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ORIGINAL ARTICLE

LACTOSE INTOLERANCE IN BULGARIA: A PRELIMINARY STUDY

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Abstract. *Lactose intolerance affects more than 65% of the world's population. Main methods for confirming this condition include hydrogen breath test, oral lactose administration and blood glucose measurements, and also biopsy. An association of lactose intolerance with genetic polymorphisms specific to certain regions is found. In Europe, genetic testing of C/T13910 is being implemented. Incidence data is available for most countries in Europe. At the time of our study, there is no data on the prevalence of lactose intolerance in Bulgaria. In this study, a questionnaire on the prevalence and awareness of lactose intolerance among the Bulgarian population was created. For the first time, the results of a lactose tolerance test and a genetic test of volunteers have been reported and systematized. The lactose test with oral administration of lactose was designed for self-testing and appeared to be a good choice to establish the current condition but was not definite enough. As many as 45% showed inconclusive results, and the patient's symptoms were the leading point for the diagnosis. The genetic test results showed a huge prevalence of the recessive allele (C13910) associated with lactose intolerance (97%). New horizons are being opened for studies of the Bulgarian population to establish their lactose resistance in the presence of the recessive allele.*

Key words: *lactose intolerance, Bulgaria, genetic test, lactose tolerance test*

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INTRODUCTION

Lactose is the main sugar in mammalian milk and it delivers the energy of the newborns. This sugar consists of glucose and galactose linked with β-1,4-glycosidic bond [1]. The lactose is linked with absorption and retention of main minerals like zinc, magnesium and essentially calcium. Also, it is associated with vitamin D levels in the organism. Furthermore, the lactose is the only meal source of galactose, which is essential for nerve cell membrane formation [2]. Consequently, lactose has a main role in the well-being of young and adults.

According to the classification of Porzi et al. [3], there are 9 types of lactase function disorders, and the terms including malabsorption, persistence, intolerance, and deficiency are clearly described. The lactase deficiency is a condition with no lactase in the intestine and it could be congenital (genetic dis-

order without lactase from birth), primary (lowering the lactase activity with age), secondary (temporally compromised lactase activity from illness or injury). Actually, lactose malabsorption is described as impossibility to digest or absorb lactose due to primary or secondary enzyme deficiency. Lactase persistence is associated with phenotype experience of active lactase throughout adulthood, while lactase non-persistence is early reduction of the enzyme throughout childhood. Lactose intolerance is a clinical syndrome manifesting typical gastrointestinal symptoms. Porzi et al. [3] single out in the ranking the people who perceive themselves as lactose intolerant, but do not have a medical analysis and diagnosis such as self-reported lactose intolerance. This term represents a large percentage of people and was also used in the current study. At the official United States government website, National Library of Medicine [4], the etiology linked to lactose intolerance is classified into 4 causes of lactase deficiency: primary, secondary, congenital and developmental. First three have similar description as already mentioned, whereas the developmental lactase deficiency appears as a new class. It is a condition of early delivered infants (28 to 37 gestational weeks) with immature intestine digestion function. It improves with time, due to maturation of the intestine and higher lactose hydrolysis.

Lactase (the full name of the enzyme is lactase-phlorizin hydrolase) enzyme activity increases even during the pregnancy and has maximum at the time of the term birth. This is vital for the newborn who is supposed to use only breast milk for food. It is interesting that after weaning, in a part of the population, this enzyme begins to decrease and, accordingly, symptoms of lactose intolerance become manifested [5]. Pienar et al. noticed exact periods and lactose intolerance could be detected in early childhood [6]. Since the age of two years, the amount of the lactase enzyme decreases and lactose intolerance symptoms begin before the age of 6. Typical intestinal symptoms of lactose intolerance are pain and swelling in the abdominal area, flatulence, diarrhea, and nausea.

The oldest and the most invasive method is the biopsy of the jejunum. Also, biopsy is the most expensive tool, but it is considered as a reference standard for primary lactase deficiency. Rarely, but it is possible to have false negative results due to enzyme expansion positions [7].

