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Comparison of Indirect Methods for Lactose Malabsorption

Abstract: Although direct determination of the lactase activity of the small intestinal mucosa is considered a "gold standard" for the diagnosis of hypolactasia leading to lactose malabsorption, less invasive and simpler indirect techniques like the "plasma glucose response test" and "breath hydrogen test" are preferred in daily practice to diagnose the disorder. The aim of this study was to compare the different indirect diagnostic methods for lactose malabsorption. The following tests were performed in 54 adults: plasma glucose response test, breath hydrogen test and urine galactose/creatinine ratio. The diagnostic variables were as follows: increased plasma glucose $\leq$ 20 mg/dl; increased exhalation of breath hydrogen $\geq$ 20 ppm, and urinary galactose/creatinine $\leq$ 0.1 mg/mg 60 min after 50 g oral lactose loading. Using the gold standard of two diagnostic variables (plasma glucose response test and breath hydrogen test) being positive, lactose malabsorption rate was found to be 85%. Positive predictive values of the plasma glucose response test and breath hydrogen test were 93.8% and 95.8%, respectively.

The discordance rate of the tests was 9.2%. We modified a qualitative galactose spot test to determine urinary galactose quantitatively, and used the galactose/creatinine ratio for the indirect detection of lactose malabsorption. The sensitivity, positive predictivity and negative predictivity of the urinary galactose/creatinine ratio at 60 min were determined to be 93.4%, 93.4% and 37.5%, respectively.

In conclusion, we suggest the use of the positivity of any two (instead of the former use of only one) of these three indirect methods in accordance with the suitability of the technical equipment of a laboratory, as a "gold standard" for diagnosing lactose malabsorption. Being noninvasive, safe and accurate, we propose the use of a combination of urinary galactose/creatinine ratio and breath hydrogen test, where available, as a convenient protocol for the diagnosis of lactose malabsorption.

Key Words: Lactose malabsorption, urinary galactose, breath hydrogen test, lactose tolerance test

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Introduction

Disaccharide lactose is split in the small bowel by enzyme lactase (a β-galactosidase) into equal molecular amounts of glucose and galactose. The activity of lactase is high at birth. In the majority of the world's population it decreases after infancy (1). The primary lactase deficiency rate was reported to be 71% among the adult population in Turkey (2).

Individuals with hypolactasia malabsorb ingested lactose, the unhydrolyzed lactose, which by osmosis draws water into the small intestine, passes to the colon and is fermented by colonic bacteria into volatile fatty acids, hydrogen, methane and carbon dioxide. Hydrogen gas (H_2) is absorbed in part and excreted in the breath of individuals with lactose malabsorption (3). This results in considerable suffering from abdominal pain, bloating, flatus and diarrhea (4).

Lactose malabsorption can not be determined from a patient's clinical history, because many patients do not recognize the symptoms and some remain asymptomatic (5). Lactose malabsorption is diagnosed by a variety of methods including plasma glucose response test (the classical lactose tolerance test) based on serial blood glucose determination (6), the breath hydrogen test (7) and the measurement of lactase from a small bowel biopsy (8).

The direct lactase assay and the determination of the lactase to sucrase ratio from jejunal biopsy is the most reliable method, but it understandably cannot be the method of choice for outpatient clinics (9,10).

The most commonly used indirect method is serial blood glucose determinations after an oral lactose load. This test is very simple to perform and standardize, and is minimally invasive. However, its sensitivity and specificity are questionnable owing to variations in, for example, gastric emptying and the glucose metabolism (11). The test is of little use in patients with diabetes (12).

The breath hydrogen test is based on the rise in exhaled breath hydrogen that is formed from unabsorbed lactose in the colon by appropriate bacteria. Serial sampling at intervals of 15 min to 1 h for 2 to 4 h is needed during the test. Although it is considered the most reliable, non-invasive and economical technique by some authors (7,13), Arola et al. (10) determined a lower sensitivity (69%) for the test in comparison with other methods (81-94%).

