The Prevalence of Primary and Secondary Lactase Deficiency in Patients with Celiac Disease

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Article

The Prevalence of Primary and Secondary Lactase Deficiency in Patients with Celiac Disease

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Abstract: Background: Celiac disease (CD) is defined as a systemic autoimmune disorder induced by gluten and other prolamins, which leads to gradual histopathological damage of the duodenum mucosa, intestinal villous atrophy, in particular. The brush border of enterocytes produces disaccharidases, including lactase. Lactase deficiency may be primary - genetically determined or secondary- due to damage to the intestinal villi. To exclude the primary cause of lactase deficiency, LCT gene polymorphism is necessary to evaluate. Objective: In patients diagnosed with CD, the intestinal villi should recover after strict adherence to the gluten-free diet, and therefore no lactase enzyme deficiency secondary to the underlying disease should be observed. Methods: The study group consisted of 38 patients, 30 women and 8 men (Group 1), who presented symptoms suggesting CD at the time of diagnosis, histology of duodenal mucosa samples revealed Marsh grade 3 and had confirmed the presence of HLA-DQ2 or DQ8 genes and had the gluten-free diet recommended. The control group consisted of 31 healthy volunteers, 18 women and 13 men. A hydrogen breath test (HBT) was performed in all groups with 50 g of lactose dissolved in 250 ml of water. Among patients with a positive HBT in all groups, a blood sample was collected to determine the C/T (-13910) and G/A (-22018) polymorphism in the promoter of the LCT gene responsible for the synthesis of the lactase enzyme. Among patients with lactase deficiency confirmed in HBT, LCT gene analysis was performed by assessing the C/T (-13910) and G/A (-22018) polymorphisms.Results: A significantly higher incidence of lactase enzyme deficiency was found in Group 1 (n=27, 71.1%) compared to the control group (n=10, 32.3%) ($p<0.01$). Severe lactase deficiency was observed more frequently in the group 1 than in the control group (n=19 (50%) vs. n=5 (16.1%); $p<0.01$), while mild and moderate lactase enzyme deficiency was observed with similar prevalence. Severe deficiency of the lactase enzyme was found significantly more frequently in the group of patient who did not strictly follow the diet than in the group declaring a strict adherence to a gluten-free diet (n=13 (65%) vs. n=6 (33%); $p<0.05$). No significant difference was found in the frequency of the C/T (-13910) and G/A (-22018) polymorphism in the promoter of the LCT between the analyzed groups with previously positive HBT test. Only in group 1, in 5 (18.5%) patients presence of LCT gene variants responsible for lactase enzyme deficiency was not detected. Conclusions: Among patients with CD, lactase deficiency was confirmed in the majority of the study participants, of whom only about half had primary lactase deficiency. In the study group, a correlation was demonstrated between severe deficiency of the lactase enzyme and non-adherence to gluten- free diet.

Keywords: celiac disease; gluten-free diet; lactose enzyme deficiency; LCT gene variants

1. Introduction

Celiac Disease

Celiac disease is defined as a systemic autoimmune disorder induced by gluten and prolamin derivatives in genetically predisposed individuals who present with a variety of gluten-dependent symptoms, tissue transglutaminase 2 specific-antibodies (TTG), histocompatibility antigens HLA-DQ2 and DQ8 (Human Leukocyte Antigen) presence and enteropathy [1,2].

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Persistent inflammation leads to gradual histopathological changes in the duodenum. The histopathological image of small intestinal mucosa was described and initially systematized by Marsh et al., and then modified by Oberhuber et al. During progressive immune reaction, an increase in the number of intraepithelial lymphocytes (IEL) >30 per 100 enterocytes is observed. In a further stage, there comes to intestinal crypt hyperplasia and the increase in the mitotic index. Then the inflammatory process leads to gradual destruction of intestinal villi until their complete atrophy [3–7].

Lactase Deficiency

Lactase is a digestive enzyme that splits lactose into D-glucose and D-galactose. It is produced in the brush border of enterocytes in the small intestine tract. A part of the population develops deficiency of the enzyme. Primary lactase deficiency, which is genetically determined, is mainly associated with the LCT gene polymorphism in two variants: C > T -13910 and G > A -22018 [8–11]. The frequency of genetic variants predisposing to the development of primary lactase deficiency varies between 21.4% and 97% depending on the studied population. Lactase deficiency is observed more frequently in Africa, Australia, Indonesia, East and North Asia, where there is no long-standing tradition of consuming dairy products [12–16]. In the studies conducted by Kuokkanen et al., and by Mądry et al., in groups of 303 and 200 randomly selected volunteers from Poland, respectively, the presence of the CC-13910 variant was confirmed in approximately 31.5-33.1% of the study participants [17,18].