The breath tests for lactose intolerance are non-invasive and cost-effective. They are based on the fact that the sources for hydrogen (H2) and methane (CH4) production in humans are carbohydrates. The hydrogen test is considered as a gold standard. However, this method has its limitations. The false-negative results are from 2.5% to 15%. Also, the test is performed in 3 hours and test samples are taken every 30 minutes [8]. The other limitation is the difficult interpretation of the results from patients with high baseline hydrogen connection (> 20 ppm). Furthermore, the breath tests are not recommended in the COVID-19 pandemic due to SARS-COV-2 transmission. Obtaining and handling breath samples is a high health risk [9]. Another test for lactose intolerance is the oral lactose tolerance test. It aims to check whether the organism is able to break down the milk sugar (lactose) to simple sugars (glucose and galactose). The test measures the levels of glucose in the blood, because during the hydrolysis of a molecule lactose is released a molecule glucose [4]. The lactose tolerant test consists of consumption of 50 g lactose and plasma glucose determination. The advantages of the test is the low cost and excellent availability. But the disadvantages are the long time of testing and the invasiveness. False-negative results of the test are possible due to fluctuations in blood glucose [10]. Domínguez Jiménez and Fernández Suárez [11] discovered some discrepancy in results when comparing capillary and venous blood. They reported that the results from capillary blood are higher than the venous blood. Therefore, it was necessary to specify the method used in the presentation of the results.

Relationship between the occurrence of lactose intolerance and genetic polymorphism was discovered. Also, Tomczonek-Moruś et al. found a good relationship between hydrogen breath test and genetic test [12]. There are single nucleotide polymorphisms (SNPs) associated with lactose intolerance, for example: C/T-13910, which is found among the populations of Europe, Middle East, parts of Asia and Africa; G/A-22018, found in Europe, South and East Africa; C/T-13914, among Eastern Europeans; G/A-13908, in Far East Asia, etc. [13]. Genetic tests are moderately invasive and quite expensive, however, the false-positive results are rare (< 5%) [10].

Geographically, lactose intolerance affects more than half of the world's population (more than 65%). At the same time, a higher prevalence is observed in Africa (almost 100%) and Asia (70%). The prevalence of cases is not necessarily related to the continent in which people are located. In America, the differences in the manifestation of lactose intolerance are large. Thus, cases in America are reported to be 50%, with 15% being white, 53% being Mexican-American, and the largest percentage (80%) being African-American. In the continent of Europe, where Bulgaria is also located, the prevalence of lactose intolerance is considered to be relatively low (28%). These lati-

tudes also vary widely among nations. Only 2% of Scandinavians have this condition, while the affected people from southern Italy are reported to be 70% [14]. To the best of our knowledge at the time of this study, no laboratory tests and statistical analyses have been performed to examine the population in Bulgaria. Consequently, we aimed our actions at filling this gap. Collected information can serve researchers, general practitioners, consultants and nutritionists, as well as the general reader.

During the research, the following tools were used: a questionnaire, an oral lactose tolerance test with capillary blood measurements (an easy self-test), as well as a genetic test of lactose intolerance., The genetic test was based on the polymorphism dominant in Europe (C/T13910) [2, 6, 15]. The present study is the first made in Bulgaria and the presented results are unique and incomparable at the moment of the publication.

MATERIALS AND METHODS

At the first part of the study, 203 volunteers took part in the questionnaire survey, and 20 of them were tested with an oral lactose tolerance test, while 31 volunteers were tested with a genetic test for lactose intolerance.

A self-test variation of the oral lactose tolerance test was made, and a commercially available glucometer Wellion Galileo Compact was used. The genetic test of lactose intolerance was conducted with test strips Genotype SugarTol (Hain Lifescience, Nehren, Germany). DNA isolation was performed using specific genecards – GenoCard (Hain Lifescience, Nehren, Germany). Every genecard was used for four different samples. PCR analyses were performed using 1000 Touch Thermal Cycler. The hybridization reaction tubes were loaded on a thermo-shaker TwinCubator (Hain Lifescience, Nehren, Germany).

Questionnaire study of lactose intolerance

An anonymous questionnaire was prepared and distributed through the accessible Google Forms. The responses of 203 volunteers were received and analyzed. Also, the parents of children, aged from 13 days to 17 years, were surveyed. The questionnaire was presented together with short information about the types and the symptoms of lactose intolerance.

Oral lactose tolerance self-test

A rapid oral lactose tolerance self-test was used for 20 volunteers and the results were obtained within 2 hours. The milk sugar intake was made by consumption of a glass of milk [16,17,18]. The glucose concentration after lactose consumption was tracked. Each volunteer was clinically healthy and was currently not prescribed or taking any antibiotics. Also, the volunteers were asked not to eat or perform any physical exercises within 8 hours prior to the test. The volunteers were also introduced to the test procedure and they completed an informed consent form for participation. Each of them was asked to share the presence or absence of any symptoms related to the consumption of lactose-containing foods. The test procedure is described on Figure 1.