The urinary galactose determination test is another approach suggested for the diagnosis of lactose malabsorption. The theory of the test relies upon the determination of the excreted amount of urinary galactose that passes into portal circulation following its absorption in the gastrointestinal canal and, that was able to break away from hepatic clearance despite its presence at low plasma concentrations. A diminution in the galactose excretion rate is expected in cases with lactose malabsorption (14). The measurement of urinary galactose using commercial test strips has been suggested, but those test strips, which use galactose oxidase, are no longer available (15). Grant et al. (16) reported that the measurement of the galactose/creatinine ratio in the first 3 h urine samples after drinking a lactose load of 50 g discriminates between healthy people and individuals with lactose malabsorption.

Buttery et al. (17) developed a "visual screening" method for urine samples after 1 h for the determination of galactose concentration used to diagnose lactose maldigestion. In the study, the patients were given 300 mg/kg of body-weight ethanol for the inhibition of galactose to glucose conversion in the liver as well as a 50 g lactose load; the sensitivity of the method was established to be 93%. On the other hand, as a non-invasive method, the diagnostic value of urinary galactose determination is thought to have been insufficiently assessed by some authors (18).

We compared the usefulness of indirect diagnostic tests concerning the evaluation of lactose malabsorption (e.g., plasma glucose response test, breath H_2 test and urinary galactose/creatinine ratio) to elucidate the etiology of disorders such as recurrent abdominal pain in children, irritable bowel syndrome and chronic diarrhea.

Materials and Methods

Fifty-four adult patients (age range: 20-59 years) who attended the Gastroenterology Department of GATA Medical Faculty and were referred to our laboratory for the assessment of lactose intolerance were recruited for this study. The members of the study group were advised to abstain from foodstuffs that delayed intestinal passage, such as leguminous food as well as cereal containing bran and fiber, and were encouraged to eat meat or rice at dinner the day before the investigation. Patients who had been treated with an antibiotic drug were not included in the study at least before 15 days following completion of the medication. Patients with active diarrhea during or shortly prior to the test were also excluded.

The below mentioned test protocol (Fig. 1) was practised after an overnight fast, during which the patients were allowed to drink some water. The patients were not allowed to exercise, smoke, eat or drink during the test period.

In brief, after an overnight fast (zero min, basal samples), a urinary sample was taken and a venous blood sample was drawn into vacuum tubes containing EDTA/flouride as well as obtaining end-expiratory breath H_2 samples immediately before the lactose challenge.
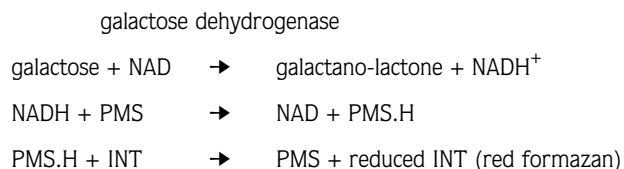
Following the administration of 50 g of lactose orally within 5 min dissolved in 400 ml water, blood (30 min and 60 min) and urine (60 min) samples were obtained. End-expiratory breath samples of 20 ml were taken at regular intervals of 30, 60, 90, 120 and 180 min after the lactose load.

The glucose levels of the plasma samples were determined on a Dax-48 autoanalyzer (Bayer, Germany) by using a commercially available glucose oxidase-peroxidase enzymmatic method at the date of sampling, after obtaining the plasma samples by centrifugation at 2,000 rpm for 10 min. Delta ($\triangle$) plasma glucose levels were calculated by subtracting the baseline glucose level from the peak glucose level after the lactose challenge.

Breath samples were taken regularly in gas sampler bags as end-expiratory samples and they were analyzed immediately by a hydrogen analyzer (Quintron CM2 Microlyzer, USA). The analyzer was calibrated daily with a 100 ppm H_2 standard and was linear between the 0 and 200 ppm H_2 level. Delta ($\triangle$) breath hydrogen was defined as the highest breath H_2 concentration minus the baseline (fasting) H_2 value.

