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Serum diamine oxidase activity is associated with lactose malabsorption phenotypic variation

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ABSTRACT

Objectives: Recently, an intermediate lactose intolerance (LI) phenotype based on the lactase gene (*LCT*) C/T$_{-13910}$ polymorphism was proposed. However, a multifactorial genesis of LI phenotypic variation including endogenous and exogenous factors cannot be ruled out. Therefore, this study was conducted to investigate a possible association between serum diamine oxidase (DAO) and LI phenotypes in individuals with lactose malabsorption (LM).

Design and methods: A total of 121 ambulatory patients with LM were included in this retrospective study. The lactose hydrogen breath test (LHBT) and serum DAO activity measurements were performed on the same day. A thorough anamnesis with respect to gastrointestinal symptoms was carried out at the initial consultation.

Results: In total, 44 (36.4%) patients with a serum DAO activity <10 U/mL showed higher H_2 levels after 60 (mean: 53.7 ± 57.6 vs 34.5 ± 31.7 parts per million [ppm], $p = 0.116$), 90 (mean: 70.3 ± 57.5 vs 52.7 ± 41.4 ppm, $p = 0.184$) and 120 min (mean: 98.9 ± 72.5 vs 67.9 ± 44.9 ppm, $p = 0.012$) during LHBT compared to 77 (63.6%) patients with a serum DAO activity ≥10 U/mL. Individuals with a serum DAO activity <10 U/mL tended to report gastrointestinal symptoms during the LHBT more often ($p = 0.091$).

Conclusions: Our findings suggest that patients with LM and a serum DAO activity level < 10 U/mL had higher end-expiratory H_2 levels and tended to be more symptomatic during the LHBT compared to LM patients with DAO activity levels ≥10 U/mL.

1. Introduction

Lactose malabsorption is a widespread gastrointestinal condition worldwide. The lactase enzyme, which is responsible for the cleavage of the disaccharide lactose into the absorbable monosaccharides glucose and galactose, is localized in the brush boarder of the small intestine [1]. This enzyme shows maximal activity during the perinatal period. After 2–12 years of age, a "lactase non-persistence" phenotype with low lactase activity and a "lactase-persistence" phenotype with normal lactase activity can be distinguished [2]. In individuals with "lactose non-persistence", lactose malabsorption (LM) is defined as an inadequate digestion of lactose, whereas lactose intolerance (LI) is defined as LM combined with gastrointestinal symptoms [3].

The lactose hydrogen breath test (LHBT) is a widely used diagnostic tool for lactose malabsorption testing [4], with about one-third of lactose malabsorbers complaining of clinical symptoms during the LHBT [5]. In contrast, genetic testing for lactase non-persistence does not allow for the assessment of clinical symptoms or different clinical presentations of LI [4].

Recently, an intermediate LI phenotype based on the lactase gene (*LCT*) C/T$_{-13,910}$ polymorphism was proposed [6]. This fact raises the question of non-genetic factors being responsible for a multifactorial genesis of different LI phenotypes. Interestingly, a previous study found significant correlations between lactase and diamine oxidase (DAO) activities in small intestinal biopsies of children [7]. Based on these results, a possible relationship of DAO activity and different clinical presentations of LI may be considered.

DAO, the main enzyme in metabolizing ingested histamine, is synthesized by mature apical cells in the intestinal mucosa localized in the upper intestinal villi [8]. It is continuously released from the

Abbreviations: LI, lactose intolerance; LM, lactose malabsorption; LHBT, lactose hydrogen breath test; *LCT*, lactase gene; DAO, diamine oxidase; HI, histamine intolerance; CO_2, carbon dioxide; H_2, hydrogen.

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enterocytes and also transported from the intestinal mucosa to the blood circulation [9]. Diseases with intestinal mucosa damage lower the serum DAO activity. The decrease is associated with the severity of mucosal damage [10].

The aim of this retrospective study was to investigate a possible association between serum DAO activity and different LI phenotypes. Therefore, LHBT and serum DAO activity measurements were performed in parallel for 121 ambulatory patients with LM.

