Comparison of Quick Lactose Intolerance Test in duodenal biopsies of dyspeptic patients with single nucleotide polymorphism *LCT*-13910C>T associated with primary hypolactasia/lactase-persistence[1]

Comparação do Teste Quick de Intolerância à Lactose em biópsias duodenais de pacientes dispépticos com polimorfismo de nucleotídeo único *LCT*-13910C>T associado com hipolactasia primária/lactase persistente

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ABSTRACT

PURPOSE: To analyze the usefulness of Quick Lactose Intolerance Test in relation to the genetic test based on *LCT*-13910C>T genotypes, previously validated for clinical practice, for primary hypolactasia/lactase-persistence diagnosis.

METHODS: Thirty-two dyspeptic patients that underwent upper gastrointestinal endoscopy entered the study. Two postbulbar duodenal biopsies were taken for the Quick test, and gastric antral biopsy for DNA extraction and *LCT*-13910C>T polymorphism analysis. DNA was also extracted from biopsies after being used in the Quick Test that was kept frozen until extraction.

RESULTS: Nine patients with lactase-persistence genotype (*LCT*-13910CT or *LCT*-13910TT) had normolactasia, eleven patients with hypolactasia genotype (*LCT*-13910CC) had severe hypolactasia, and among twelve with mild hypolactasia, except for one that had *LCT*-13910CT genotype, all the others had hypolactasia genotype. The agreement between genetic test and quick test was high ($p<0.0001$; Kappa Index 0.92). Most of the patients that reported symptoms with lactose-containing food ingestion had severe hypolactasia ($p<0.05$). Amplification with good quality PCR product was also obtained with DNA extracted from biopsies previously used in the Quick Test; thus, for the future studies antral gastric biopsies for genetic test would be unnecessary.

CONCLUSION: Quick test is highly sensitive and specific for hypolactasia diagnosis and indicated those patients with symptoms of lactose intolerance.

Key words: Lactose Intolerance. Endoscopy. Biopsy. Nutrition, Public Health.

RESUMO

OBJETIVO: Analisar a aplicabilidade do Teste Quick de Intolerância à Lactose em relação ao teste genético baseado nos genótipos *LCT*-13910C>T, previamente validado para a prática clínica, para diagnóstico de má digestão primária de lactose/digestão de lactose.

MÉTODOS: Trinta e dois pacientes dispépticos submetidos à endoscopia digestiva entraram no estudo. Duas biópsias duodenais pós-bulbares foram empregadas no Teste Quick, e biópsia do antro gástrico para extração de DNA e análise do polimorfismo *LCT*-13910C>T. DNA também foi extraído de biópsias depois de terem sido usadas no teste Quick, e conservadas congeladas.

RESULTADOS: Nove pacientes com genótipo de lactase persistente (*LCT*-13910CT ou *LCT*-13910TT) tinham normolactasia, onze pacientes com genótipo de hipolactasia (*LCT*-13910CC) tinham hipolactasia severa, e entre doze com hipolactasia leve, com exceção de uma que tinha genótipo *LCT*-13910CT, todos os demais tinham genótipo de hipolactasia. A concordância entre o teste genético e o Quick Teste foi alta ($p<0,0001$; Índice Kappa=0,92). A maioria dos pacientes que relataram sintomas com ingestão de alimentos com lactose tinham hipolactasia severa ($p<0,05$). Amplificação com produto de PCR foi obtido com DNA extraído das biópsias usadas no teste Quick; portanto, nos trabalhos futuros seria desnecessário coletar biópsia do antro gástrico para o teste genético.

CONCLUSÃO: O Teste Quick é altamente sensível e específico para diagnóstico de hipolactasia e indicou aqueles pacientes com sintomas de intolerância à lactose.

Descritores: Intolerância à Lactose. Endoscopia. Biópsia. Nutrição em Saúde Pública**.**

Introduction

Lactase or lactase-phlorizin hydrolase, located in the brush border of intestinal mucosa, is responsible for the hydrolysis of lactose, the major carbohydrate present in milk, into galactose and glucose. The maximal activity of lactase is during perinatal period; however, in most humans of different ethnic groups, it declines at some point during life, emerging two groups: lactase-persistence (normolactasia, lactose digestion) and lactase non-persistence (hypolactasia, lactose maldigestion). Undigested lactose is fermented by the intestinal flora, producing hydrogen, methane, carbon dioxide and short-chain fatty acids, which cause symptoms of lactose intolerance, flatulence, bloating, abdominal pain and diarrhea, depending on the amount of ingested lactose[1].

