Title: High Prevalence of Small Intestinal Bacterial Overgrowth in Lactose Intolerance Patients: is it a chicken and egg situation?

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Introduction

Lactose Intolerance is the most common intestinal disorder that is associated with the absence or drastically reduced level of intestinal lactase (1,2). Two types of Lactase deficiency are known: a primary inherited hypolactasia, characterized by an autosomal recessive trait (3) and a secondary hypolactasia, an acquired disorder associated with various intestinal diseases (4). In this group, celiac disease, Crohn's disease, acute gastroenteritis and eosinophil gastroenteritis are the most common. Lactose Intolerance is a common condition in European countries (25%), with a low

incidence in Northern Europe and a much higher incidence in the Mediterranean area. Although it cannot be considered a real disease, being an environmental enzymatic adaptation, it may cause considerable clinical annoyances, eventually leading to malabsorption and social disabilities, with impairment of quality of life. Moreover, a substantial portion of patients treated by strict lactose-free diet remains symptomatic or show only partial improvement. It has been concluded, from previous studies, that lactose malabsorption plays a definite but minor role in the etiology of irritable bowel syndrome (IBS) (5, 6, 7, 8). Lactose intolerance refers to gastrointestinal symptoms including pain and diarrhea, related to fermentation of undigested lactose in the gastrointestinal tract, usually in the colon. However, the ingestion of lactose by an individual with lactose malabsorption does not always result in lactose intolerance . We noted that some of these patients presented with concomitant small intestinal bacterial overgrowth (SIBO). The gold standard for diagnosis of hypolactasia is the measurement of lactase activity in intestinal biopsies (9). Genotyping characterization is reported to perfectly correlate with the level of lactase activity in intestinal biopsy samples: genotype C/C-13910 is associated with adult-type hypolactasia; genotypes C/T-139 10 and T/T-13910 are associated with lactase persistence (10, 11). Since these techniques are invasive and relatively expensive, therefore not suitable for screening, the diagnosis of hypolactasia is currently based on the lactose hydrogen breath test (LHBT), a reliable, non-invasive, simple and repeatable test (12, 13). The aim of this population-based study in patients with chronic abdominal complaints is to verify: 1) the prevalence of lactose intolerance; 2) the ability of LHBT to diagnose SIBO alongside lactose malabsorption; 3) the frequency of association between SIBO and lactose intolerance; 4) the role of treatment with rifaximin.

Materials and Methods

From November 2011 to July 2012, 500 consecutive patients referred for abdominal complaints to the Gastroenterology Unit of Mauriziano U.1° Hospital, Turin, Italy, were enrolled in this study. The study was performed in accordance with the Helsinki declaration, approved by the Ethic Committee. All the patients have given informed consent to the study. All the patients fitted in the diagnosis of IBS, mostly of diarrhea variety, with a minority (20%) being of constipation variety, according to Rome III diagnostic criteria. Patients who used antibiotics in the last 6 months,

currently using laxatives or eukinetics, or who have been submitted to colonoscopy or barium enema in the last month before test were excluded. Neoplasia, malabsorption diseases, previous gastrointestinal surgery and metabolic/hormonal disturbances were also exclusion criteria. Malabsorption was excluded on the ground of personal and family history, clinical examination, current biochemical tests (vitamins A,D,E,K, B_{12}, abtTG), endoscopy and imaging procedures. All the patients underwent hematological routine tests for liver, kidney and thyroid function, faecal microbiology and LHBT. An upper gastrointestinal endoscopy was performed within 2 years of the study. The first series of one hundred consecutive patients, whose LHBT peaked at the 120^{th} minute or earlier, underwent a subsequent glucose hydrogen breath test (GHBT) one week later. Fifty asymptomatic subjects, comparable for age and genre, in absence of clinical history of chronic and acute diseases, were used as control. In consideration of the complete absence of symptoms, both past and present, this relatively small group of healthy subjects seems to us sufficient for a comparison with patients.

Gastrointestinal Symptoms

At the entry of the study, soon after treatment with rifaximin and 3 months after the completion of treatment, patients were asked to grade the intensity of abdominal symptoms on a Visual Analogue Scale (VAS). The VAS consisted on a line of 10 cm long with 0 cm indicating “no sensation” and 10 cm indicating “the strongest sensation ever felt”. Symptoms recorded were epigastric pain and burning, post-prandial fullness, bloating, belching, nausea, early satiety, constipation and diarrhea.

