, subjective and objective LI were the main outcome parameters in our analysis. LM can be diagnosed in different ways, the non-invasive and inexpensive LBT, and LTT being the most common methods. Testing of lactase activity in mucosal biopsy samples from duodenum or jejunum is the gold standard method in the diagnosis of LM, but due to the invasiveness, high costs, and patchy expression of the enzyme it is performed less frequently, compared to the tests mentioned above. The availability of genetic testing of the genes associated with lactase non-persistence (C/T_13910 with CC genotype; G/A_22018 with GG genotype) is variable, its costs are relatively high, and the sensitivity depends on the patients'genetical origin (the different regional mutations are not examined). Participants with

LM who had abdominal symptoms during or shortly after lactose test were defined as objectively lactose intolerant. Participants reporting before any tests, that their symptoms can be in connection with the ingestion of lactose-containing products, were defined as subjectively lactose intolerant.

Statistical analysis

Pooled odds ratios (OR) were calculated with 95% confidence intervals (CI). Random effects and fixed models were applied at all of the analyses with DerSimonian-Laird estimation. Statistical heterogeneity was analyzed using the I^2 and the chi-square test to gain probability-values; $p < 0.1$ was defined to indicate significant heterogeneity. Statistical analyses were performed using the Comprehensive Meta-Analysis Software (CMA). Forest plots were used to present the results of the meta-analyses. To check for publication bias, the visual inspection of funnel plots and Eggers' tests were performed.

Methods for AIM 2

The key points of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guideline were followed in planning and reporting this study. We retrospectively analyzed data from adult symptomatic patients who underwent the LBT and LTT in parallel at our center (Division of Gastroenterology, First Department of Medicine, University of Pécs) between 15 February 2016 and 14 February 2017. The LBT and LTT were carried out with 50 g lactose, H_2 levels were measured with Micro H_2 instrument (Micro Medical Limited, P.O. Box 6. Rochester, Kent ME1 2AZ ENGLAND). Before lactose ingestion, baseline end-alveolar H_2 and blood glucose levels were measured (0 min). Then patients drank the set amount of lactose dissolved in 250 ml water. After this process, end-alveolar H_2 and blood glucose levels were measured every 30 minutes over three hours (in the case of glucose over two hours). Depending on the clinical situation and patients' compliance, in clinically uncertain (but not in all) cases, a lactulose breath test with 10 g lactulose was carried out to prove or reject the diagnosis of SIBO or slow oro-cecal transit. A significant, ≥20 ppm elevation of H_2 level during the LBT and/or less than 1.1 mmol/l rise of blood glucose during the LTT was diagnostic for LM. Patients with negative LBT and LTT are lactose

digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Patients with an early (≤90 min) significant (≥20 ppm) rise of H_2 during the LBT and/or lactulose breath test were determined to have SIBO. We excluded patients with inappropriate preparation for the test (baseline H_2 level >20 ppm) and those with suspected rapid or slow oro-cecal transit (clinical symptoms of gastroparesis and a negative LBT with a positive LTT or no significant rise of H_2 during a 180-min lactulose breath test compared to the baseline value). We collected data on the baseline characteristics of the analyzed population (mean age, gender differences, and their correlation with the outcome measures), the diagnostic tests (baseline and maximum H_2 and glucose levels, time of glucose and H_2 peak, and the presence of LM), the presence and type of symptoms occurring during the test, and the presence of LI and SIBO.

Statistical analysis

Data were analyzed using SPSS 25.0 software. Means, standard deviation, minimum and maximum values, and relative frequency were calculated for descriptive statistics. The Pearson correlation, the Mann–Whitney test, and ORs with 95% CI were used for other analyses. A p-value of less than 0.05 was accepted as statistically significant.