Symptoms suggestive of lactose intolerance are unspecific and may be related to other causes such as irritable bowel syndrome, cow's milk protein allergy, bacterial overgrowth, celiac disease, and inflammatory bowel disease, or other dietary sources of intestinal gas, such as beans, which contain two indigestible sugars, stachyose and raffinose[1]. Self-perceived lactose intolerance may lead to unnecessary avoidance of milk and dairy products, the main source of calcium in the diet, with future consequences on bone health[1], hypertension, and diabetes[2]. Thus, diagnosis of lactose maldigestion imposes for adequate clinical management and correct lactose-restriction diet.

The diagnosis of lactose maldigestion was initially performed by lactase activity in jejunal biopsies[3], being replaced by duodenal biopsies; nonetheless, the mean lactase activity in the duodenum is lower than in the jejunum, being less reliable[4]. Quick Lactose Intolerance Test in postbulbar duodenum samples showed sensitivity and specificity of 95% and 100%, respectively[5], being more sensitive than lactose breath test[6,7], and highly sensitive in a pediatric population[8].

Lactose tolerance test, based on blood glucose levels measured before and after an oral load of lactose, indicates lactose tolerance when the rise of serum glucose is ≥20 mg/dL, although widely performed is less sensitive than the lactose tolerance hydrogen breath test, considered the gold standard. Both tests provoke symptoms in those with lactose maldigestion, are long-standing, and cumbersome; the hydrogen breath test depends on the activity of the intestinal flora to ferment undigested lactose[9].

In a Brazilian population, using the single nucleotide *LCT*-13910C>T polymorphism of lactase-persistence, only 24.7% of Europeans descent and 18.3% of African origin subjects had lactase-persistence genotype[1], and showed an excellent correlation with lactose tolerance test results[10]. Thus, the purpose of this study was to compare the results of Quick Lactose Intolerance Test with *LCT*-13910C>T genotypes to achieve its clinical application for hypolactasia diagnosis.

Methods

This prospective research protocol was approved by the Research Ethics Committee of the Clinics Hospital of Ribeirão Preto Medical School of the University of São Paulo (Protocol 8171/2012). Subjects that were routinely enrolled to perform the upper digestive endoscopy for some clinical investigation were invited to participate, and gave written informed consent. Exclusion criteria were signs of bleeding, fistulas, or recent high digestive sutures (<7 days), and celiac disease.

A questionnaire of dietary lactose containing food ingestion and related symptoms was applied (Chart 1).

CHART 1 – Dietary lactose intolerance survey applied before the endoscopic procedure.

The amount of ingested food and periodicity	YES	NO
Milk (all types)		
Condensed milk		
Cream milk		
Ice cream and milk shakes		
Chantilly		
Puddings in general (including flans)		
Butter		
Margarine		
Cheese (all types)		
Yogurt in general (including curd)		
White sauce		
Cream soups ready to use		
Cakes		
Pizzas/pasta and homemade doughnuts		
Cookies		
Pancake		
Chocolate		
Chocolate milk (and other flavored milk)		
Others (candies, caramel, potato puree, some salad sauces, soufflés)		

Thirty-two dyspeptic patients (mean age 50.2±17.5 years, 59.4% females), submitted to routine upper digestive endoscopy in the Digestive Endoscopy Center of Clinics Hospital of Ribeirão Preto Medical School of the University of São Paulo, entered the study. Twenty-three (72%) subjects were Caucasian and 9 were African-Brazilians.

Quick lactose intolerance test

Two specimens of biopsy of the postbulbar region (2 mm each) were taken to perform the Quick Test (Biohit, Helsinki, Finland) for lactase activity assay, according to the manufacturer instruction. The specimens were placed into the well of the test plate immediately after its collection; two drops (80 µl) of a substrate solution were added and incubated for 15 minutes. After this step 2 drops of chromogen solution (10 µl) in acetic acid and 1 drop (80 µl) of signaling enzymatic solution were added with incubation time of 5 minutes. The total reaction time was 20 minutes. The test result was colorimetric: a dark blue coloring, indicating normolactasia (lactase activity), light blue color mild hypolactasia and colorless severe hypolactasia.