Physical Examination and Safety Parameters

Each patient underwent a thorough physical examination. Peripheral blood cell count within the last 3 months and any variation in body weight in the last 6 months were recorded at the entry of the study. Any new symptom during rifaximin treatment was also recorded.

Lactose Malabsorption Evaluation

Each subject was submitted to LHBT, by means of Breath Tracker digital microlyzer (Quin Tron Instrument Company, Milwaukee, WI 53215, USA), after low carbohydrate diet, an overnight fasting and chlorhexidine mouthwash. Breath samples were collected before oral administration of 20 g of lactose in 250 mL of water and after every 30 minutes for 240 minutes. This dosage was used as it is nearer to the daily intake of the Italian population (FAO statistics, 1981). The accuracy of the instrument was ± 1%; the sensor sensitivity was ±1 ppm, with correction factor for CO_2. The test was considered positive for lactose malabsorption when an increase of H_2 expirate over the baseline level was > 20 ppm. An increase >12 ppm at 120^{th} minute or earlier was considered suspected for SIBO and the patient was reassessed by GHBT one week after. Patients with early positivity of LHBT were considered lactose malabsorbers only if subsequent values of H_2 expirate over baseline level were >20 ppm, at least 2 times after 120 minutes. An increase of CH_4 >15 over the baseline level was also considered diagnostic for lactose malabsorption.

SIBO Evaluation

The first one hundred subjects suspected to have SIBO based on LHBT results were submitted to GHBT according to the procedure described elsewhere (14). Breath samples were collected before administration of 50 g of glucose in 250 mL of water and after every 15 minutes for 120 minutes. The test was considered positive and diagnostic for SIBO when the increase of H_2 expirate over the baseline level was >12 ppm.

SIBO Eradication

Half of the patients affected by SIBO were randomly treated with rifaximin 400 mg 3 times per day for 2 wks, on an open label, 1 to 1 basis, the remaining patients being treated only with a lactose-free diet. GHBT was re-assessed 6 months after the completion of treatment and symptoms were simultaneously recorded. Symptom improvement was defined as a reduction of at least 75% over the value at the entry of the study.

Statistics

Statistical analysis of data was carried out by SPSS software, version 12 for Windows (SPSS Inc., Chicago, IL). For quantitative variables the Mann-Whitney test was used. The X^2 test with Yates correction was performed to evaluate SIBO prevalence (difference between groups).

Results

Demographics

From November 2011 to July 2012 500 patients underwent LHBT. The first one hundred consecutive patients whose LHBT sowed a peak of at least 12 ppm over the baseline level at 120th minute or earlier underwent GHBT as well, in order to validate SIBO diagnosis. Three hundred and sixty were female (mean age 45±23 years, range 18-78), 140 were male (mean age 46±26, range 18-79). Fifty asymptomatic subjects, in absence of clinical history of chronic and acute diseases, recruited from January 2010 to July 2012, comparable for genre and age (30 female; mean age 43±24, range 18-75 years), were used as control.

Prevalence of Lactose Malabsorption

Two hundred ninety five patients (59%) tested positive for lactose malabsorption. No difference between males (210/360 =58%) and females (85/140=60%) was noted. Three out of 50 controls (2 females) tested positive at LHBT (6%). The difference between patients and controls was statistically significant (*P*<.001).

Comparison between LHBT and GHBT

The first 100 patients whose LHBT showed a value ≥12 ppm over the baseline level at 120th minute or earlier were suspected of having SIBO and underwent GHBT. All these patients but two showed a positive result for SIBO at GHBT, with a concordance of 98%. The negative patients showed a value of 10 over the baseline level, with only a trend toward positivity. Respectively 89 % and 92% of LHBT and GHBT peaked at 90th minute, with a value of at least 12 ppm over the baseline level. A significant association was found between the time at which H2 excretion peaked (at least 12 ppm over the baseline level) (r=0.662; *P*<.001), maximum H2 concentration measured (r = 0.634*; P*<.001) and overall H_2 excretion over the first 120 minutes (r = 0.668, *P*<.001) during the lactose and glucose HBTs.