2. Materials and methods

2.1. Study design and study population

A total of 121 consecutively-examined ambulatory adult patients with verified LM were included in this retrospective study. The inclusion criteria were: a minimum age of 18 years, the parallel performance of the LHBT and serum DAO activity measurements, a thorough anamnesis of gastrointestinal symptoms at the first consultation, and a twelve-hour overnight fasting state. Furthermore, patients were asked to refrain from smoking. Individuals with incomplete case histories or colonoscopy and/or antibiotic-based therapy at least 4 weeks before the LHBT were excluded from the study. None of the patients showed signs of active or chronic inflammatory bowel disease or were on drug intake (i.e., acetylcysteine, verapamil, cimetidin, metoclopramide) at study entry.

2.2. Ethics

The study was approved by the ethics committee of the Johannes Kepler University of Linz (Linz, Austria [trial registration number: K-107-16]) and is in accordance with the latest version of the Declaration of Helsinki.

2.3. LHBT

Stationary gas-chromatography was employed to measure the end-expiratory H_2 concentrations (Gastrolyzer, Bedfont Scientific inc., Kent, United Kingdom). The baseline (0 min) concentration was determined after an overnight fasting state of 12 h. After the oral ingestion of 50 g of lactose dissolved in 200 mL of water the end-expiratory breath H_2 concentration was measured at 30, 60, 90, and 120 min [11,12]. The results were expressed in parts per million (ppm). The patients were instructed to report clinical symptoms and to avoid smoking, eating or physical activity during the test. The LHBT was interpreted to be positive if the H_2 peak was ≥20 ppm over the baseline value [11,13].

2.4. Serum DAO activity measurements

The serum DAO activity was determined using a quantitative radio extraction assay (DAO-REA®; Sciotec Diagnostic Technologies GmbH, Tulln, Austria). According to the literature [14,15], a serum DAO activity <10 U/mL was considered to be associated with a higher risk of histamine intolerance (HI) compared to a serum DAO activity ≥10 U/mL. The intra- and inter-assay reproducibility of the DAO radio extraction assay was <10 and <15% (information of the manufacturer).

2.5. Statistical analysis

For the statistical analysis, descriptive statistics were used to summarize and present the study variables. For subgroup comparisons of categorical parameters, the exact Chi-Square test was used. For subgroup comparisons of metric variables in the case of normal distribution (verified with the Kolmogorov-Smirnov test) the paired Student's *t*-test was calculated. For metric non-normal distributed variables the exact Mann-Whitney *U* test was used. A p-value < 0.05 was considered statistically significant. Analyse-it® software version 2.30 (Analyse-it Software, Ltd., Leeds, United Kingdom) was used for statistical analysis.

3. Results

3.1. Patient characteristics

In total, 121 patients were included, of whom 80 (66.1%) were female and 41 (33.9%) were male with a mean age of 47 years. The demographic and clinical data are presented in Table 1.

3.2. Serum DAO activity

Serum DAO measurements of all patients ($n = 121$) showed a median value of 13.6 (range: 1.5–65.7) U/mL. Of these, 77 (63.6%) individuals had a serum DAO activity ≥10 U/mL (median: 18.4 [range: 10.3–65.7]), whereas 44 (36.4%) patients demonstrated a serum DAO value <10 U/mL (median: 5.7 [range: 1.5–9.5]).

3.3. LI phenotypic variation

LM patients with a serum DAO activity <10 U/mL showed higher end-expiratory H_2 levels after 60 (mean: 53.7 ± 57.6 vs 34.5 ± 31.7 ppm, $p = 0.116$), 90 (mean: 70.3 ± 57.5 vs 52.7 ± 41.4 ppm, $p = 0.184$) and 120 min (mean: 98.9 ± 72.5 vs 67.9 ± 44.9 ppm, $p = 0.012$) during LHBT compared to LM patients with a serum DAO activity ≥10 U/mL. Box-and-whisker plots of end-expiratory H_2 levels during the LHBT in 77 and 44 individuals with serum DAO activity ≥10 and DAO activity <10 U/mL, respectively, are shown in Figs. 1 and 2.

3.4. Self-reported symptoms during the LHBT

Taken together, 82 (67.8%) patients with LM reported one or more gastrointestinal symptoms (i.e., abdominal pain, diarrhea, bloating, heartburn, burping, nausea) during the lactose LHBT. Interestingly, self-reported gastrointestinal symptoms tended to be more frequent in patients (34 out of 44 [77.3%]) with a serum DAO activity <10 U/mL compared to those patients (48 out of 77 [62.3%]) with a DAO activity ≥10 U/mL ($p = 0.091$).

