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A biopsy-based quick test in the diagnosis of duodenal hypolactasia in upper gastrointestinal endoscopy

Background and study aims: The usefulness of a new quick test for endoscopic diagnosis of adult-type hypolactasia was tested in duodenal biopsies. In this test, an endoscopic biopsy from the postbulbar duodenum is incubated with lactose on a test plate, and a color reaction develops within 20 min as a result of hydrolyzed lactose (a positive result) in patients with normolactasia, whereas no reaction (a negative result) develops in patients with severe hypolactasia.

Patients and methods: Two postbulbar duodenal biopsies were taken from 80 prospectively enrolled adult outpatients with dyspepsia. The biopsies were used for the Quick Lactase Test (Biohit PLC, Helsinki, Finland) and in biochemical disaccharidase (lactase, sucrase, and maltase) assays. In addition, the C/T_{-13910} genotype was determined from DNA extracted from gastric antral biopsies using polymerase chain reaction sequencing in genomic analysis of adult-type hypolactasia.

Results: Twenty-one of 22 patients (95%; 95% CI, 87–100%) with biochemical lactase activity < 10 U/g protein, but none of the 58 patients with lactase activity of 10 U/g protein or more had a negative result in the Quick Lactase Test. Seven of the 80 patients (9%; 95% CI, 3–15%) had a Quick Lactase Test result that indicated mild hypolactasia (a mild color reaction). All patients with celiac disease (n = 6) had a negative Quick Lactase Test result. Nine of 74 patients (six patients with celiac disease were excluded) had a CC_{-13910} genotype in genomic testing, indicating adult-type hypolactasia. All of them had negative test results with the Quick Lactase Test. Twenty-six patients had a TT genotype, indicating normolactasia, and none of these patients had a negative test result in the Quick Lactase Test. Six of 39 patients (15%; 95% CI, 4–27%) with a CT genotype had a negative result in the Quick Lactase Test.

Conclusions: The Quick Lactase Test effectively identifies patients with severe duodenal hypolactasia. In comparison with CC (adult-type hypolactasia) and TT individuals (normolactasia), the sensitivity and specificity of the Quick Lactase Test result was 100%. In comparison with biochemical lactase assays, the sensitivity and specificity of a negative Quick Lactase Test for indicating hypolactasia (lactase activity < 10 U/g protein) were 95% (95% CI, 87–100%) and 100%, respectively.

Introduction

A decline in lactase activity in the intestinal mucosa after childhood leads to a condition known as adult-type hypolactasia or lactase nonpersistence. If an affected individual is exposed to dairy products, clinical symptoms such as abdominal pain, distension, cramp, flatulence, nausea, and diarrhea may develop due to unhydrolyzed lactose in the intestine [1]. Adult-type hypolactasia represents a normal physiological condition after weaning [2]. However, approximately half of the world's popula-

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tion have the ability to maintain lactase activity in adulthood, and they digest lactose throughout life (lactase persistence, "normolactasia"). Lactase persistence is a dominantly inherited state and is frequent in populations adapted to dairy products, especially in Northern Europe [3–5]. A variant, C/T_{-13910}, located about 14 kb upstream from the initiation codon of lactase on chromosome 2q21, has been shown to be associated with lactase persistence/nonpersistence [6–9].

The diagnosis of adult-type hypolactasia is usually based on the lactose tolerance test, the specificity of which has been reported to be in the range of 77–96%, with a sensitivity of 76–94% [10]. For the breath hydrogen test, the specificity is reported to be 89–100% and the sensitivity 69–100%. [10]. A classic reference method has been direct biochemical assay of disaccharidase activity in duodenal or intestinal biopsies [11]. The most recent test involves determination of the C/T_{-13910} genotype from blood lymphocytes [6].

Patients who undergo a diagnostic upper gastrointestinal endoscopy may have abdominal symptoms that are related to hypolactasia in the small intestine and to intolerance of dairy products. A novel biopsy-based method for rapid endoscopic diagnosis of duodenal hypolactasia has recently been developed. This method is based on incubation of a postbulbar endoscopic duodenal biopsy with lactose in a plate in which a strong color reaction develops within 20 min if the lactase activity is "normal" and if glucose appears in the test plate from hydrolyzed lactose.