Secondary lactase deficiency associated with damage to the brush border of enterocytes leads to a decrease in enzymatic activity. Diseases that may lead to secondary lactase deficiency include nonspecific inflammatory bowel disease, mainly Crohn's disease, CD, malabsorption syndrome and irritable bowel syndrome [19–21]. Secondary lactase deficiency is also observed in systemic diseases, including systemic sclerosis and cystic fibrosis [22–25]. It may also be of a temporary nature, secondary to bacterial and viral infections of the gastrointestinal tract, chronic use of nonsteroidal anti-inflammatory drugs and antibiotic therapy [26].

However, the presence of genetic variants contributing to lactase deficiency does not directly translate into a decrease in enzymatic activity. Further diagnostic tests are necessary to assess the actual enzyme deficiency presence. The hydrogen breath test (HBT) is the least invasive and at the same time not expensive diagnostic technique that provides reliable results [27,28].

Treatment of lactase deficiency is currently based mainly on an elimination diet excluding milk and dairy products. Moreover, oral enzyme supplementation is a common and recognized treatment method [29,30]. A correlation has also been demonstrated between the degree of lactase enzyme deficiency and the necessary dose of oral supplementation [31]. There are also ongoing studies evaluating the effectiveness of probiotics that split lactose into galactose and glucose, but their use does not bring more advantageous over the enzyme supplementation [32,33].

2. Assumptions and Objectives

According to the pathophysiology of celiac disease, enterocytes are damaged during its course, leading to a gradual villous atrophy and expansion of crypts. Disaccharidases, including lactase, are produced in the enterocyte brush border, located in the distal part of the intestinal villi. In patients diagnosed with celiac disease, the intestinal villi should recover after strict adherence to a gluten-free diet, and therefore no secondary lactase enzyme deficiency symptoms should be observed. In order to exclude regular, unconscious diet non-adherence, a detailed patient history should be taken and the serum concentration of anti-TTG antibodies should be determined. A gluten-free diet can be extremely problematic to follow and patients often non-intentionally miss the diet. To exclude the primary cause of lactase deficiency, it is necessary to assess the LCT gene polymorphism predisposing to its development. The study will assess the frequency of co-occurrence of celiac disease and primary lactase deficiency as well as the correlation between the gluten-free diet non adherence and the prevalence of lactase enzyme deficiency.

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3. Aim

1. Assessment of the prevalence of lactase deficiency and its severity among patients with celiac disease.
2. Searching for the correlations between the lactase deficiency presence, non- adherence to gluten-free diet and high levels of the tissue transglutaminase 2 specific-antibodies.

4. Material and Methods

Study and Control Groups

The study group consisted of 38 patients, 30 women and 8 men (Group 1), who presented symptoms suggesting celiac disease at the time of diagnosis, histology of duodenal mucosa samples revealed Marsh grade 3 and current examinations confirmed the presence of HLA-DQ2 or DQ8 genes. Those patients were presumably observing the gluten-free diet recommended by the treating physicians.

The control group consisted of 31 healthy volunteers, 18 women and 13 men.

The mean age of patients in the study group was 35.87 ± 10.74 years, whereas in the control group 40.13 ± 15.66 years.

Hydrogen Breath Test

A hydrogen breath test was performed in all groups with 50 g of lactose dissolved in 250 ml of boiled mineral water. None of the subjects had taken antibiotics for two weeks preceding the study, had no infection, nor took analgesics or laxatives for a few days prior to the study. The study participants were asked not to eat high-fiber, dairy or flatulence-inducing foods such as peas, beans, onions or cabbage the day before the test. The subjects were allowed to eat their last light meal approximately 14-16 hours before the test. They had not smoked cigarettes since the evening hours. On the day of the test, everyone was required to brush their teeth. Until the start of the test, the subjects were only allowed to drink still mineral water in moderate amounts and during the test they were not allowed to consume any liquids or food [34].