Methods for AIM 3

Search for articles

Our work was planned according to the PRISMA 2009 statement. We used the PICO format to formulate our question (P: patients with IBS; I: low-FODMAP diet; C: high-FODMAP/standard IBS diet; O: IBS Symptom Severity Score [IBS-SSS]). The search covered three databases (PubMed, EMBASE, and the Cochrane Library) with the terms ‘FODMAP AND irritable bowel syndrome’. For better targeting of synonymous phrases, we used the search terms: ‘FODMAP’ OR ‘FODMAPS’ OR ‘Fermentable poorly absorbed short-chain carbohydrates’ OR ‘Fermentable oligosaccharides disaccharides monosaccharides and polyols’ as was done in a recent meta-analysis by Marsh et al.

Study selection

We included randomized controlled trials (RCT), non-randomized controlled trials, and non-controlled prospective trials in our meta-analysis. Retrospective studies were excluded. By

definition, adult IBS patients had to be diagnosed according to the Rome II, Rome III, Rome IV, NICE criteria. As a standard, validated output measure, we searched for studies reporting the IBS-SSS.

Quality assessment of the individual studies

The quality of RCTs was assessed with the frequently used Jadad score, while non-randomized and non-controlled prospective studies were evaluated according to the Methodological Index for Non-Randomized Studies (MINORS).

Data extraction

At the end of the screening process, relevant data were independently extracted from studies by the two reviewers. These included: IBS-SSS as the main outcome parameter, study design, basic characteristics of the study population (age, percentage of females, and IBS subtypes), length of follow-up, diagnostic criteria for IBS, and the size of the low-FODMAP and control (high-FODMAP) groups.

Outcome measure

Irritable bowel syndrome symptom severity score (IBS-SSS)

This score provides a measure of overall IBS severity. It was validated by Francis et al. in 1997 and consists of five questions that measure abdominal pain severity, abdominal pain frequency, abdominal bloating, bowel habit dissatisfaction, and interference with quality of life on a 100 mm VAS. Patients should rate every symptom with a score from 0–100, so the theoretical range is 0–500 mm, with higher scores indicating a more severe disease. A final score of less than 175 indicates mild disease, 175–300 shows moderate severity, and >300 points refer to severe IBS.

Statistical Analysis

Data analysis was conducted with the CMA (Version 3.0, Biostat Inc.). In the forest plot analysis, mean differences with 95% confidence intervals were calculated from studies that contained means, standard deviation (SD) or mean differences, and SD of differences and p-values. The studies we included in the meta-analysis indicated that there is a considerable heterogeneity (different clinical methods, diverse participants, etc.), so the random-effects

model was used according to the DerSimonian and Laird method. Statistically, heterogeneity was tested by Q test ($\chi 2$) and I^2 indicator. The Q test was considered significant when $p < 0.1$. We used subgroup analysis, with a p-value of less than 0.05 indicating a significant difference to compare the differences in the IBS-SSS between the control and low-FODMAP diet groups. The potential for "small study effects", including publication bias, was examined by visual inspection of funnel plots, in which the standard error was plotted against the net change for each study.

RESULTS

Results for AIM 1

Search results

Altogether 14 case-control studies met the inclusion criteria and remained for quantitative analysis. The systematic literature search was based on the PRISMA 2009 guideline. At the time of the literature search, we found no eligible paper that used the most recent diagnostic criteria (Rome IV) for IBS.

Lactose intolerance

Our results showed that subjective LI was more common in IBS compared to HCs, patients reported more often that their abdominal symptoms can be related to lactose-containing products (OR = 3.499; 95% CI: 1.622 – 7.551; p = 0.001). The examined population was significantly heterogeneous (I^2 = 86.774%; p = 0.000).

Significantly more maldigester IBS patients reported abdominal symptoms during or shortly after the diagnostic test compared to controls (OR = 2.521; 95% CI: 1.280 – 4.965; p = 0.008), but our result is limited by the heterogeneity of the analyzed population (I^2 = 74.866%; p = 0.003).