LCT-13910C>T single nucleotide polymorphism analysis

DNA was extracted by salting out from specimens of the antrum[11], and used in the polymerase chain reaction and restriction fragment length polymorphism analysis, as was previously described[10]. Two biopsies after being used in the Quick Test were frozen for DNA extraction in order to see viability to avoid collection of biopsies from the gastric antrum for the genetic test. Digested PCR products with *Hinf*I were visualized on a 3% low melting point agarose gel stained by ethidium bromide. Samples showing a single band of 201 bp were classified as the *LCT*-13910CC genotype (hypolactasia or lactase non-persistence), a single band of 177 bp as the *LCT*-13910TT genotype (normolactasia, lactase-persistence), and two bands of 201 bp and 177 bp the *LCT*-13910CT genotype (normolactasia, lactase-persistence).

Statistical analysis

Statistical analysis was performed by Kappa index measure of agreement of diagnostic tests, and Chi-square or Likelihood ratio using SPSS version 15.0 for Windows (Chicago, Illinois, USA). A p value of <0.05 was considered statistically significant.

Results

There was no significant association of ethnicity and gender with *LCT*-13910C>T genotypes, the Quick Test results, and the presence of symptoms with milk and/or dairy products consumption, despite Caucasians being less symptomatic (Chart 2).

CHART 2 – Demographic data, symptoms with lactose containing food, Quick Test and *LCT*-13910C>T genotypes results.

Case	Age	Gender	Ethnicity	Symptoms with lactose	Quick Test	Genotype
1	71	F	Caucasian	No	Normo	TT
2	66	M	Caucasian	No	Severe	CC
3	27	F	Caucasian	Milk, dairy products	Severe	CC
4	37	F	African	No	Normo	CT
5	38	F	African	Milk, dairy products	Severe	CC
6	27	M	Caucasian	Milk, dairy products	Severe	CC
7	65	M	Caucasian	No	Normo	TT
8	54	M	Caucasian	No	Severe	CC
9	38	M	Caucasian	No	Mild	CC
10	78	F	Caucasian	No	Mild	CC
11	58	F	Caucasian	No	Mild	CC
12	46	M	African	Milk	Severe	CC
13	55	F	African	Milk, dairy products	Severe	CC
14	51	F	Caucasian	Milk, dairy products	Mild	CC
15	46	F	Caucasian	Milk	Normo	CT
16	18	F	Caucasian	No	Normo	CT
17	31	M	Caucasian	No	Severe	CC
18	88	F	Caucasian	No	Mild	CC
19	59	M	Caucasian	No	Mild	CC
20	53	M	Caucasian	No	Normo	CT
21	61	M	Caucasian	No	Mild	CC
22	28	F	Caucasian	No	Normo	CT
23	58	F	African	Cheese, Pasta	Severe	CC
24	53	F	Caucasian	No	Normo	CT
25	17	M	Caucasian	No	Mild	CC
26	58	F	African	No	Mild	CC
27	44	F	Caucasian	Milk	Severe	CC
28	71	F	African	Milk, great amount	Severe	CC
29	39	F	African	No	Mild	CT
30	73	M	Caucasian	No	Mild	CC
31	59	M	Caucasian	No	Normo	TT
32	39	F	African	No	Mild	CC

Nine patients (41%) with hypolactasia genotype and one (10%) with lactase-persistence genotype reported symptoms with milk and/or dairy products consumption, $p>0.05$ (Table 1).

TABLE 1 – Presence of symptoms with milk and/or other lactose-containing food consumption in patients with hypolactasia and lactase-persistence genotypes, and in groups according to the Quick Test results.

LCT-13910C>T Genotypes	With symptoms	No symptoms	Total
Hypolactasia genotype	9 (41%)	13 (59%)	**22 (100%)**
Lactase-persistence genotype[a]	1 (10%)	9 (90%)	**10 (100%)**
Total	10 (31.2%)	22 (68.8%)	**32 (100%)**
Quick Test Results			
Normolactasia	1 (11.1%)	8 (88.9%)	**9 (100%)**
Mild Hypolactasia	1 (8.3%)	11 (91.7%)	**12 (100%)**
Severe hypolactasia[b]	8 (72.7%)	3 (27.3%)	**11 (100%)**
Total	10 (31.2%)	22 (68.8%)	**32 (100%)**

[a] p>0.05, [b]p<0.05

The association of symptoms with the distribution of the patients according to the Quick Test results was significant (p<0.05), showed that 8 (72.7%) of the patients with severe hypolactasia, one with mild hypolactasia and one with normolactasia had symptoms with lactose-containing food ingestion (p<0.05). One Caucasian woman 46 years old with lactase-persistence and normolactasia had symptoms with milk ingestion (Chart 2).

