Urinary lactose tolerance test for the detection of lactose malabsorption

İdrarda laktoz tolerans testi ile saptanan laktoz malabsorpsiyonu sıklığı

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SUMMARY: *We investigated the frequency of hypolactasia by determining galactose excretion and galactose/creatinine ratios in two-hour urine collections after a standard loading of lactose in 47 healthy adult volunteers. Following overnight fasting, a solution containing 50 g of lactose was given. A urinary galactose level of less than 0.9 mmol/L was taken to indicate hypolactasia. Lactose malabsorption was determined in 83% of the volunteers. Urinary galactose levels were consistent with galactose/creatinine ratios.*

Key words: Lactose malabsorption, lactose tolerance test, urinary galactose, urinary galactose/creatinine ratio

ÖZET: *47 sağlıklı erişkin gönüllüde standart laktoz yüklemesini izleyen iki saatlik idrarda atılan galaktozu ve galaktoz/kreatinin oranını saptayarak hipolaktazya sıklığını araştırdık. Bir gece boyu aç kaldıktan sonra 50 g laktoz içeren bir eriyik içirildi. İdrarda 0.9 mmol/L'nin altındaki galaktoz düzeyleri hipolaktazya belirteci olarak kabul edildi. Gönüllülerin % 83'ünde laktoz malabsorpsiyonu saptandı. İdrar galaktozu ölçümleri, galaktoz/kreatinin oranlarıyla uyumlu idi.*

Anahtar sözcükler: Laktoz malabsorpsiyonu, laktoz tolerans testi, İdrar galaktoz/kreatinin oranı

Activity of lactase is high at birth, but in the majority of the world population it decreases after infancy. Autosomal recessive inheritance in primary lactase deficiency, and autosomal dominant inheritance in ability to digest lactose, have been documented. The prevalence of hypolactasia varies widely among different populations, and it is known to be higher in southern and eastern Europe; 70% of adults in Turkey are reported to suffer from lactose malabsorption (1, 2). Lactose malabsorption should be considered in recurrent abdominal pain, irritable bowel syndrome, chronic diarrhea and dyspepsia. Although various methods of diagnosis exist; no gold standard is available. Breath hydrogen excretion after lactose ingestion is used commonly to diagnose lactose malabsorption, although the technology for this test is not available in Antalya. The objective of our study was to determine the frequency of lactose malabsorption and lactose intolerance on the basis of an absorption test by measurement of urinary galactose (3-6).

Lactose cannot be absorbed as a disaccharide, but is hydrolyzed into glucose and galactose in the brush border membrane of enterocytes. Hepatic galactose clearence in normal subjects is 94% (7); in another study, it has been shown that 88% of galactose is cleared by the liver and excreted in urine, even when the galactose concentration delivered by the portal vein is below 30 mg/dl (8). The tubular reabsorption of galactose is less efficient than that for glucose. Galactose is excreted unchanged in urine, as it does not have a renal threshold. The determination of urinary galactose levels after taking lactose orally is defined as a reliable method of diagnosis of lactose malabsorption. The presence of 0.9 mmol/L or less of galactose in urine indicates a diagnosis of hypolactasia (3-6).

MATERIALS AND METHODS

Fourty seven healthy volunteer adults participated in the study. We explained the objectives of the study and the details of the tests.

They were asked not to eat all night, including breakfast, and empty the bladder. A fasting urine sample was obtained before lactose ingestion. A 20% lactose solution containing 50-g lactose in 400 mls water was given orally. A 5 ml sample was taken from urine excreted during the following two hours, and stored at -20 °C. The volunteers

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Table 1. Urinary galactose levels and galactose/creatinine ratios in lactose absorbers

	Urinary galactose (mmol/L)	*Urinary galactose (mg/dL)*	*Urinary galactose/creatinine (mg/dL)*
1	1.205	21.73	0.0855
2	1.566	28.24	0.2603
3	0.995	17.94	0.1604
4	0.934	16.84	0.2365
5	2.098	37.84	0.1143
6	3.333	60.1	0.4739
7	1.693	30.53	0.0987
8	1.142	20.59	0.0847

did not eat during the test, but drank water to increase diuresis. A galac MPR 1 (Boehringer Mannheim) test kit and a spectrophotometer set at a wavelength of 365 nm were used according to the manufacturers instructions to measure galactose in urine.