The measurement of hydrogen in exhaled air was performed using the Gastrolyzer Gastro+® device. The first measurement was performed before consumption of the lactose solution and then after it was consumed at 30-minute intervals for a total of 240 min.

An increase in exhaled hydrogen by 20 ppm was defined as lactase deficiency according to the applicable guidelines [35]. It was assumed that an increase in hydrogen in the exhaled air by 20-40 ppm after at least 60 minutes from the start of the test indicated a mild deficiency of the lactase enzyme, an increase between 40 and 80 ppm indicated moderate enzyme deficiency and a severe deficiency was diagnosed when the concentration of hydrogen in exhaled air exceeded 80 ppm [36,37].

Genetic Polymorphism in the LCT Gene

Among patients with a positive HBT test in all groups, a blood sample was collected to determine the C/T (-13910) and G/A (-22018) polymorphism in the promoter of the LCT gene responsible for the synthesis of the lactase enzyme.

Statistical Analysis

In the analyzed study, independent groups were compared. Nominal variables are presented as percentages. To compare two nominal variables, the Chi^2 test with Yates' correction or the exact two-tailed Fisher test was used, depending on the size of the study groups.

Continuous variables were tested for normality of distribution using the Shapiro-Wilk test. In the case of a normal distribution, variables are presented as means and standard deviations. Student's t-test was used to compare continuous variables in two groups of normal distribution. In the case of non-normal distribution, continuous variables were reported as medians and interquartile ranges

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(IQR). Mann-Whitney U test was used to compare two groups with non-normally distributed continuous variables.

The level of statistical significance was $p<0.05$.

Statistical analyses were performed using the Statistica 10 (Statsoft, Tulsa, USA).

The study protocol was approved by the Ethics Committee of the Medical University of Lodz No RNN/215/12/KE. All the participants provided informed consent.

5. Results

Assessment of the Prevalence of Lactase Enzyme Deficiency in the Study Group and the Control Group

Compliance and scrupulous adherence to the recommendations necessary for the correct performance of the hydrogen breath test described above, was declared by 100% of the study participants. A significantly higher incidence of lactase enzyme deficiency was found in Group 1 (n=27, 71.1%) compared to the control group (n=10, 32.3%) ($p<0.01$) (Figure 1)

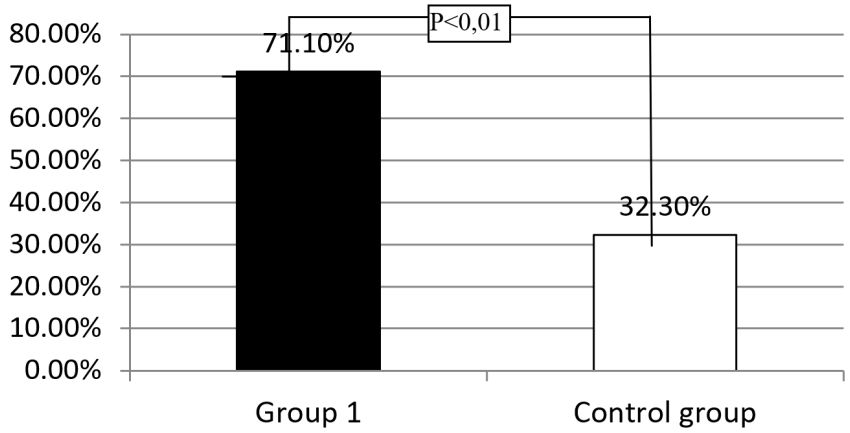

Figure 1. The prevalence of lactase enzyme deficiency in Group 1 and control group.

Further analysis showed that severe lactase deficiency was observed more frequently in the Group 1 than in the control group (n=19 (50%) vs. n=5 (16.1%); $p<0.01$). Mild and moderate lactase enzyme deficiency was observed with similar prevalence in all analyzed groups (Figure 2).