Nine patients with *LCT*-13910CT or *LCT*-13910TT genotypes had normolactasia, eleven patients with *LCT*-13910CC genotype had severe hypolactasia and among twelve with mild hypolactasia, except for one that had *LCT*-13910CT genotype, all the others were *LCT*-13910CC (Table 2). Agreement between the Quick Test and the genetic test was high (p<0.0001; Kappa index 0.92), considering *LCT*-13910CC as hypolactasia genotype, and *LCT*-13910CT or *LCT*-13910TT as lactase-persistence genotype, the sensitivity and the negative predictive value of the Quick Test were 100%, and the specificity and the positive predictive value were 90% and 95.6%, respectively.

TABLE 2 – Association of the *LCT*-13910C>T genotypes with the Quick Test results.

Genotypes	Normolactasia	Mild hypolactasia	Severe hypolactasia	Total
***LCT*-13910CC**		11	11	22
***LCT*-13910CT**	6	1		7
***LCT*-13910TT**	3			3
Total	9	12	11	32

P<0.0001; Kappa Index 0.92 considering CC (hypolactasia genotype) and CT and TT (lactase-persistence genotype)

Biopsies that were previously used in the Quick Test and used for DNA extraction, gave good quality PCR products; thus, gastric antrum biopsies could be avoid for the genetic test.

Discussion

The most important finding of this study is that comparing the genetic test with Quick lactose intolerance test, the latter indicates individuals with severe hypolactasia that are more prone to present symptoms of lactose intolerance, agreeing with previous report[5], rather than those with mild hypolactasia, even both presenting primary hypolactasia genotype. Another finding was that mild hypolactasia was associated with hypolactasia genotype rather than with lactase-persistence genotype, as was previously described by Kuokkanen *et al*.[5], although they showed that these individuals had intermediate levels of duodenal lactase activity, between those levels detected in the normolactasia group and in the severe hypolactasia group. This finding may explain the fact that some individuals with hypolactasia genotype are asymptomatic even ingesting a great amount of milk, suggesting that the levels of the physiological decline of lactase vary among different populations[12].

The association of primary hypolactasia genotype with symptoms after lactose-containing food ingestion was not significant, in contrast to previously reported in 1900 Finnish adults that showed a significant association of hypolactasia genotype, less milk intake, and the presence of gastrointestinal symptoms[13], the explanation for that is 50% of patients with hypolactasia genotype had mild hypolactasia. One Caucasian woman 46 years old with lactase-persistence and normolactasia, unexpectedly reported symptoms with milk ingestion that may

be caused by irritable bowel syndrome (IBS), as was suggested there may be other components of milk besides lactose that cause symptoms among subjects with IBS[14].

Even though the number of the patients analyzed in this study is lower than previous reported by other authors that compared the Quick Test with the genetic test, the results of sensitivity (100%) was similar[5]. Although presenting a high sensitivity the Quick Test has limitations, one is the size of the biopsies that if larger or shorter than 2 mm may give false negative or false positive results of hypolactasia, respectively. Another is the dependence on an invasive exam for collection of samples that not always is accepted by the patients that prefer a blood collection test. A biopsy-based gastrointestinal endoscopy exam is limited by the coagulation status, bleeding risks, and clinical conditions of the patient. Another point to consider is the Quick Test has to be performed immediately after collection of duodenum samples, requiring a laboratory technician in the endoscopy suite, as the incubation times have to be rigorously followed, and even being a very simple device, skillful in laboratory handling is necessary to perform the Quick Test.

The Quick Test is expensive (USD 30/individual test) for the Public Health Service compared to the genetic test, being more suitable for dyspeptic patients already undergoing endoscopy examination in private clinics for some clinical investigation. Additionally, the genetic test indicates primary hypolactasia and lactase-persistence[8]. However, those with lactase-persistence genotype may also present transitory hypolactasia along with celiac disease, Crohn's disease, or infectious enteritis diagnosis [1].

Outside the setting of tertiary referral centers genetic test may be considered more cumbersome than a gastrointestinal endoscopy with duodenal biopsy for the Quick Test analysis. Nonetheless, blood samples may be shipped to this referral centers for DNA-based genetic tests that have advantages: absence of lactose intolerance symptoms, single testing, non-invasive, and low cost[8].

In these case series an association of hypolactasia genotype with ethnic groups was not observed in contrast with our report in 567 brazilians[8], maybe the effect of the low number of patients.

Conclusions

Quick test is highly sensitive and specific to indicate subjects with hypolactasia and lactase persistence, and those more prone to present lactose intolerance symptoms. The genetic test is simple, non invasive, highly sensitive, and has a low cost.

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