0.2 ml of urine was diluted in 1.8 ml of distilled water. 0.2 ml of this mixture was pipetted into a disposable test tube. 0.1 ml of a solution containing 3 ml of phosphate buffer solution and 13 mmol/L of NAD (nicotinamide adenine dinucleotide) was added and the mixture stirred with a plastic spatula. Absorption was measured. This measurement before the addition of an enzyme was taken as blind (A1). Absorption was measured again and incubated at room temperature for 40 minutes (A2); 0.02 ml of a suspension containing at least 20 units of galactose dehydrogenase per milliliter was then added. While the free galactose in the urine sample was oxidizing to the galactonic acid, the resulting reduced NAD directly affected the concentration of galactose.

galactose + NAD + Gal-DH (galactonic acid + NADH + H)

The difference between A1 and A2 was calculated, multiplied by predetermined constants based on wavelength, and concentration was determined in mmol/L units.

The volunteers were questioned for lactose intolerance symptoms after the test and three hours after drinking lactose. Any one of the symptoms of abdominal pain, nausea or vomiting, bloating or diarrhea was accepted as lactose intolerance. They were notified of the test results.

Urinary creatinine concentrations were measured in the samples using an auto analyzer (Technicon Axon, France). The ratio of urinary galactose to urinary creatinine was calculated in two hours urine collections from every subject.

RESULTS

Of the 47 participants, 20 were female and 27 male. Participants were classified as lactose malabsorbers if they had a two-hour urinary galactose concentration of <0.9 mmol/L. Eight subjects (three females and five males) were classified as lactose absorbers and 39 subjects (17 females and 22 males) were lactose malabsorbers. The frequency of lactose malabsorption did not vary with sex (p= 1.0).

Urinary galactose/creatinine ratios were significantly different between lactose absorbers (0.1929+/-0.1253) and malabsorbers (0.0524+/-0.0382) (t= 6.055 and p< 0.001) (Table 1 and 2).

Intolerance symptoms developed in 26 of the 47 volunteers (55%), and these symptoms developed in one absober. Abdominal pain was experienced 21 out of 39 malabsorbers (54%), but none of the absorbers (p= 0.0029). Nausea and vomiting was experienced nine out of 39 malabsorbers (23%), and one out of the eight absorbers (11%) (p= 0.66). Diarrhea was experienced by four out of 39 malabsorbers (10%), and none of the 8 absorbers (p= 1.0).

DISCUSSION

Whilst the prevalence of lactose malabsorption has been studied in most European and Asian countries, the validity of the prevalence estimates for the Turkish population is questionable. We found the frequency of lactose malabsorption to be 83% in healthy adult volunteers.

Althought the hydrogen breath test has gained widespread clinical use, no direct tests are cur-

Table 2. Urinary galactose levels and galactose/creatinine ratios in lactose malabsorbers

	Urinary galactose (mmol/L)	*Urinary galactose (mg/dL)*	*Urinary galactose/creatinine (mg/dL)*
1	0.176	3.17	0.0195
2	0.302	5.44	0.0275
3	0.449	8.09	0.0413
4	0.278	5.01	0.0497
5	0.288	5.19	0.0283
6	0.195	3.51	0.0730
7	0.190	3.42	0.0269
8	0.234	4.21	0.0281
9	0.732	13.19	0.0351
10	0.576	10.38	0.0405
11	0.498	8.98	0.0450
12	0.820	14.78	0.1548
13	0.102	1.83	0.0139
14	0.546	9.84	0.0567
15	0.689	12.42	0.0655
16	0.625	11.27	0.0927
17	0.507	9.14	0.0581
18	0.630	11.36	0.0369
19	0.239	5.22	0.0414
20	0.786	14.17	0.2020
21	0.883	15.92	0.1039
22	0.834	15.03	0.0803
23	0.732	13.19	0.0426
24	0.566	10.20	0.0395
25	0.703	12.67	0.0557
26	0.439	7.91	0.0335
27	0.161	2.90	0.0216
28	0.395	7.12	0.0602
29	0.502	9.05	0.0473
30	0.342	6.16	0.0221
31	0.576	10.38	0.0136
32	0.654	11.79	0.0356
33	0.771	13.90	0.0855
34	0.478	8.61	0.0863
35	0.527	9.50	0.0383
36	0.615	11.09	0.0792
37	0.346	6.23	0.0270
38	0.229	4.12	0.0228
39	0.136	2.45	0.0120

rently used to assess lactose absorption. Since there is no other metabolic production of hydrogen, pulmonary excretion may be used as a measure of bacterial fermentation of non-absorbed lactose by the colonic microflora. Usually 50-gram of lactose is administered after an overnight fast. The hydrogen breath test requires gas liquid chromatography; other disadvantages are the dependency on an appropriate colonic flora and fecal pH, the need for low fasting hydrogen concentrations and the consideration of previous use of antibiotics. The possibility of a false negative breath test due to inability of the colonic microflora to produce H2 is still a matter of discussion, moreover it may be caused by hyperventilation, gastric emptying time, antibiotics, and reduction of hydrogen-generating bacteria due to acute diarrhea (3, 9). The prevalence of non H2 -producing subjects has been described from nearly absent to about 10% (10-13). Also, false positive findings my occurr in long and deep sleepers, smokers, and patients who ate beans the previonus night (3, 9).