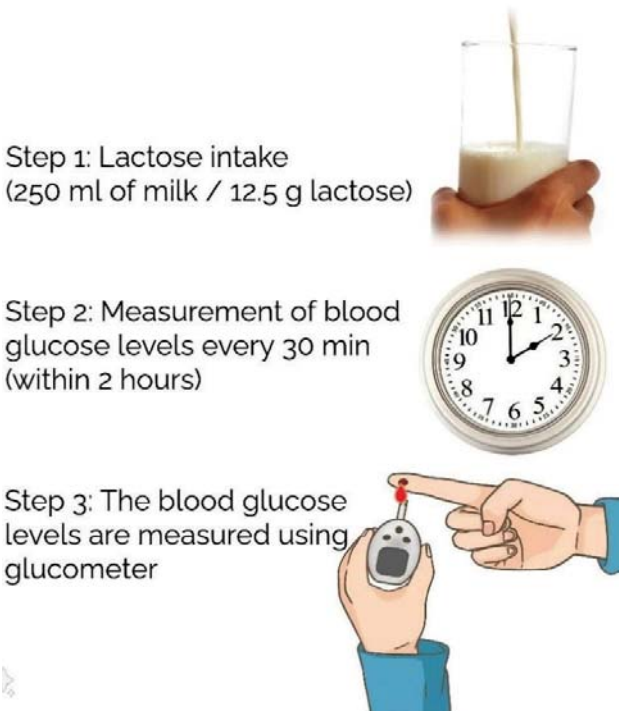

Fig. 1. Oral lactose tolerance self-test steps

Steps of the oral self-test procedure:

- The puncture site was cleaned with an alcoholic cotton swab.
- The initial glucose concentration was performed before the lactose intake.
- Ingestion of one glass of lactose containing milk (~ 5 g of lactose in 100 g of milk) within 10 minutes.
- The puncture site was cleaned with an ethanol cotton swab.
- Blood samples were taken after 30 minutes, 1 hour and 2 hours.
- The test results were reported and recorded.
- The blood results and shared symptoms were analyzed.

Genetic test Genotype SugarTol

A quick survey on the medical laboratories in Bulgaria was performed to record ones conducting genetic tests for lactose intolerance. The results showed that

only three laboratories in the country perform such kind a test. Furthermore, it was established that only one of the laboratories has the equipment and personnel to carry out the test in our country, while the rest only take the blood samples and send them for analyses abroad.

Based on the recommendations of the medical laboratory performing the test in the country, the genetic test of lactose intolerance that we have chosen was Genotype SugarTol (Hain Lifescience, Nehren, Germany). The test showed polymorphism for lactose intolerance and also for fructose intolerance. Nobody from the tested volunteers showed fructose intolerance. Consequently, the test part and the results for lactose intolerance are described and discussed below. The method could be divided into several main steps, presented in Figure 2.

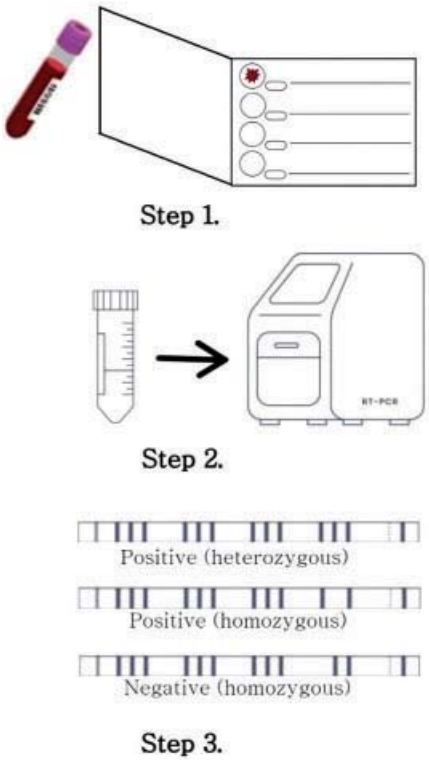

Fig. 2. Main steps of the genetic test of lactose intolerance using GenoType SugarTol

DNA isolation

First, 25 µL of blood was pipetted, then spotted on the labelled GenoCard paper (the DNA was retained on the membrane). The sample was left to air dry for approximately 30 minutes. When the blood sample had dried, three spots of the membrane (with retained DNA) were removed from the GenoCard using a puncher and were placed in a vial (Figure 3). The puncher was washed 3-4 times between samples, first with bleach (to remove DNA residues) and then with distilled water.