Lactose maldigestion and irritable bowel syndrome

Based on the ingested lactose dose used in the different studies three subgroups were made: (1) 10 g-18 g; (2) 20-25 g; (3) 40-50 g. Overall there was no significant difference in the prevalence of LM between IBS and HC groups (OR = 1.122; 95% CI: 0.929 – 1.356; p = 0.232). The I^2 test showed no significant heterogeneity (I^2 = 0.000%; p = 0.479). We did not find significant

difference either between (p = 0.121), or within the subgroups: (1) OR = 1.420, 95% CI: 0.873 – 2.309, p = 0.158 (I^2 = 0.000%; p = 0.810); (2) OR = 0.926, 95% CI: 0.711 – 1.206, p = 0.568 (I^2 = 11.037%; p = 0.338); (3) OR = 1.356, 95% CI: 0.977 – 1.882, p = 0.068 (I^2 = 0.000%; p = 0.651). There was no significant heterogeneity within the subgroups.

According to the test methods, three subgroups were made: (1) genetic test; (2) LBT, and (3) LTT. Overall, there was no significant difference in the prevalence of LM between IBS patients and HCs (OR = 1.156; 95% CI: 0.985 – 1.356; p = 0.077) and the analyzed studies were homogeneous (I^2 = 0.548%; p = 0.590). We did not find significant difference either between (p = 0.548) or within the subgroups: (1) OR = 1.243, 95% CI: 0.922 – 1.677, p = 0.154 (I^2 = 0.000%; p = 0.664); (2) OR = 1.159, 95% CI: 0.948 – 1.416, p = 0.150 (I^2 = 4.977%; p = 0.396); (3) OR = 0.868, 95% CI: 0.492 – 1.533, p = 0.626 (I^2 = 0.000%; p = 0.561). There was no significant heterogeneity within the subgroups.

Based on the test type and ingested amount of lactose, four subgroups were made: (1) 20-25 g LBT; (2) 40-50 g LBT; (3) 40-50 g LTT and (4) 10 g-18 g LBT. Overall there was no significant difference between IBS and control groups in the prevalence of LM (OR = 1.122; 95% CI: 0.929 – 1.356; p = 0.232) and the analyzed studies were homogeneous (I^2 = 0.000%; p = 0.479). LM was more frequent among IBS patients who underwent LBT with 40-50 g lactose (2) compared to HCs (OR = 1.692; 95% CI: 1.134 – 2.527; p = 0.010; I^2= 0.000%; p = 0.938). Between (p = 0.051) and within the other subgroups there was no significant difference: (1) OR = 0.926, 95% CI: 0.711 – 1.206, p = 0.568 (I^2 = 11.037%; p = 0.338); (3) OR = 0.868, 95% CI: 0.492 – 1.533, p = 0.626 (I^2 = 0.000%; p = 0.561); (4) OR = 1.420, 95% CI: 0.873 – 2.309, p = 0.158 (I^2 = 0.000%; p = 0.479). There was no significant heterogeneity within the subgroups.

Results for AIM 2

A total of 310 patients were assessed in the period noted above. Twenty-four of them were excluded because of inappropriate preparation and 22 (7.6% of the well-prepared patients) were ruled out because of slow oro-cecal transit, leaving 264 patients, 185 females (F: 70.1%), and 79 males (M: 29.9%), for statistical analysis. No patient had rapid transit in our study group. The mean age of the analyzed study group was 40.3 years (F: 40.6 years; M: 39.5 years).

Based on the LBT and/or LTT results, 49.6% (131/264) of the study population had LM (LBT and/or LTT positivity). Seventy-eight (78/131, 59.5%) of them had symptoms after lactose ingestion and were therefore defined as lactose intolerant (78/264, 29.5%). Combined positivity (LBT+LTT) was found in 30.7% (81/264) of the patients. There was no significant difference between females and males in the prevalence of normal lactose digestion, LM, and LI ($p > 0.05$). There was no significant correlation between age and digester ($p = 0.352$), maldigester ($p = 0.352$), and LI ($p = 0.098$) status.

Lactose maldigestion and intolerance based on the lactose breath test

Based on the LBT only, 39.8% of the tested study population (105/264) were LM, and 73 of them (69.5%) had symptoms during the test; therefore, 27.7% (73/264) of the population was defined as lactose intolerant. The majority (159/264, 60.2%) of the patients had a negative LBT, however; 13.8% (22/159) of them had symptoms after lactose ingestion, meaning that 8.3% (22/264) of the analyzed patients had symptoms without a positive test result. There was a weak negative correlation between age and baseline H_2 ($p = 0.009$; $r = -0.161$). There was no significant connection between gender, age, and LBT positivity (gender: $p > 0.05$; age: $p = 0.792$).