4. Discussion

In the present study, 121 adult individuals with LM were investigated using LHBT and serum DAO activity measurements. To the best of our knowledge, this is the first study addressing the possible association between serum DAO activity levels and different LI phenotypes.

The serum DAO activity has been recognized as a marker of intestinal mucosal maturation and integrity [16] with low serum DAO levels reflecting mucosal injury [17,18]. Similarly, a reduced lactase activity in the small intestine may also indicate mucosal damage [18,19] associated

Table 1
Demographic and clinical characteristics of the patients.

	Study population ($n = 121$)
Gender:	
Female	80 (66.1%)
Male	41 (33.9%)
Mean age in years (±SD)	47 (±17)
Gastrointestinal symptoms:	
Abdominal pain	39 (32.2%)
Bloating/flatulence	82 (67.8%)
Diarrhea	36 (29.7%)
Obstipation	6 (4.9%)
Heartburn	4 (3.3%)
Burping	2 (1.6%)
Nausea	1 (0.8%)

SD = standard deviation.

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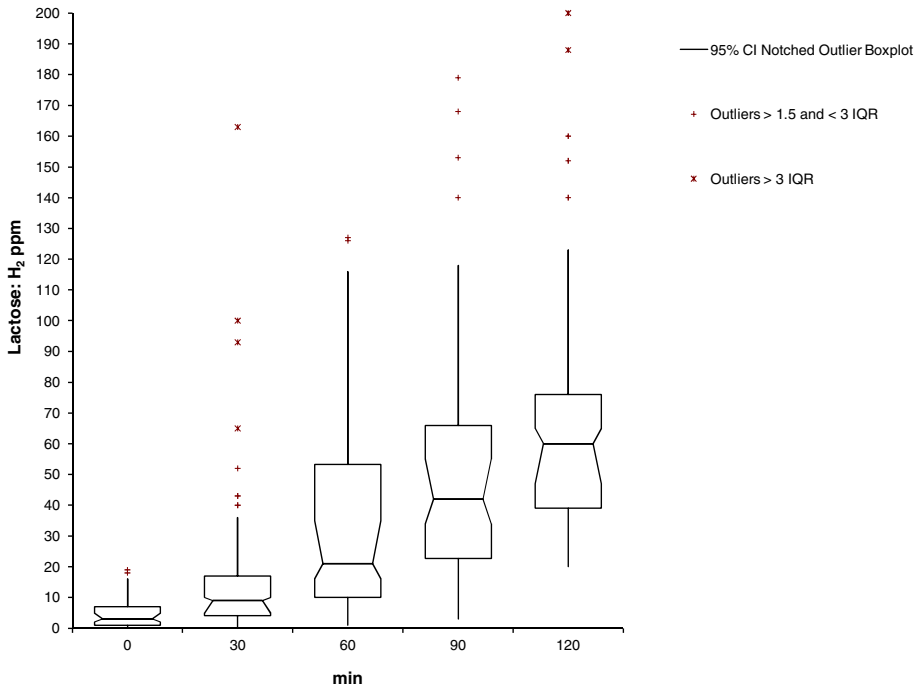

Fig. 1. Box-and-whisker plot of exhaled H_2 levels during the LHBT in 77 patients with serum DAO activity ≥10 U/mL. The boxes represent the 25th to 75th percentile range. The line inside the boxes represents the median value of H_2 concentrations expressed in parts per million (ppm).

with conditions such as gastroenteritis, short bowel syndrome, celiac and tropical sprue, infectious enteritis, radiation enteritis, gastrointestinal surgery or drugs [12]. As a consequence non-absorbable lactose reaches the large intestine, where it is fermented into short-chain fatty acids, carbon dioxide (CO_2), and hydrogen (H_2) by colonic bacteria [12,20]. In turn, the H_2 production causes GI symptoms such as abdominal pain, bloating and/or diarrhea [6,20,21].