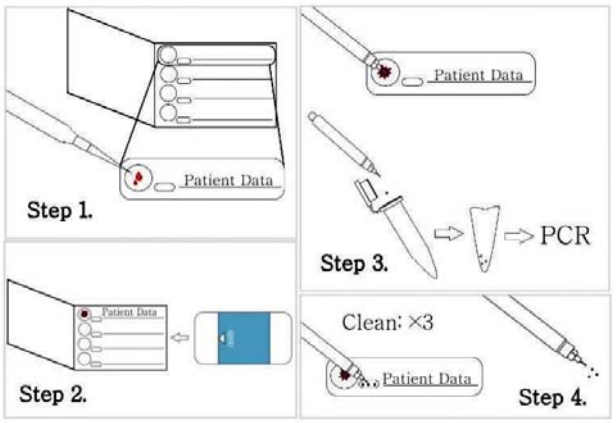

Fig. 3. Schematic representation of the GenoCard usage.

– DNA amplification

The DNA amplification was conducted using solutions provided by the manufacturer. Thus, 10 µL of AM-A buffer (containing specific primers, nucleotides and Taq polymerase) and 35 µL of AM-B buffer (containing dye) were added to the vial with DNA. The tubes were placed in a PCR amplifier and the necessary parameters were set. The amplification of DNA is presented in Table 1.

Table 1. DNA amplification parameters

Number of cycles	Temperature	Duration
1	95ºC	15 min
10	95ºC	30 sec
	58ºC	2 min
25	95ºC	25 sec
	53ºC	40 sec
	70ºC	40 sec
1	70ºC	8 min

– DNA hybridization

The HYB (Hybridization Buffer) and STR (Stringent Wash Solution) solutions were heated up to 37-45ºC until they became ready for the hybridization step. The remaining reagents, except CON-C (Conjugate Concentrate) and SUB-C (Substrate Concentrate), were warmed to room temperature: CON-D (Conjugate Buffer), SUB-D (Substrate Buffer), DEN (Denaturation Solution), HYB (Hybridization Buffer), STR (Stringent Wash Solution) and RIN (Rince Solution). The concentrates were diluted to a working concentration of 1:100 with the respective buffer CON-D (Conjugate Dilution) for CON-C and SUB-D (Substrate Dilution) for SUB-C. The working solutions were homogenized well and brought to room temperature.

Hybridization steps:

- 20 µL of DEN (Denaturation Solution) was pipetted in a corner of each of the wells used.
- 20 µL of the amplified sample was added to the solution, the mixture was homogenized and then incubated at room temperature for 5 minutes. Meanwhile, the required number of test strips were taken with tweezers and labelled with a pencil underneath the colored marker.
- Carefully, 1 ml of pre-warmed HYB was added to each well. The tray was shaken gently until the solution colored homogeneously.
- One test strip was placed in each well. The strips were completely covered by the solution and the marked sides were faced upward. The tray was incubated for 30 minutes at 45ºC on a shaking water bath (TwinCubator) and after that the HYB was completely aspirated (Figure 4).

Fig. 4. Test strips incubated with amplified DNA and Hybridization Buffer on a TwinCubator shaker

- 1 ml of STR was added to each strip and was incubated for 15 minutes at 45ºC on a shaking water bath. After that the STR was discarded completely and the remaining fluid was removed by turning the tray upside down on an absorbent paper.
- Each strip was washed with 1 ml of RIN (Rinse Solution) for 1 minute at room temperature.
- 1 ml of the working solution of the conjugate was added to each strip and was incubated for 30 minutes at room temperature on the shaking water bath.
- The conjugate solution was removed, and each strip was washed twice for 1 minute with 1 ml of RIN and once for 1 minute with 1 ml of distilled water on the shaking water bath. Any traces of water were removed after the last wash.
- 1 ml of the working solution of the substrate was added to each strip and was incubated protected from light without shaking. The incubation time (the time until the bands are clearly visible) was 5 minutes.
- The reaction was stopped as soon as the bands were clearly visible by rinsing twice with distilled water. The strips were removed from the tray using tweezers and were dried between two layers of absorbent paper.