Lactose maldigestion and intolerance based on the lactose tolerance test

Based on an analysis of the LTT alone measured in parallel, 40.5% of the same study population (107/264) were maldigesters and 65 of them (60.7%) had symptoms during the test. Therefore, 24.6% (65/264) of the population was defined as lactose intolerant. The majority (157/264, 59.5%) of the patients had a negative LTT; however, 19.1% (30/157) of them had symptoms after lactose ingestion, meaning that 11.4% (30/264) of the analyzed patients had symptoms without a positive test result. Men had a significantly higher baseline ($p < 0.001$) and maximum ($p = 0.015$) glucose level. There was a moderate positive correlation between age and glucose levels (baseline: $p < 0.001$; $r = 0.338$; maximum: $p < 0.001$; $r = 0.222$). There was no significant connection between gender, age, and LTT positivity (gender: $p > 0.05$; age: $p = 0.378$).

Combined lactose breath test and lactose tolerance test positivity

Combined positivity (LBT+LTT) was found in 30.7% (81/264) of the patients, 74% of them (60/81) had symptoms. Therefore, 22.7% (60/264) of the study population was lactose intolerant based on the combined results. In the majority (183/264, 69.3%) of the population, one or both tests were negative; however, 19.1% (35/183) of them had symptoms meaning that 13.3% (35/264) of the analyzed patients had symptoms without combined test positivity.

Clinical symptoms

Thirty-six percent (95/264) of the patients had symptoms after lactose ingestion, bloating being the most frequent (60/264; 22.7%). There was no statistically significant difference between females and males in the presence of symptoms ($p > 0.05$). Those who had nausea/vomiting were significantly older ($p = 0.014$). Otherwise, there was no statistically significant correlation between age and symptoms ($p = 0.204$). 12.8% (17/133) of the lactose digester patients (the LBT and LTT are negative) and 59.5% (78/131) of the maldigester patients (at least one of the tests is positive) had clinical symptoms. Based on the latest meta-analysis conducted by our workgroup, we hypothesize that IBS may be a contributing factor in LI among lactose maldigesters.

The role of small intestinal bacterial overgrowth

Approximately one-third (92/264; 34.8%) of the study population and 60% (57/95) of the symptomatic patients had SIBO based on the definition. There was no significant difference in the presence of SIBO between females and males (F: 68/185, 36.8%; M: 24/79, 30.4%, $p > 0.05$); furthermore, there was no significant correlation between age and SIBO ($p = 0.848$). SIBO patients had significantly higher maximum H_2 levels ($p < 0.001$), and they reached the H_2 peak later ($p < 0.001$). Moreover, they had lower maximum glucose levels ($p < 0.001$), and LTT positivity was significantly more frequent in this patient group (OR = 5.833; 95% CI: 3.356–10.138). Symptoms were more common in SIBO patients compared to non-SIBO patients (OR = 5.743; 95% CI: 3.300–9.994), especially abdominal discomfort (OR = 3.201; 95% CI: 1.196–8.565), bloating (OR = 4.798; 95% CI: 2.606–8.833), diarrhea (OR = 6.443; 95% CI: 2.737–15.168), and other symptoms (OR = 5.825; 95% CI: 2.193–15.469). Other

symptoms comprise increased bowel motility, flatulence, belching, sensation of fullness in the stomach, headache, burning sensation in the stomach, or increased sensation for defecation.

In 90.9% (240/264) of the patients, the LBT gave correct diagnosis (30.7% true positive: 81/264, 60.2% true negative: 159/264) of LM (or the lack of it) using combined LBT and LTT as reference. False-positive results were found in 9.1% (24/264) of the cases; however, there are no false negatives in this setting. LBT in this setting has 100% sensitivity, 86.9% specificity, 77.1% positive predictive value, and 100% negative predictive value. SIBO was found in 76.5% (62/81) of the true positive and 75% (18/24) of the false-positive patients.