This study investigated the usefulness of this novel Quick Lactase Test by comparing it with direct assays of lactase activity from duodenal biopsies biochemically, and with those obtained by assaying the C/T_{-13910} variant.

Patients and methods

Patients

Eighty outpatients (mean age 47 ± 16 years; 30 men, 50 women) were prospectively enrolled and underwent diagnostic upper gastrointestinal endoscopy (esophagogastroduodenoscopy) for dyspeptic symptoms at Helsinki University Central Hospital/Jorvi Hospital in 2003. In addition to routine biopsies for microscopy (postbulbar and bulbar duodenum, antrum, and gastric body), two extra biopsies were taken from the postbulbar duodenum. One of these biopsies was used in the Quick Lactase Test and the other in biochemical assays of disaccharidase activity [11].

Symptom recordings

Before endoscopy, all of the patients were asked about their use and tolerance of dairy products. Those who did not use dairy products or had symptoms caused by them, and those with a positive lactose intolerance test in their medical history, were regarded as symptom-positive patients (with "milk intolerance", symptom grade 1). Those who used dairy products without subjective symptoms were regarded as symptom-negative patients (with "milk tolerance", symptom grade 0).

Reagents

Peroxidase, hexokinase, glucose-6-phosphate dehydrogenase, and adenosine triphosphate (ATP) were obtained from Roche Diagnostics, Mannheim, Germany. Glucose oxidase was obtained from Fluka BioChemica, Buchs, Switzerland, and lactose from Merck, Darmstadt, Germany. NADP, maltose, sucrose and TMB2HCl (3,3',5,5'-tetramethylbenzidine dihydrochloride) were obtained from Sigma-Aldrich Chemie, Steinhem, Germany.

Preparation of homogenate samples

Biopsy specimens were stored at –70 °C and weighed in a cold room with a temperature of –20 °C. Glass homogenizers were used, and homogenization was carried out in crushed ice to avoid warming of the sample. Each biopsy specimen was homogenized in 200 µl of 0.9% NaCl.

Disaccharidase activity measurement

Disaccharidase measurement was carried out in a water bath at 37 °C in thin-walled polymerase chain reaction tubes principally in accordance with the method described by Dahlqvist [11]. The disaccharidase reaction was started by adding a substrate buffer solution (lactose, sucrose, or maltose in a 0.1-mol/l sodium maleate buffer, pH 6.0) to a final concentration of 0.26 mol/l. In addition, a blank sample was prepared by adding water instead of the substrate solution. The total reaction time at 37 °C was 60 min. Thereafter, the reaction was stopped by placing the tubes in ice and adding perchloric acid at a final concentration of 0.43 mol/l and shaking. The samples were then neutralized with 0.34 mol/l KOH – 0.075 mol/l imidazole base – 0.075 mol/l KCl. The precipitate that was formed in neutralization was centrifuged down, and liberated glucose in supernatant was measured fluorometrically with a Transcon 102 FN analyzer (Biohit PLC, Helsinki, Finland) using a hexokinase/glucose-6-phosphate dehydrogenase reaction with NADP as a cofactor [12]. The samples were diluted to 40 times the original volume in a reaction buffer solution containing 0.14 U/ml glucose-6-phosphate dehydrogenase, 0.1 mmol/l $NADP^+$, 0.5 mmol/l dithiothreitol (DTT), 0.3 mmol/l ATP, 1 mmol/l EDTA, 5 mmol/l $MgCl_2$ and 50 mmol/l Tris-HCl buffer pH 8.1. The buffer blank was measured and the reaction was started by adding hexokinase (in 20 mmol/l Tris-HCl buffer pH 8.1) to a final concentration of 0.35 U/ml. Biochemical duodenal lactase activity correlated well with the lactase–sucrase ratio (L/S ratio) (Figure **1**). The lower the lactase activity was, the smaller was the L/S ratio as well. There were six patients with celiac disease and all of them had lactase activity < 10 U/g protein and low L/S ratios < 0.25.