Statistical data processing

The obtained results were analyzed using publicly available Microsoft Excel. The dependencies between the results were established and converted into a graphic form. A chi-square test was used to establish or reject the overlap of the results of the different tests. The chi-square test uses the formula:

$$\chi^2 = \Sigma . \frac{(Observed\text{-}Expected)^2}{Expected}$$

The degrees of freedom were defined as: df = n – 1. That represented the number of rows of values (n) and one was subtracted. For the purposes of the study, the degrees of freedom were df = 1. The level of significance (α) was 0.05. The lower the obtained chi-square value, the greater the relationship between the data presented. The obtained result was compared to the critical value from a publicly available table, including the significance level. If the obtained chi-square value is greater than the critical value from the table, then there was a statistically significant difference between the presented data (tests).

RESULTS

The interpretation of the results from the oral self-test was based on literature data [19, 20]. The test was considered normal if the glucose level rises by more than 30 mg/dL (1.665 mmol/L) within 2 hours after the intake of lactose solution. A rise of 20-30 mg/dL (1.11-1.665 mmol/L) within 2 hours of the lactose solution intake was inconclusive. A result lower than 20 mg/dL (1.11 mmol/L) within 2 hours of the lactose solution intake suggests that the volunteer was lactose intolerant, and further tests should be done.

Results from the questionnaire study

The majority of volunteers (93.3%) rejected the possibility of having lactose intolerance, while the rest (6.7%) shared that they suffer this condition. The parents reported that 83.2% of their children had not shown any symptoms of the condition. However, only a small part of the volunteers (13.4%) had tested their child for lactose intolerance. Moreover,

a larger percentage of the surveyed said that they had not had such tests (67.0%), or that they had only consulted a medical doctor (23.2%). According to the laboratory tests that volunteers mentioned for lactose intolerance, only 4.14% used a genetic test, the same amount (4.13%) – a blood test, and 1.53% marked „other food intolerance test". The results are presented on Figure 5.

Results from the oral lactose tolerance self-test

Blood samples (peripheral blood) were taken before and after lactose intake within up to 2 hours (minimum 30 min). In the used test conditions, the rising of the glucose levels with 1 mmol/L within 1 h (strongly manifested at the 30th minute) rejected the possibility of lactose intolerance [18] (Figure 6).

The results from the oral lactose tolerance self-test conducted on 20 volunteers (aged 13 to 55 years) are shown in Table 2. Among those tested, 40% showed a convincing ability to break down lactose, i.e., they were lactose tolerant, 45% had inconclusive results and they were advised to undergo other tests, and the remaining 15% had lactose intolerance, according to the oral test. Also, a relationship between those conditions and the age of the volunteers was noticed. Lactose intake with successful absorption was observed among the younger volunteers (average age 23 years). In the older population (average age 41 years) inconclusive oral test results were noted. According to the test, the oldest group of volunteers (average age 52 years) have lactose intolerance. It could also be explained by the possible presence of heterozygotes among the volunteers, whose gene for lactose intolerance manifests itself with age and leads to difficulty in breaking down lactose in the organism [3, 4].