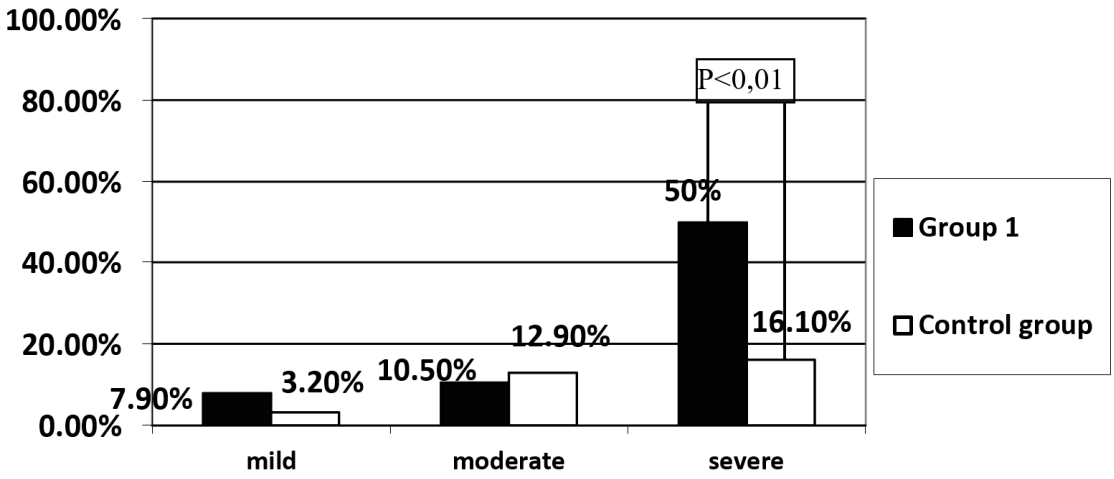

Figure 2. The severity of lactase deficiency in the investigated groups.

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When assessing the group of patients with celiac disease (Group 1), a severe deficiency of the lactase enzyme was found significantly more frequently in the group intentionally or non-intentionally non-adherent to the gluten- free diet than in the group declaring the strict adherence (n=13 (65%) vs. n=6 (33%); p<0.05) Figure 3).

Figure 3. The prevalence of severe lactase enzyme deficiency depending on the degree of dietary control among patients with celiac disease.

C/T(-13910) and G/A(-22018) Polymorphisms in the Promoter of the LCT Gene

Among patients with lactase deficiency confirmed in HBT, LCT gene analysis was performed by assessing the C/T (-13910) and G/A (-22018) polymorphisms in order to differentiate the primary and secondary lactase deficiency. Patients with variant C at position 13910 and G at position 22018 have the genetic predisposition to develop primary lactase deficiency. In Group 1, 15 (39.5%) and in the control group 8 (25.8%) patients had primary enzyme deficiency. In 7 (18.4%) Group 1 patients and 2 (6.5%) from the control group, the heterozygous form of the LCT gene polymorphism was found. Only in Group 1, in 5 (18.5%) patients the presence of LCT gene variants responsible for lactase enzyme deficiency was not detected (Figure 4). As shown in Figure 4, among the CD patients with the positive HBT, only 55% had primary lactase deficiency.

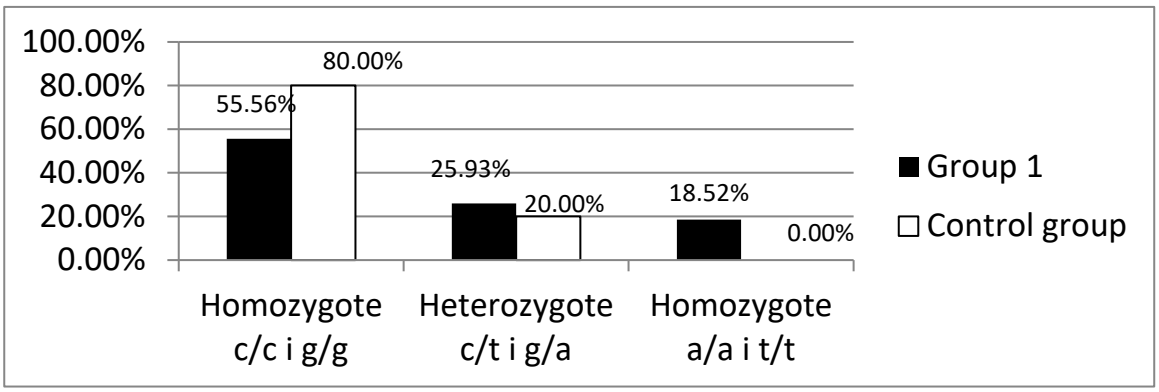

Figure 4. The prevalence of LCT gene variants in patients with lactase deficiency in HBT test.