Protein determination

Protein concentrations in the homogenate samples were determined using the BioRad Dc Protein A Assay with BSA as a standard (BioRad Laboratories, Hercules, California, USA) in accordance with the manufacturer's instructions.

Calculation of the results and statistics

The results are expressed as a U (µmol substrate/min at 37 °C) disaccharidase/g protein. In the statistical analysis, both parametric tests (Student's *t*-test after logarithmic transformation if necessary) and nonparametric tests (the chi-squared test) were used. In addition, 95% confidence intervals (95% CI) were calculated when appropriate.

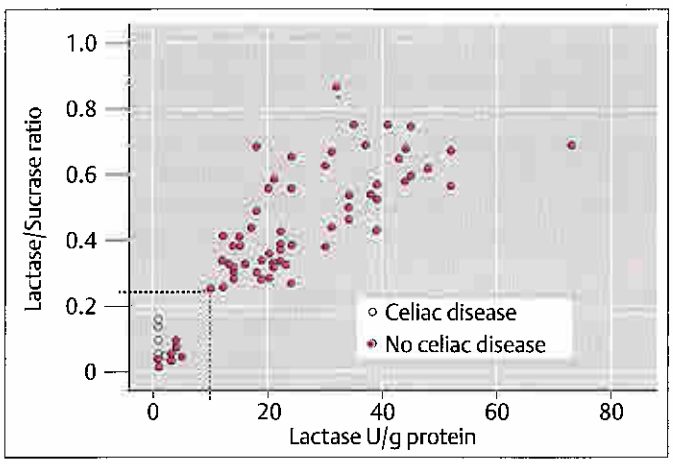

Figure **1** Biochemically assayed lactase activity and ratio of lactase–sucrase (L/S) activity in endoscopic biopsies from the postbulbar duodenum in the study population (80 patients). Patients with lactase activity < 10 U/g protein and an L/S ratio < 0.25 are indicated by dotted lines.

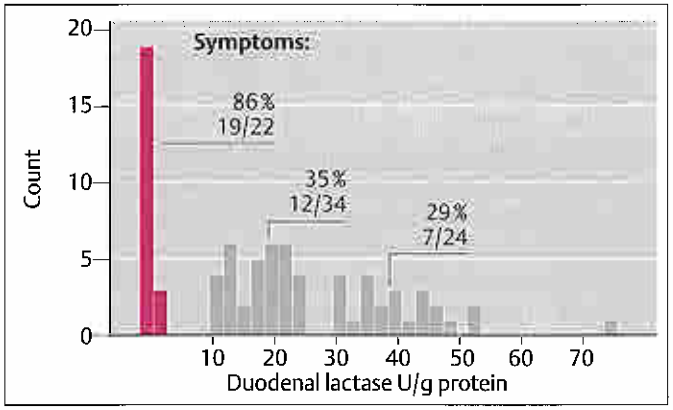

Figure **2** Histogram of the distribution of biochemical lactase activity in the whole study population. The prevalence of milk-related symptoms in three separate subgroups is shown.

Quick test assay of lactase activity from duodenal biopsy specimens

The Lactose Intolerance Quick Test Kit (Biohit PLC, Helsinki, Finland) was used in endoscopic assays of duodenal lactase activity. The kit includes a plate for a biopsy specimen and drop bottles containing reagent solutions. The test was carried out in accordance with the manufacturer's instructions by placing the biopsy sample in the well and adding each of the reagent solutions into the well with the specified volume, sequence, and time. Briefly, the reaction was carried out in two steps at room temperature as follows: a 15-min lactase reaction, followed immediately by a 5-min signal reaction in which liberated glucose is measured by the glucose oxidase/peroxidase reaction.