The measurement of H_2 in the end-expiratory breath is considered to be the most clinically significant parameter of bacterial lactose fermentation in the colon [6]. Nevertheless, the interpretation of the LHBT is limited because this method is not standardized yet [12]. Moreover, in individuals with carbohydrate malabsorption, the acidic microclimate in the colon may affect the bacterial H_2 production and cause false negative LHBT results [12,22]. A longer orocecal transit time may

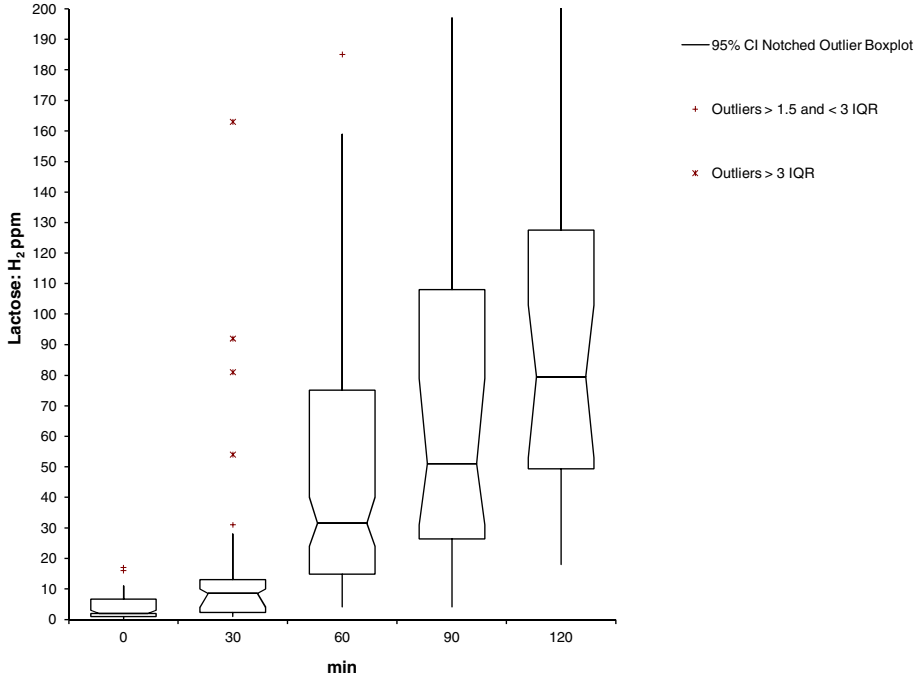

Fig. 2. Box-and-whisker plot of exhaled H_2 levels during the LHBT in 44 patients with serum DAO activity <10 U/mL. The boxes represent the 25th to 75th percentile range. The line inside the boxes represents the median value of H_2 concentrations expressed in parts per million (ppm).

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be another possible reason for false negative LHBT results, because breath testing may be finished before a measurable H_2 increase is established [11,12]. Pre-analytical poor patient preparation during the alveolar air collection may also influence LHBT measurements in patients with LI [20].

In this study serum DAO activity discriminates between two distinct LI phenotypes as defined by LHBT: lactose malabsorbers with higher end-expiratory H_2 breath levels (DAO activity <10 U/mL) and those with lower H_2 breath levels (DAO activity ≥10 U/mL). Of note, individuals with LM and a DAO activity <10 U/mL tended to be more symptomatic during LHBT ($p = 0.091$).

In a recent study, individuals with the *LCT* C/T$_{-13,910}$ genotype tended to have clinical symptoms (i.e., abdominal pain) more often during breath testing compared to those with an *LCT* T/T$_{-13,910}$ genotype [6]. Therefore, the genotype–phenotype correlation may be one explanation for the existence of different LI phenotypes. However, our data demonstrate that serum DAO activity levels also segregate into varying clinical presentations of LI. Nevertheless, the factors responsible for triggering symptoms in individuals with LI are not yet completely understood [5,20]. Furthermore it is known that the prediction of LI based on the subjective perception of reported symptoms remains difficult [12,23].

Two shortcomings of this study may be described. Firstly, intestinal lactase activity measurements and *LCT* genotyping (C/T$_{-13,910}$ polymorphism) were not performed. Secondly, this study is retrospective and cross-sectional in nature, representing the serum DAO activity determinations only at a single measuring point.

In conclusion, our findings suggest that patients with LM and a serum DAO activity level < 10 U/mL had higher end-expiratory H_2 levels and tended to be more symptomatic during the LHBT compared to LM patients with DAO activity levels ≥10 U/mL. However, prospective longitudinal studies incorporating follow-up measurements of DAO activity are required to fully elucidate the association with LI.

Conflict of interest

Wolfgang J. Schnedl is co-founder of GedoMed GmbH (Bruck an der Mur, Austria). The other authors disclose no conflict of interest regarding the publication of this article.

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