In 90.2% (238/264) of the patients, the LTT gave correct diagnosis (30.7% true positive: 81/264, 59.5% true negative: 157/264) of LM (or the lack of it) using combined LBT and LTT as reference. False-positive results were found in 9.8% (26/264) of the cases; however, there are no false negatives in this setting. Therefore, LTT has 100% sensitivity, 85.8% specificity, 75.7% positive predictive value, and 100% negative predictive value. SIBO was found in 76.5% (62/81) of the true positive, but surprisingly in 0% (0/26) of the false-positive patients.

Based on these findings the combination of the LBT and LTT and the careful monitoring of results (e.g. early H_2 rise, parallel performed lactulose breath test) can decrease false results caused by e.g. SIBO.

Results for AIM 3

Searching results

After the searching and screening process, ten articles reporting on IBS-SSS eligible for further evaluation were found. Of these studies, six were available in full-text format, and four were short abstracts or supplements. At the time of the literature search, we found no eligible paper that used the most recent diagnostic criteria (Rome IV) for IBS.

Irritable bowel syndrome symptom severity score

First, we wanted to see if a low-FODMAP diet is an effective treatment for IBS. We compared the pre- vs. post-intervention IBS-SSS in control groups (four publications) and low-FODMAP groups (eight publications). There was a significant reduction in IBS-SSS in both control (difference in means [DIM], post- minus pre-values: -59.816 (95% CI: -108.922 – -10.710); p

= 0.017) and low-FODMAP groups (DIM: -105.339 (95% CI: -140.773 – -69.905); p = 0.000). This means that both standard (high-FODMAP) and low-FODMAP diets are effective in improving symptoms and quality of life among IBS patients. The forest plot suggests that a low-FODMAP diet is more effective, but we cannot prove this statistically because of the overlapping CIs. Significant heterogeneity was found between the studies: control group IBS-SSS values: Q = 9.837; df = 3; p = 0.02; I^2 = 69.504%; low-FODMAP group IBS-SSS values: Q = 26.321; df = 7; p < 0.001; I^2 = 73.405%.

We compared the pre- and post-intervention scores between the control and low-FODMAP groups in the controlled trials (six publications for each group). This shows that there is no statistically significant difference in pre-values between the groups (DIM: control minus low-FODMAP values: -8.675 (95% CI: -40.043 – +22.693); p = 0.588), but a significant difference between post-values (DIM: +51.537 (95% CI: +18.891 – +84.183); p = 0.002) could be observed. These results confirm that the therapeutic effect of a low-FODMAP diet is better than standard dietary advice in patients with IBS. The meta-analysis also showed a significant heterogeneity: pre-IBS-SSS values: Q = 21.242; df = 5; p = 0.001; I^2 = 76.462; post-IBS-SSS values: Q = 20.675; df = 5; p = 0.001; I^2 = 75.816.

DISCUSSION

The connection between irritable bowel syndrome and lactose consumption-related disorders (meta-analysis)

A growing number of studies have shown that intolerance to lactose-containing products and other food types is more frequent among patients with IBS than among healthy subjects, but to our best knowledge, no meta-analysis investigated the association between these two conditions so far. Only two recent reviews by Borghini and Bayless et al. discuss the correlation between IBS and LI. The underlying mechanism remains unknown, but common etiological factors like psychological (e.g., anxiety) and gastrointestinal dysfunctions (e.g., visceral hypersensitivity and altered gut transit) might play a role. The visceral hypersensitivity can also be in connection with altered gut microbiome. Gut microbiota of IBS patients is generally reduced and has lower diversity, compared to HCs. It has been shown that potentially pathogenic bacteria (e.g.