Urinary creatinine levels were determined on a Dax-48 autoanalyzer (Bayer, Germany) by using the kinetic Jaffe method. Urinary galactose levels were determined by improving the visual spot test of Buttery et al. (17) into a quantitative method by our group.

The chemical principle to determine urinary galactose was as follows:

galactose dehydrogenase

galactose + NAD ➔ galactano-lactone + $NADH^+$

NADH + PMS ➔ NAD + PMS.H

PMS.H + INT ➔ PMS + reduced INT (red formazan)

The procedure can briefly be explained as follows:

To determine the amount of galactose in urine samples, to 50 µl of urine sample (test) or standard (18 mg/dl galactose) in a cuvette were added 50 µl pooled plasma, 0.5 ml of buffer (phosphate buffer 0.2 mol/l, pH 7.5), 50 µl of color reagent (containing 80 mg of p-iodo nitrotetrazolium violet, 200 mg of NAD and 5 mg of phenazine methosulfate in 20 ml of water), and 0.075 U of galactose dehydrogenase, followed by mixing. Distilled water rather than was used for blanking. After incubation for 15 min at room temperature in a dark chamber, test and standard absorbances were measured against the blank at 500 nm with a spectrophotometer. The results were evaluated according to prepared calibration graphics.

A corrected galactose concentration was established by subtracting the baseline galactose value from galactose concentration at 60 min., and this figure was used to calculate the urinary galactose/creatinine ratio; the results of the corrected galactose concentration were expressed per creatinine concentration (mg/mg).

Following the lactose challenge, a peak glucose rise of ≤ 20 mg/dl, or an exhalated H_2 increment of ≥ 20 ppm (11), or a galactose/creatinine ratio of ≤ 0.1 mg/mg (16) in comparison with baseline levels were all accepted as predictors of lactose malabsorption.

Statistical Analysis

Statistical analyses were carried out by using SPSS and EXCEL for Windows. Relationships between the variables were detected by Pearson's correlation test; a p-value less than 0.05 was considered statistically significant.

Results

The results of different analyses performed on the 54 adult patients with probable lactose intolerance are depicted in Figure 2. Positivity rates for the plasma glucose response test, breath H_2 test and urinary galactose/creatinine ratio were 90%, 89% and 85%, respectively.

Taking the plasma glucose response test and the breath H_2 test into consideration, both of the tests were positive in 46 patients while only one alone was positive in five patients and no positivity was achieved in three patients (Table).

When the positivity of the plasma glucose response test plus the breath H_2 test was accepted for the two-positive criterion as suggested by Peuhkuri et al. (19), lactose malabsorption frequency was established to be (46/54) 85%. The positive predictive values of the tests were calculated to be 93.8% and 95.8%, respectively (Table).
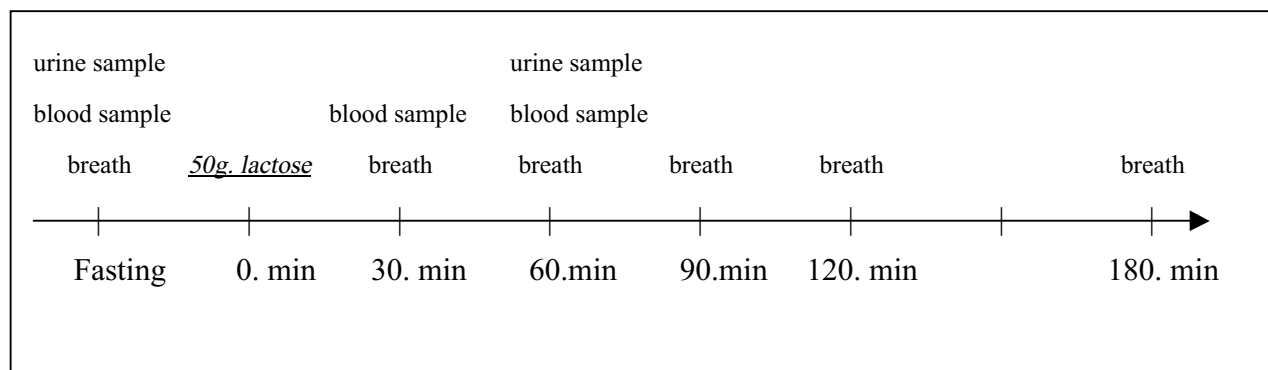