In order to quantify the Quick Lactase Test, a color chart was constructed and calibrated with simultaneous biochemical determination of enzyme activity (see above) and the Quick Test in biopsy specimens from the same area in the postbulbar duodenum of the same patient. No color reaction corresponds to lactase activity less than 10 U/g protein (severe hypolactasia); a mild blue reaction corresponds to 10–30 U/g protein (mild hypolactasia); and a deep blue reaction corresponds to 30 U/g protein or more (normolactasia).

DNA isolation, polymerase chain reaction, and direct sequencing

The C/T_{-13910} genotype was determined by direct sequencing. DNA was isolated from paraffin-embedded blocks (biopsies from the gastric antrum) using a genomic DNA purification system kit (Puregene, Gentra Systems, Minneapolis, Minnesota, USA), as described previously [7].

Ethics

The study was approved by the Ethics Committee of Helsinki District University Hospital, Helsinki, Finland. The purpose of the study was explained to all of the patients before the endoscopic examinations, and all of the patients provided written consent before enrolment in the study.

Results

Prevalence of symptoms from dairy products

The histograms of the biochemical lactase activity in duodenal biopsies from all 80 patients in the study population are shown in Figure **2**. At least two, possibly three, different subpopulations appear: those with lactase activity < 10 U/g protein and those with lactase activity of 10 U/g protein or more. In the first subgroup, 19 of 22 patients (86%; 95% CI, 72–100%) did not use dairy products or had symptoms caused by milk more often than the rest of the patients with duodenal lactase 10 U/g protein or more (19 of 58 patients, 33%; 95% CI, 21–45%; $P < 0.001$).

Biochemical lactase activity and Quick Lactase Test

Among the 80 patients, there were 22 patients with duodenal lactase activity < 10 U/g protein, all of whom had a lactase–sucrase ratio (L/S) of < 0.25 (see also Figure **1**). Of these, 21 (95%; 95% CI, 87–100%) had a negative result ("severe hypolactasia") in the Quick Lactase Test. None of the 58 patients with lactase activity of 10 U/g protein or more showed a negative result in the Quick Lactase Test. However, in seven of the 80 patients (9%; 95% CI, 3–15%), the Quick Lactase Test produced a slightly positive reaction ("mild hypolactasia"). In these patients, the mean and median figures for biochemical lactase activity lay between those of the Quick Lactase Test-negative ("severe hypolactasia") and Quick Lactase Test-positive groups ("normolactasia") (Figure **3**).

Six patients were found to have celiac disease and atrophy of the duodenal villi on microscopy. All of them had biochemical lactase activity < 10 U/g protein and an L/S ratio < 0.25. All of them had negative results (severe hypolactasia) with the Quick Lactase Test (Figure **1**).

C/T_{-13910} polymorphism and Quick Lactase Test

A comparison of the Quick Lactase Test results with the C/T_{-13910} polymorphism findings is presented in Table **1**. The six patients with celiac disease were excluded from this analysis. All nine patients with the C/C_{-13910} genotype (indicating down-regulation of the lactase gene in adulthood) had a negative result, but none of

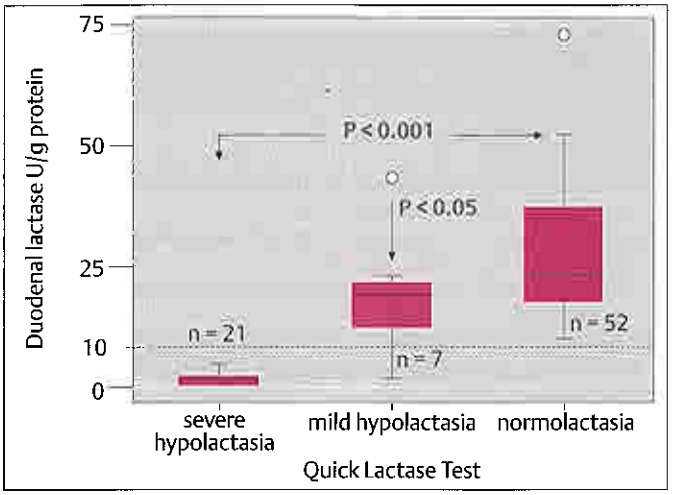

Figure **3** Box-plot presentation of the relationship between the Quick Lactase Test and biochemical activity of duodenal lactase. The boxes present the intermediate 50% of cases. The vertical line indicates the median case. The dotted line shows the duodenal lactase activity level (10 U/g protein) used as the cut-off value for the presence of milk-related symptoms (see Figure **2**).