Clostridium spp., Ruminococcus spp., Streptococcus spp., Enterobacteriaceae members) are more concentrated in IBS patients than in controls. A recent MRI (magnetic resonance imaging) study concluded that visceral hypersensitivity, rather than excessive gas production is responsible for carbohydrate associated symptoms in patients with IBS. The hypersensitivity to colonic distension can be transferred to mice by fecal transplantation which highlights the role of the microbiome. Moreover, gut microbiota produces many neuroactive or neuromodulatory metabolites (histamine, serotonin, gamma-aminobutyric acid, brain-derived neurotrophic factor, etc.), which can potentially lead to peripheral or central neural sensitization.

The strength of our study is that standardized, well-defined, rigorous outcome measures were used to assess the role of lactose consumption-related disorders in IBS patients, and a sufficient number of articles were found to carry out a detailed statistical analysis. Only full-text papers were enrolled, where IBS patients with appropriate control groups were present. Our meta-analysis is the first to provide evidence for the connection between IBS and LI and our former data suggests that a lactose-free or lactose-restricted diet (low-FODMAP) in the treatment of IBS could improve the therapeutic effect on IBS symptoms and might decrease healthcare-related and societal costs.

There are some limitations to our study. Firstly, we focused on the prevalence of LM and subjective/objective LI, and due to the lack of detailed, uniform, controlled, published data, we could not perform a statistical analysis of individual symptoms. Uniform, consensus-based, well-comparable measurement of symptom severity, for example, VAS is suggested for use in future studies. Because of the same reasons, we could not analyze the role of lactose-restricted diet or lactase replacement in this patient group; therefore, a network meta-analysis could be a useful future perspective to establish which treatment is better in IBS. Secondly, because of the lack of data in the different IBS subtypes, it is not clear which subgroup is mostly affected by LI. Moreover, the diagnostic criteria for IBS and the diagnostic thresholds of LBT and LTT were different in some studies which could influence the results. The sensitivity and specificity of these noninvasive tests are relatively high, however, false positive or negative results could affect our findings. It should be taken into account that similar activity of lactase in two persons might result in different LBT results due to the different activity and composition of the

intestinal microbiota and the lactase non-persistence allele is not always associated with LM. Another difficulty is that it is hard to identify the food, responsible for the symptoms. The correlation between self-reported and objective LI increases with the ingested lactose dose. Finally, we found significant heterogeneity in the analysis of the subjective and objective LI. We could not perform subgroup analysis with different amounts of lactose in LI, however, it can influence the frequency and severity of the abdominal symptoms and therefore the prevalence of objective LI, as presented by Yang et al.

The role of small intestinal bacterial overgrowth and false-positive diagnosis of lactose intolerance (retrospective observational study)

LI is a relatively common problem in the white population, affecting approximately 47% of Eastern European adults. There are widely-used, inexpensive, non-invasive, diagnostic methods based on the measurement of end-alveolar H_2 concentration (LBT) or blood glucose (LTT). The sensitivity and specificity of these tests are relatively high, but they depend on the ingested lactose dose (25 g LBT: 82% and 95%; 25 g LTT: 78% and 93%; 50 g LBT: 92% and 83%; 50 g LTT: 94% and 90%). Other circumstances, such as SIBO, antibiotic usage, lung diseases, inappropriate preparation, and abnormal gastric emptying can influence their diagnostic accuracy. A combination of these tests and careful evaluation of the results can reduce the false positive or negative cases; however, due to the lack of evidences, in most studies, they are used separately.

The gold standard diagnostic method is the testing of lactase activity in duodenal and jejunal biopsy samples taken from the mucosa. However, due to the invasiveness, high costs, and patchy enzyme expression, it is less frequently performed compared to the tests noted above. Moreover, it should be considered that similar lactase activity in two patients might result in different LBT results due to the different activity and composition of the intestinal microbiota. There are several genes associated with lactase non-persistence (C/T_13910 with CC genotype; G/A_22018 with GG genotype), but the availability of genetic testing is variable, and its costs are relatively high. Moreover, the lactase non-persistence allele is not always associated with LM. A Hungarian study, published by Nagy et al., determined the applicability of the LBT in comparison with genetic screening (C/T_13910). They found that 37% of the analyzed

population had lactase non-persistence, which correlated well with positive LBT results in symptomatic children. We found similar LBT positivity among symptomatic adults. Another retrospective study from Hungary, conducted by Buzás et al., also underlined that both genetic and breath tests are sufficiently accurate.