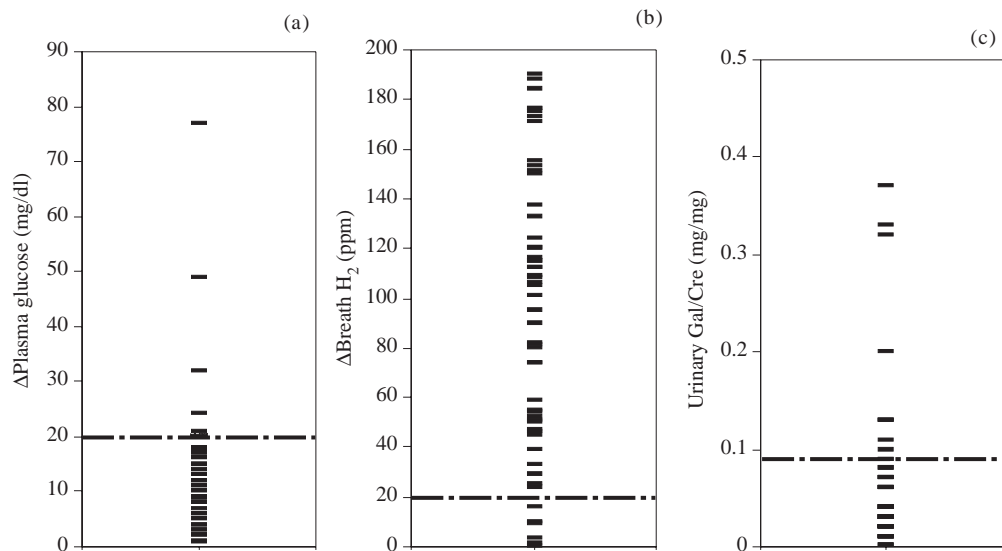Figure 1. Lactose loading test protocol.

Figure 2. The distribution spectra of lactose tolerance tests.

(a) Plasma glucose response test   (b) Breath H$_2$ test   (c) Urinary galactose/creatinine ratio

Table   Results of different tests in the study group.

	Plasma glucose response test: (+) Breath H$_2$ test: (+) (n = 46)	Plasma glucose response test: (-) Breath H$_2$ test: (-) (n = 3)	Plasma glucose response test: (-) Breath H$_2$ test: (+) (n = 2)	Plasma glucose response test: (+) Breath H$_2$ test: (-) (n = 3)
Urinary galactose/creatinine ratio: ≤ 0.1 mg /mg	43		1	2
Urinary galactose/creatinine ratio: ≥ 0.1 mg /mg	3	3	1	1

No correlation was established between the results of the plasma glucose response test and those of the breath H$_2$ test (Fig. 3); however, a moderate correlation was found between the plasma glucose response test and the urinary galactose/creatinine ratio (r = 0.594, p < 0.05) (Fig. 4).

Scanning the absorption spectrum of the final color reached at the end of the quantitative urinary galactose determination test, a peak maximum of around 500 nm was obtained (Fig. 5).

The method was found to be linear between galactose concentrations of 0 and 54 mg/dl; thus, allowing the determination of urinary galactose quantity following a lactose challenge (Fig. 6).