Table **1** Relationship between the Quick Lactase Test and C/T_{-13910} polymorphism (six patients with celiac disease are excluded). Differences in the frequency of patients with hypolactasia and normolactasia are significant ($P < 0.001$; contingency table for chi-squared test) between different polymorphic genotypes

Genotype	*Quick Lactase Test*			
	Hypolactasia			
	Severe	*Mild*	*Normolactasia*	**Total**
CC	9	0	0	9
CT	6	5	28	39
TT	0	2	24	26
Total	15	7	52	74

the 26 patients with the T/T_{-13910} genotype (indicating persistence of lactase activity in adulthood) had a negative result in the Quick Lactase Test. Six of 39 patients (15%; 95% CI, 4–27%) with the C/T_{-13910} genotype showed a negative result in the Quick Lactase Test ("severe hypolactasia"). All of these patients had biochemical duodenal lactase activity less than 10 U/g protein and an L/S ratio < 0.25.

Discussion

The present study indicates that the biopsy-based Quick Lactase Test provides good differentiation between patients with severe hypolactasia and those with normal lactase activity. The sensitivity and specificity of the negative Quick Lactase Test for indicating severe hypolactasia (lactase < 10 U/g protein) were 95% (95% CI, 87–100%) and 100%, respectively, in comparison with biochemical lactase activity in the duodenum. When the results of

In brief

The Quick Lactase Test is intended for rapid endoscopic diagnosis of adult-type hypolactasia in endoscopic biopsies from postbulbar duodenum. The test shows a very high accuracy when compared with the biochemical lactase activity in additional duodenal biopsies and with the genomic C/T_{-13910} polymorphism known to associate with adult type hypolactasia.

the Quick Lactase Test were compared with C/T_{-13910} polymorphism, the sensitivity and specificity of the Quick Lactase Test for indicating hypolactasia were 100%. All nine patients with the C/C_{-13910} genotype (indicating adult-type hypolactasia) and none of the 26 patients with the T/T_{-13910} genotype (indicating normolactasia) had a negative Quick Lactase Test result. In addition, all six patients with celiac disease had severe hypolactasia (duodenal lactase activity < 10 U/g protein and L/S ratio < 0.25), and all showed a negative Quick Lactase Test.

There were seven patients (9%) in the present study population in whom the Quick Lactase Test showed a slightly positive test result ("mild hypolactasia" in the Quick Lactase Test). In these individuals, the mean and median values for biochemical lactase activity were intermediate to those seen with clearly negative or positive Quick Lactase Test findings. These individuals also tended to be heterozygous for the C/T_{-13910} polymorphism. This suggests that heterozygosity of the C/T_{-13910} allele results in half of the ability to down-regulate the lactase gene in adulthood in comparison with C/C_{-13910} homozygotes. However, in the present series, milk-related symptoms appeared to be associated in particular with severe hypolactasia – i.e., with patients in whom duodenal lactase activity was less than 10 U/g protein, the cut-off that is commonly used to indicate severe and symptomatic hypolactasia [13].

In summary, the present investigation shows that a negative result in the Quick Lactase Test indicates severe hypolactasia with a high degree accuracy and that a positive test result indicates normolactasia with a high level of sensitivity and specificity. The test is handy and practical. Its use and costs correspond to those of the quick urease test that is commonly used for rapid and endoscopic diagnosis of *Helicobacter pylori* infection in gastric biopsies.

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Competing interests: Prof. Pentti Sipponen and Prof. Matti Härkönen are scientific advisors of Biohit PLC.

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