It should be mentioned that in our study the population had a very large female representation (185 vs 79); however, there were no statistically significant gender-related differences regarding LM, LI, LBT/LTT positivity, symptoms frequency, and prevalence of SIBO, which underlines the literature data in case of LI. Moreover, despite the literature data, we did not find any age-related correlations in the outcomes mentioned above.

The limitations of our results should be considered for a correct interpretation, thus possibly influencing outcomes. Firstly, our results are based on a single-center retrospective medical database analysis. Secondly, we only analyzed the results in one year; therefore, the number of enrolled patients is relatively low. Thirdly, the amount of ingested lactose can influence the prevalence of LM and LI, and the frequency of symptoms. We used a relatively high dose of lactose and we did not perform blinded testing with placebo. Based on the retrospective character, follow-up after antibiotic treatment or low-lactose diet could not be performed to confirm the diagnosis of SIBO and LI based on symptom relief. Moreover, the lactulose breath test was performed only in clinically uncertain cases, not on all patients. Therefore, the true diagnosis and prevalence of LI and SIBO could not be assessed correctly. Only patients with high initial end-alveolar H_2 concentration got antiseptic mouthwash. Another significant limitation is that our study group comprises symptomatic patients referred to our clinic, thus potentially leading to sampling bias. It also should be considered that we did not measure methane levels in the end-alveolar gas samples to determine false-negative LBT caused by methane-producing bacteria. Based on the recent results false-negative LBT (5-15%) is mainly caused by methane production. Finally, the symptoms of the patients are subjective, thus possibly prompting inaccurate conclusions. Interpretation of patient-reported symptoms will differ between clinicians; therefore, standardized symptom definitions should have been used to minimize errors. According to the Oxford Centre for Evidence-Based Medicine 2011, the evidence level of our findings is level 3.

The role of low Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols diet (meta-analysis)

The standard dietary approach for IBS dietary therapy (high-fiber, low-fat, etc., as detailed above) recommended by guidelines only improves IBS symptoms to a limited extent. A growing number of recent studies have shown a beneficial effect of a low-FODMAP diet on IBS symptoms. Several of them have compared its efficacy to a standard IBS diet and challenged us to review the latest literature on the issue. A recent meta-analysis by Marsh et al. analyzed the beneficial effect of a low-FODMAP diet on symptoms and quality of life in adult and pediatric patients with IBS and IBD in the literature up to 24 March 2015. They only investigated the complex IBS-SSS only in four articles, and it was not stated whether the low-FODMAP diet is significantly better than a control diet or not. We carried out our analysis on IBS-SSS, using more (ten) articles, and we only focused on adult patients with IBS. A previous meta-analysis by Khan et al. and systematic review by Rao et al. also proved the efficacy of this diet on symptom improvement and suggested its introduction as a baseline treatment, but they could not state clearly whether it is better than standard dietary advice or not. Rao et al. also investigated the high-fiber diet on chronic constipation and IBS. They performed a literature search up to September 2014 and did not conduct a statistical analysis due to heterogeneity and methodological quality. To our knowledge, this is the first meta-analysis to compare the effectiveness of low-FODMAP foods to a regular IBS diet recommended by the guidelines.

FODMAPs are poorly absorbed carbohydrates that cause an increase of water content in the bowel based on the osmotic effect and increased gas production by colonic bacterial flora. These effects of FODMAPs induce several symptoms in patients with IBS and numerous patients with functional gastrointestinal disorders mainly by distension and the osmotic laxative effect.

Because of the considerable heterogeneity of the expressed data, the random-effects model was used with the DerSimonian and Laird method for analysis. This is possible because of the similar effect of non-investigated variables such as food intolerances and functional digestive tract disorders other than IBS that could cause IBS-like symptoms as well. It would be important

to study the effect of a low-FODMAP diet in these groups to better understand the role of a food challenge in provoking uncompliant symptoms of functional digestive tract disorders, as in IBS. Nevertheless, the beneficial effects of a low-FODMAP diet on IBS-SSS were statistically significant even in the heterogeneous population analyzed, thus supporting the high impact of this diet on IBS symptoms. Study data reflected some publication bias based on heterogeneity.