Taking the plasma glucose response test plus the breath H$_2$ test as the "diagnostic standard", two positive
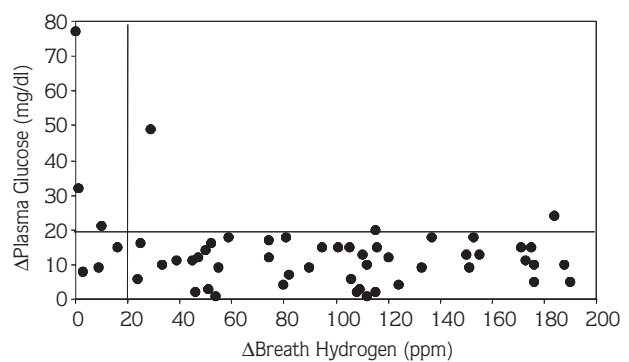

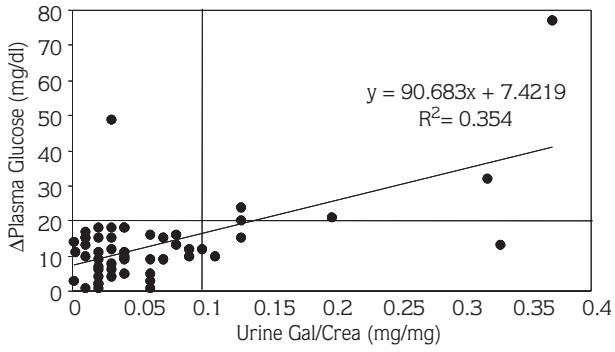Figure 3.   The correlation between the plasma glucose response test and the breath H$_2$ test.

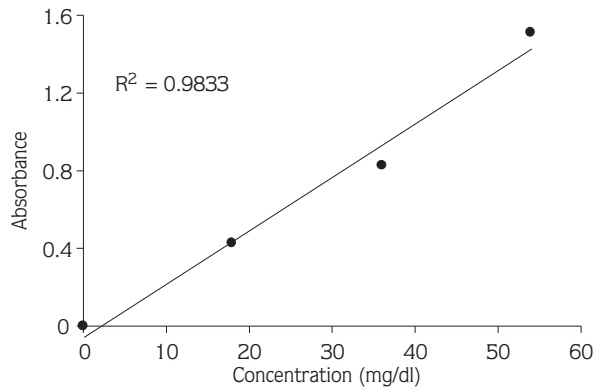Figure 4. The correlation between the plasma glucose response test and urinary galactose/creatinine ratio.

$$y = 90.683x + 7.4219$$
$$R^2 = 0.354$$

Figure 6. The calibration graphics of the quantitative urinary galactose level determination method.
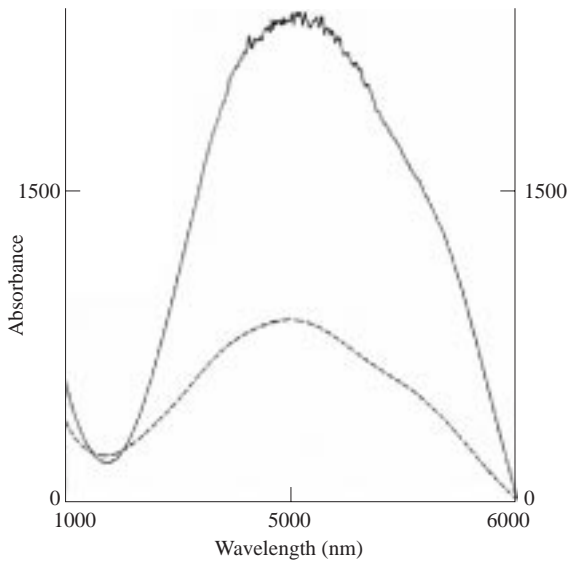
$$R^2 = 0.9833$$