6. Discussion

As described in the Introduction, in the course of celiac disease, as a result of the progressive inflammatory process, enterocytes are damaged, including the cells producing disaccharidases in the brush border, leading to their deficiency.

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Lactase deficiency is common in society. As described above, in Poland, genetically determined lactase deficiency is observed with the prevalence of 31-37.5%. The basic test for diagnosing lactase enzyme deficiency is the hydrogen breath test using lactose in accordance with the applicable guidelines [35].

According to the described physiological process of lactase enzyme production, damage to enterocytes leads to a decrease in the production of this enzyme. Therefore, in the course of CD, the incidence of lactase deficiency should be higher than in the general population. Basen on HBT, in the study group, lactase enzyme deficiency occurred significantly more frequently than in the control group (71.1% vs. 32.3%). Severe lactase deficiency was more common among patients intentionally or non- intentionally non-adhering to the gluten- free diet than those following the diet (65% vs. 33.3%). Tursi et al., observed lactase deficiency in 13% of adult patients with celiac disease on a gluten-free diet [38]. In the study by Ojetti et al., among adults diagnosed with celiac disease, 33% had a persistently positive hydrogen breath test result for lactose, despite following a gluten-free diet for 12 months [39]. Already in the 1980s and 1990s, numerous studies indicated a correlation between the degree of villous atrophy in single biopsies and the enzymatic activity of disaccharidases [40–43]. Ojetti et al. showed that proper adherence to a gluten-free diet leads to gradual regeneration of intestinal villi [39]. Lactose malabsorption is common among children with untreated celiac disease and the application of a restrictive diet results in the return of normal lactase secretion [44]. Although studies have shown that despite the regeneration of intestinal villi, the enzymatic activity of lactase is reduced [45], and even minor histopathological changes (Marsh 1 and 2) lead to its significant deficiency [46]. In turn, Prasad et al. demonstrated in 71 children with celiac disease a significant correlation between the degree of villous atrophy and a decrease in the disaccharidase concentration. In this study, significantly lower enzymatic activity was found in patients with significant villous atrophy (Marsh 3b and 3c) than in the group of patients without celiac disease [47]. Based on histopathological evaluation, Nieminen et al. also confirmed the correlation between the degree of villous regeneration and disaccharidase activity [48]. According to Ojetti et al., celiac disease occurs more frequently in the group of patients with lactase enzyme deficiency than in the general population [49].

In Group 1, primary lactase deficiency occurred with a prevalence similar to the average in Poland (mutations in the LCT gene were found in 39.5% of the study group) and similar to the Control group (25.8%). However, it was only responsible for the development of lactase deficiency in 55% of cases. In the studies conducted so far, the prevalence of primary lactase deficiency was similar in patients with celiac disease compared to healthy individuals [50,51]. The differentiation between primary and secondary lactase deficiency affects the treatment and prognosis of patients with celiac disease. In the case of primary lactase deficiency, the patient must, in addition to a gluten-free diet, follow a low-lactose diet or apply enzyme supplementation [27,52–54]. In turn, patients with secondary deficiency should be informed that if a gluten-free diet is followed correctly, the symptoms may gradually disappear after consuming meals containing lactose [39,44]. However, according to Mones et al., despite the regeneration of intestinal villi, the level of disaccharidases is significantly lower than in the general population [46]. Lactase deficiency among celiac disease patients may be responsible for more than 8% of non-responsive celiac disease cases [55,56]. Moreover, it has been shown that many gastrointestinal symptoms such as diarrhea, flatulence, abdominal pain in patients with celiac disease are related to the deficiency of the enzyme lactase [54,57].

7. Summary

Celiac disease is a common autoimmune disease of the gastrointestinal tract. In celiac patients, diagnostics for lactase enzyme deficiency should also be performed. This is even more important because lactose is currently widely used in the food industry and its digestion disorders lead not only to numerous gastrointestinal complaints complicating the celiac disease diagnosis and management.

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8. Conclusions:

1. Among patients with celiac disease, lactase deficiency was confirmed in the majority of the study participants, of whom only 55% had primary lactase deficiency.
2. In the study group, a correlation between severe lactase deficiency and non- adherence to the gluten- free diet was demonstrated.

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