The strength of our study is that a standardized complex outcome score (IBS-SSS) was used to measure the therapeutic effect. A sufficient number of articles were found to carry out an accurate statistical analysis, using this important outcome score. With this work, we proved not only the positive effect of a low-FODMAP diet on IBS-SSS, but also its superiority to a high-FODMAP standard IBS diet.

There are some limitations to our study. First, we focused on the complex IBS-SSS, and due to the lack of detailed published data, we did not perform a statistical analysis of the individual symptoms in the symptom score. Therefore, it is not clear which of the five elements play a key role in the improvement of IBS symptom severity toward better personalization of this dietary approach. The main reason was the lack of data and control groups, as well as the heterogeneity in the literature in measuring symptom severity (e.g. VAS and different types of Likert scale). A uniform, consensus-based, well-comparable measurement of symptom severity (e.g. IBS-SSS) is suggested for use in future studies. Second, we included not only full-text articles, but also four short supplements in our analysis, thus increasing the quantity of data on control groups. Third, because of the lack of data in the different IBS subtypes, it is not clear which subgroup experienced the greatest symptom improvement. Finally, the standard IBS diet group was not homogeneous. The control diet always contained a significant number of FODMAPs; however, only two out of ten studies detailed exact food contents. Others probably used IBS dietary guidelines; thereafter, some differences were realized between contents, thus potentially influencing our results.

CONCLUSIONS

I. Our meta-analysis is the first to confirm that:

a) **LM has the same prevalence, but**

b) **objective LI and**

c) **subjective LI**

are more common in IBS patients compared to the healthy population.

Based on these findings and literature data, **IBS can be a contributing factor of LI among people with LM**. Further studies are needed to determine whether a confirmed diagnosis of IBS is an etiological factor in determining whether LM patients present with LI.

II. Based on our results, we can conclude that:

a) **the prevalence of LI is lower in South-West Hungary compared to the Eastern European values** (29.5% vs 47%) and that is worth to perform a population-based prospective analysis in this area. During the provocation tests, **59.5% of lactose maldigesters had IBS-like symptoms (LI)**, but the role of IBS in the background is unknown.

b) **SIBO was relatively common among symptomatic patients (60%)**, **which may significantly influence the H_2 breath test based diagnostic accuracy of LM.**

c) **To use a combination of LBT and LTT testing can be a reasonable alternative of H_2 lactose- and H_2 lactulose breath test combination to exclude false positivity (caused by e.g. SIBO)**, that approach needs further validation.

III. Our meta-analysis confirms that **a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) significantly improves general symptoms and quality of life in patients with IBS**. Our analysis of the appropriate literature data also confirms that **a low-FODMAP diet is more effective than standard IBS dietary therapy** in patients diagnosed with IBS. However, a low-FODMAP diet raises certain issues, such as the alteration of gut microbiota and inadequate nutrient intake without dietitian assistance. The possible health advantages of a low-FODMAP diet – when it is effective – compared to medical treatment require further evaluation. In consideration of its possible

limitations and based on findings from this meta-analysis, **a low-FODMAP diet could be a potential first-line and supplementary dietary therapeutic approach with the aid of a dietitian for patients with IBS to improve abdominal discomfort, abdominal pain, bloating and quality of life.** Because of the lack of published data, it is not possible to prove the effectiveness of a low-FODMAP diet on bowel movement frequency in IBS patients. It also remains unclear which IBS subgroup could profit most from this diet. More RCTs are needed to analyze these effects of dietary approaches.

PUBLICATIONS AND CITATIONS

First author

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Summarized IF: 9.909

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Summarized IF: 51,568

Summarized IF from all of the publications: 61,477

Citations: 238 (independent citations: 202)
Hirsch-index: 10