Co-presence of SIBO and Lactose Intolerance

Comment [LL1]:

Based on above results we considered positive for SIBO any subsequent LHBT showing a value of at least 12 ppm over the baseline level at 120th minute or earlier. With these criteria, 72% of the patients affected by lactose intolerance resulted to be also affected by SIBO (213/295). No difference was registered between males and females. The mean age of these patients was 54±14 years. Only 28% of patients with lactose intolerance was SIBO-negative (83/295), with a mean age of 35±19 years ($p<0.05$). None of the 3 subjects in the control group who tested positive for lactose malabsorption at LHBT resulted positive for SIBO. On the other hand, SIBO was registered in 3.4% of Lactose Intolerance –negative patients (7/205; mean age 36±15 years). One hundred ninety eight patients resulted negative for Lactose Intolerance and SIBO (39.6%; mean age 42±20 years).

SIBO Eradication

Patients affected simultaneously by Lactose Intolerance and SIBO were divided into 2 groups: group A (106 patients, mean age 53±15, 40 males) was treated with lactose free diet + rifaximin 1200 mg/day for 2 weeks; group B (107 patients, mean age 55±16 years, 45 males) was treated only with lactose-free diet. After 6 months, all the 213 patients underwent GHBT and symptoms reassessment: 96% of patients treated with lactose-free diet and rifaximin (group A) and 1.8% of patients treated only with lactose free diet (group B) showed negative GHBT ($p<0.001$). In the A group all the patients but two (104/106: 98 %,), and in the B group 34/107 (32%) presented completely asymptomatic after 6 months ($p<0.001$). Mean symptom score, evaluated by VAS method, was 3±1 in group A and 8±2 in group B, with a statistically significant difference ($p<0.001$).

Discussion

It is known that the response to lactose-free diet may be elusive even when the patients affected by lactose intolerance are very careful selecting appropriate food (5,6,7,8). From these English, Danish, Scottish and Irish studies, it was concluded that lactose malabsorption plays a definite but minor role in the etiology of IBS. In fact, some patients may be unresponsive because of the presence of traces of

lactose hidden within the food, especially tinned and chemically preserved food**.** Tursi et al described 15 cases of celiac disease unresponsive to gluten-free diet in whom SIBO or lactose intolerance was the cause of unresponsiveness (15). Having observed a remarkable number of patients with lactose intolerance complaining persistent symptoms notwithstanding accurate lactose free diet, we tested the hypothesis of SIBO as being the cause for the partial failure of treatment. To date, the LHBT is the most widely used procedure in the diagnostic work-up of lactose malabsorption, being the genotyping procedure and the assessment of lactase activity in intestinal biopsy samples complicated, relatively expensive or invasive (16). The specificity of LHBT is reported to be 89-100% and the sensitivity 69-100% (17). The gold standard for the diagnosis of SIBO is yet to be defined, as direct tests of culture have substantial limitations for accessibility and performance difficulties (18). HBTs are indirect diagnostic methods based on the detection of hydrogen in expired breath, considered a measure of the metabolic activity of enteric bacteria, because mammalian tissues do not generate hydrogen. They are noninvasive, easy to perform, sensitive enough and highly specific for SIBO diagnosis (17**,**19). The problem of the methanogenic bacteria, non-producing H_2 during the breakdown of carbohydrates, was overcome in our study because the instrument we used, the Quin Tron, is able to detect both H_2 and CH_4 gases. From the diagnostic point of view, our study supports the use of LHBT for the contemporary diagnosis of lactose malabsorption and SIBO with a single, simple test, when rightly interpreted. In a practical way, it comes with no surprise that a molecule of sugar, like lactose, is methabolised by bacteria, when present, also in the upper tract of the gut, being they supplied with the appropriate enzyme, beta-galactosidase (20,21). From the epidemiological point of view, the prevalence of lactose intolerance varies considerably trough the Continents, ranging from 5% in North-west Europe, where lactase persistence is a dominantly inherited state (22) to almost 100% in some Asian population, where a vast majority of adults have genetically determined lactase deficiency (23, 24,25). The Asian studies propose that fermentation of lactose in the small bowel due to SIBO increases the likelihood of lactose intolerance symptoms to occur. Scanty data are available on the epidemiology of coexistence of lactose intolerance and SIBO in the Mediterranean area. The present study shows a high prevalence of lactose intolerance among Northern Italian patients complaining of chronic abdominal symptoms: 59%, with a statistically significant difference *vs* the control group (P<.001). The prevalence of 6% of lactose malabsorption in the control group (asymptomatic) is lower than the prevalence of hypolactasia generally