Figure 5. The absorption spectrum of the final color obtained during urinary galactose level determination. A peak maximum around 500 nm wavelength was determined.
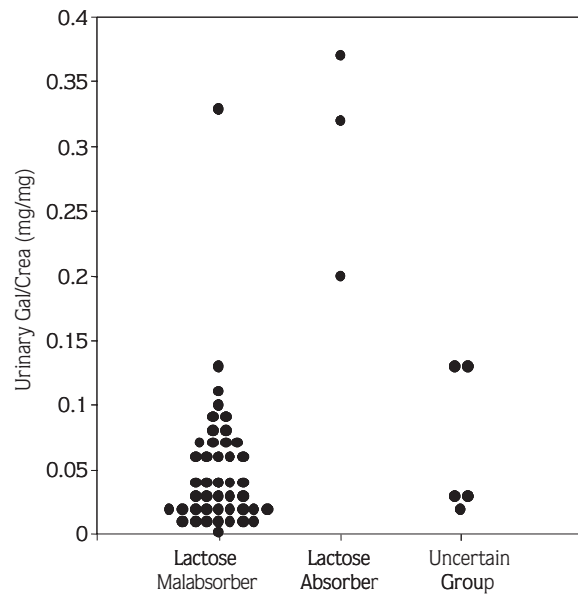

Figure 7. Results of urinary galactose/creatinine ratio of the study group.

(LA: lactose absoption [normal]; LM: lactose malabsorption)

results, two negative results and a positive plus a negative result of any combination were evaluated as lactose malabsorber (LM), normal lactose absorber (LA) and equivocal result, respectively. Regarding this evaluation, the sensitivity of the urinary galactose/creatinine ratio test was found to be 93.4%, and a distribution plotting of the results is depicted in Figure. 7. The positive predictive value was established to be 93.4% with the negative predictive value being 37.5% (Table).

Discussion

In the present study, the results of a classical lactose tolerance test (plasma glucose response test) and two non-invasive, indirect tests (breath H_2 and urinary galactose/creatinine ratio) used in the diagnosis of lactose malabsorption are compared with each other.

Despite the determination of lactase activity from jejunal biopsy being the most reliable method, with it being used as a "gold standard" for comparing indirect

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diagnostic methods of lactose malabsorption, this step was excluded from the study owing to the difficulty in using it in all the patients. On the other hand, lactase activity from the jejunal biopsy was speculated to not be a "gold standard" owing to the uneven dissemination of lactase activity throughout the small intestine mucosal structure (18,20). Thus, we preferred the "two positive parameter" criterion of two recent studies (19,21) as a "gold standard" for the diagnosis of lactose malabsorption rather than the direct determination of lactase activity.

By using this criterion, we found the lactose malabsorption rate to be ≈ 85% for adult individuals among the study group. This finding was consistent or even a little higher than the result established by Flatz et al. (2), who found the lactase deficiency rate to be 71% in 471 Turkish soldiers by utilizing the breath H_2 test. Although the 85% might cover some patients with secondary malabsorption or generalized malabsorption disorders (22), in our opinion, this figure reflects the predominant lactose malabsorption due to primary adult lactase deficiency with great probability. These findings suggest that lactase malabsorption is a gastrointestinal disorder that is common in the adult Turkish population, and thus predicts necessity of using the lactose challenge test in the differential diagnosis of disorders with comparable signs and symptoms like irritable bowel syndrome and chronic diarrhea.

There was a discordance of 9.2% between the plasma glucose response test and the breath H_2 test results within the study group; i.e. the result of any kind of test was positive while the other kind being negative for lactose malabsorption. The same discordance rate was reported to be 14.5% by Van Krughen et al. (23) in a series of 40 patients, while Hermans et al. (20) expressed the same rate as 42% in their series of 309 cases. On the other hand, no correlation was established between the results of these parameters (Fig. 3). The discordance may be explained by the fact that different parameters regarding lactose malabsorption are measured by each test. When the plasma glucose increment rate is directly proportional to the amount of lactose being hydrolyzed by the lactase enzyme, the rise in the level of breath H_2 is proportional to the quantity of unhydrolyzed lactose. Nevertheless, some factors influencing parameters may contribute to each result; e.g., plasma glucose level is affected by an alteration in hormone concentration and by