14C-labelled 50-gram lactose is given at another test. Absorbed lactose is metabolized and the 14C atom is exhaled as 14CO2. Disadvantages are its technical complexity, associated cost and radiation (3, 9).

Small bowel lactase activity is determined according to the method of Dahlqvist (14). However, because of variable mucosal lactase activity along the small bowel, this method also cannot be

Table 3. The prevalence estimates for lactose malabsorption in selected European and Asian countries (1, 15, 16)

Country	*%*	*Country*	*%*
Türkiye (Turkey)	71-84	Finland (Finnish)	17
Lebanon	78	Finland (Swedish)	8
Jordan (Arabs)	75	Finland (Lapps)	25-60
Jordan (Bedouins)	24	Denmark	4
Israel (Jews)	71	Great Britain	5
Israel (Arabs)	81	Germany (West)	15
Afghanistan	83	Germany (East)	22
Pakistan (Punjabi)	60	Poland	37
India (North)	27	Hungary	37
India (Central)	63	Austria	20
India (South)	67	Slovenia	35
Thailand (North)	100	France	37
Thailand (Central)	97	France (North)	23
Taiwan (ROC)	89	Italy (North)	51
China (Beijing)	92	Italy (Central)	19
China (Shanghai)	86	Italy (South)	41
China (Mongols)	88	Sicily	71
China (Kazakhs)	76	Estonia	23-32
Greece	75	Lithuania	38
Japan	90		

regarded as the gold standard of hypolactasia (3, 9).

Since the methods described are relatively labor-intensive, researchers and clinicians have been searching for alternative ways of measuring hypolaclasia. Probably the most attractive appears to be the measurement of total galactose and galactose/creatinine ratios in a two-hour-urine collection (1, 2). Measurement of galactose concentration in a urine sample has been reported as a suitable screening test for lactose malabsorption in two different settings (5, 6). We assayed both galactose concentration and galactose/creatinine ratio simultaneously. We found the galactose concentration is consisted in the galactose/creatinine ratio, and was not dependent on the volume of urine produced.

When the cut-off limit of the urinary galactose/creatinine ratio has been accepted as 0.08; we found the sensitivity and specificity to be 100% and 82% respectively. One can say that the ratio of urinary galactose to creatinine does work well in screening lactose malabsorption, however, urinary galactose concentration is useful in mass screening. Grant et al. compared the ratio of urinary galactose to creatinine for lactose absorbers (0.23+/-0.02) with malabsorbers (0.04+/-0.004), and showed good discrimination between the two groups ($p<0.001$) (6).

The findings of our study are consistent with the known lactose malabsorption prevalence of 70% for adults in Turkey, and are close to adult values reported in southeastern Europe, the Near East and Middle East (Table 3). Adult-type lactose malabsorption is the normal state for most humans. The marked differences between different countries have been explained by the domestication of dairy animals. With the domestication of these animals, the adult ingested more milk. In cultures with more recently domesticated dairy animals, the prevalence of adult lactase deficiency might be higher (9).

A recent study of the prevalence of lactase deficiency in Turkey showed the same rates. The hydrogen breath test (after a maximum of 50 g lactose load) was used in the study and the prevalence of lactase deficiency was found to be 84% in adults (15).

It is known that there is no effect of age or sex on the prevalence of lactose malabsorption in adults and childhood (9, 16). Differences between lactase deficiency, lactose malabsorption and lactose intolerance can be explained by variations in the

emptying time of the stomach, lactose load, intestinal motility, and bacterial flora. Lactose malabsorption can also be identified in patients other than those with lactose intolerance symptoms. This is possible with patients suffering from an unexplained dyspeptic complaint or recurrent abdominal pain. Questioning of intolerance symptoms is not sufficient, given the frequency of lactose malabsorption found in our study and others. The lactose absorption test, by determining galactose excretion and/or galactose/creatinine ratios in urine is an easy and non-invasive screening tool.

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