reported in the Mediterranean area: this could be due partly to differences in methods and partly to geographic reasons, in that Northwest Italy does not necessarily present the same specific genotypic characteristics as the South and Central Italy. Moreover, nearly 3 out of 4 patients with lactose intolerance present with a coexistence of SIBO: 72%.). This percentage is higher than that observed in IBS patients (variably reported between 20% and 60%), but it is to highlight that it refers to a selected subset of patients, affected by lactose intolerance and not to generic IBS patients. It is also conceivable that a post- infectious etiology of IBS can play some role (26). Although an early peak of hydrogen during LHBT can be theoretically due to an accelerated oro-caecal transit time, this seems unlike in our series because of the timely comparable results obtained by GHBT in the same patients. On the other hand, it has been found that small intestinal bacterial overgrowth increases the likelihood of lactose intolerance, as a direct fermentation of lactose in the small intestine, independent of oro-caecal transit time and visceral sensitivity (27). Although the criteria for SIBO diagnosis are far from well-defined even nowadays, the occurrence of complete recovery from symptoms in coincidence of treatment with rifaximin and negativization of GHBT seems to us more than evocative. The clinical role of SIBO, in this clinical setting, seems to be of crucial importance, since, in our experience, the well-being, symptom-free status 6 months after treatment, is significantly superior in patients successfully treated for SIBO than in those on lactose-free diet only. This information can assist in helping the disoriented patients demotivated for the uselessness of their diet efforts. Our data definitively confirm, on a much larger scale, the association between lactose intolerance and SIBO, observed in China by Zhao (27) on a small number of patients (9/14: 64%). At an etiologic level, without genetic tests, it is difficult to assess if SIBO is responsible for lactose intolerance or, vice versa, if lactose intolerance promotes SIBO. We think that the two possibilities may exist. Primary lactase deficiency is considered uncommon (28). Clinical conditions currently known as causing secondary hypolactasia have been excluded from our study. Factors responsible for SIBO (drugs as proton pump inhibitors, antibiotics, neuroleptics etc., as well as functional, anatomic and surgical conditions) have also been excluded. Although it is difficult to draw definitive conclusions, in absence of genetic studies, it is conceivable that, in a portion of cases, lactose intolerance could be secondary to SIBO, in our series. On the other hand, unabsorbed foods may promote the growth of bacteria in small intestinal lumen. In this setting, the egg and chicken situation is clearly hard to define. The crucial clinical action for the patient long-lasting well-

being is, in our opinion, to eradicate the SIBO alongside a strict adherence to the diet rules, irrespective of lactose intolerance as being primitive or secondary in nature. In conclusion, this study shows that lactose intolerance is very common in Northern Italian patients with chronic abdominal complaints and that SIBO may coexist with a very high frequency. Rifaximin treatment has proved to be effective and safe to eradicate SIBO, allowing a good lasting response to the lactose- free diet.

Summary

Background: Lactose intolerance is highly prevalent in Mediterranean area. A substantial portion of patients remain symptomatic in spite of fair lactose-free diet.

Aims: Assess in a series of IBS consecutive patients: 1) the prevalence of lactose intolerance; 2) the frequency of association of lactose intolerance with SIBO; 3) the possibility of SIBO as a cause of symptom persistence in patients with lactose intolerance on lactose-free diet; 4) the ability of LHBT to diagnose SIBO.

Place and duration of the study: Patients were recruited from November 2011 to July 2012 at the Gastroenterology Unit of Mauriziano Hospital U.Ist, Turin, Italy.

Methodology: Lactose malabsorption was assessed by means of LHBT and SIBO by means of GHBT and LHBT, using Breath Tracker digital microlyzer on 500 IBS patients and 50 controls. SIBO was treated, with rifaximin 1200 mg a day for 2 weeks, randomly, on 1 to 1 basis.

Results: Prevalence of lactose intolerance resulted to be 59% in IBS patients and 6% in controls, with a statistically significant difference ($p<0.001$). SIBO was present in 72% of patients with lactose intolerance in IBS group, ad in none of the subjects with lactose malabsorption (3) in control group. After 6 months, 105 out of 106 patients affected by LI + SIBO treated with rifaximin + lactose free diet, and 34 out of 107 patients affected by LI + SIBO treated only with a lactose free diet resulted completely asymptomatic. Concordance between LHBT and GHBT for SIBO diagnosis was 98%.

Conclusions: Lactose intolerance is a common condition in patients with IBS in Northwest Italy (59%) very frequently associated with SIBO (72%). This association turned out to be a major cause of symptom persistence in patients on lactose-free

diet until successful eradication of SIBO was achieved. LHBT is a simple test able to diagnose simultaneously lactose malabsorption and SIBO.

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