a delay in stomach evacuation; factors like H_2 generation capacity (2-20% of patients may be colonized with bacteria incapable of producing hydrogen), small bowel passage time, bacteria overgrowth within the small bowel and H_2 consumption by the colon may all manipulate breath H_2 levels (22). Although variations in renal excretion function and/or hepatic galactose to glucose conversion capacity may theoretically be expected to lead to alterations in the urinary galactose excretion rate, we observed a significant corelation between the results of the urinary galactose/creatinine ratio and plasma glucose response test (Fig. 4).

When we used the positivity of the both tests (plasma glucose response and breath H_2) as an indicator of lactose malabsorption, we determined that the positive predictive values of the plasma glucose and breath H_2 tests were close to each other (93.8 and 95.8, respectively). Negative predictive values were not taken into consideration due to insufficient data.

The urinary spot test for qualitative galactose determination of Buttery et al. (17) was improved into a quantitative method by us. The color of formazan, which is the final product during the test procedure, was measured at the visible area of a spectrophotometer owing to its maximum absorbance at 500 nm. This method allows the quantification of urinary galactose concentration with the use of less expensive optic devices like an ordinary spectrophotometer in comparison with the determination of urinary galactose at ultraviolet wavelength (16) by the use of a sophisticated one.

Although ethanol administration along with lactose load had been suggested for the inhibition of hepatic galactose to glucose conversion in some lactose challenge methodologies (9,17), ethanol has been considered to be useless for this purpose (16, 24). Thereupon, we utilized the lactose challenge protocol without ethanol administration.

When the galactose/creatinine ratio of ≤ 0.1 mg/mg in spot urine sample 1 h following the lactose load was considered a predictor of lactose malabsorption, as proposed by Grant et al. (16), 43/46 patients (diagnostic sensitivity = 93.4%) were diagnosed to be lactose malabsorbers according to the results of two positive parameters. Although the number of participants with normal lactose absorption was insufficient (n = 3), the urinary galactose/creatinine ratio was found to be

negative in these individuals. The sensitivity of the test was slightly lower in comparison with the results of Grant et al. (16), suggesting the 3 h urinary galactose/creatinine ratio as discriminating (sensitivity = 100%) patients with lactose malabsorption diagnosed with the breath H_2 test from normal individuals. This discrepancy may be related to diagnostic indicators of lactose malabsorption, sampling intervals or methodological differences.

The positive predictive value of 93.4% showed the possible use of a urinary galactose/creatinine ratio of $\leq$ 0.1 mg/mg as an indicator of lactose malabsorption. This value was 94% in a study protocol (10) during which a 40 min urinary galactose concentration was considered to be a diagnostic criterion following a lactose challenge with ethanol load. As the negative predictive value was low, negative values cannot be used for the exclusion of lactose malabsorption.

The results of the three available indirect diagnostic tests for the prediction of lactose malabsorption were concordant amongst > 90% of the participant individuals and the positive predictive values were > 90%. These results provide a larger scale of test selection in compliance with the technical efficiency of a laboratory for the diagnosis of lactose malabsorption. The need for special equipment to conduct a breath H_2 test and the partially invasive nature of plasma glucose determination make the urinary galactose/creatinine ratio an attractive alternative for diagnosing lactose malabsorption disorders.

In conclusion, due to the difficulty of performing direct lactase activity determination in all patients, we suggest the use of two-positive parameter criterion to improve the diagnostic accuracy of indirect tests concerning lactose malabsorption, which is a frequently faced disorder in Turkey. Being non-invasive approaches, the breath H_2 test and the urinary galactose/creatinine ratio protocol carried out following a 50 g lactose load without ethanol administration seems to be a convenient protocol for that purpose.

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