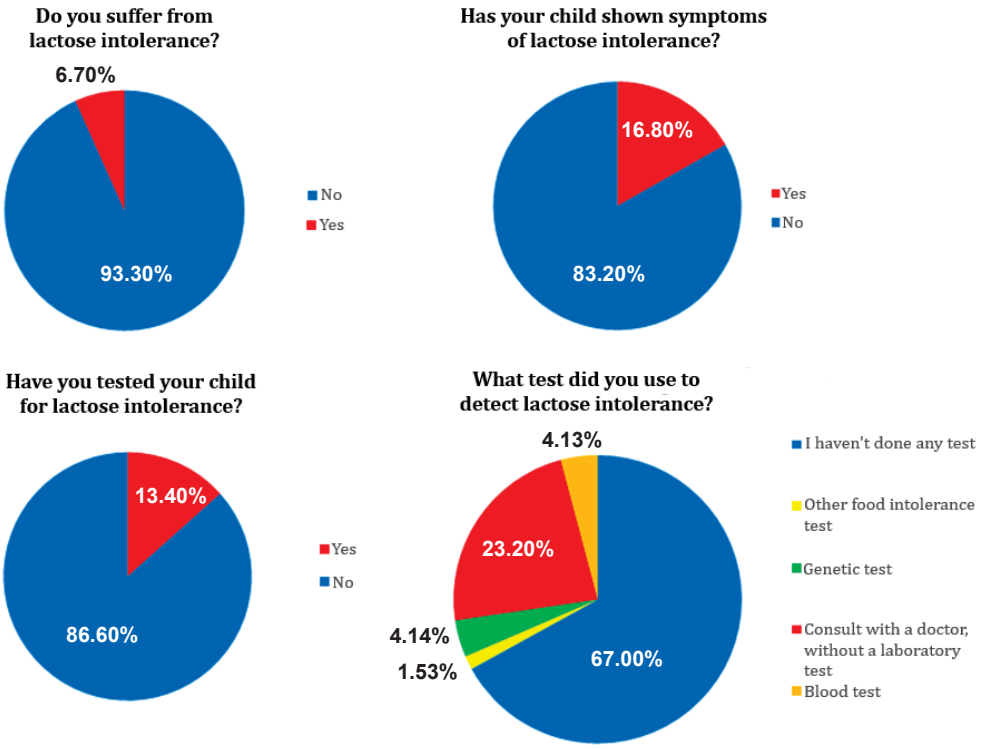

Fig. 5. A questionnaire survey about lactose intolerance in the Bulgarian population

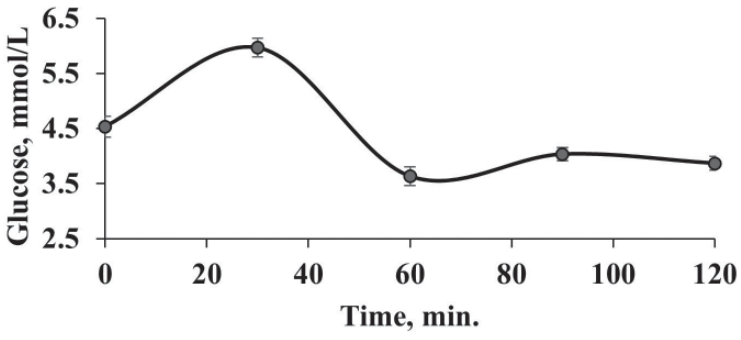

Fig. 6. Average changes in blood glucose levels in case of a positive oral lactose tolerance self-test

Table 2. Results from the oral lactose tolerance self-test

Characteristic	lactose intolerant	lactose tolerant	Inconclusive results
Number of volunteers	3 (15%)	8 (40%)	9 (45%)
Male	0 (0%)	1 (5%)	2 (10%)
Female	3 (15%)	7(35%)	7(35%)
Average age	50 (43-55)	27 (13-41)	35 (13-51)
Median age	52 (43-55)	23 (13-41)	41 (13-51)

The oral lactose tolerance self-test was presented together with questions about possible symptoms, as a result of lactose-containing food consumption. The volunteers who describe symptoms typical for lactose intolerance made up 40% and their average age was 34 years. The same number of volunteers had no symptoms, with an average age of 41. The rest of the volunteers (20%) showed conflicting results in reporting symptoms. Their average age was 23 years (Table 3).

Table 3. Results according to lactose intolerance symptoms

Characteristic	Lactose intolerant	Lactose tolerant	Inconclusive results
Number of volunteers	8 (40%)	8 (40%)	4 (20%)
Male	1 (5%)	1 (5%)	1 (5%)
Female	7 (35%)	7 (35%)	3 (15%)
Average age	32 (13-49)	38 (21-55)	30 (22-51)
Median age	34 (13-49)	41 (21-55)	23 (22-51)

Results from the genetic test Genotype SugarTol

Geno Type SugarTol test strips were used. The purpose of the test was to detect the presence or absence of the polymorphism associated with the synthesis of the lactase enzyme in the human organism. A part of the 31 volunteers in the genetic test previously participated in the oral lactose tolerance test. Contrary to expectations, as many as 97% of the volunteers showed a positive result for the C13910 polymorphism causing lactose intolerance; only one of the volunteers was homozygous for T13910 (Table 4). Hence, as the volunteers age, they should be monitored for symptoms and avoid consuming lactose-containing foods [21].

Dependence between the results of the conducted tests

To compare the performed tests, the so-called chi-square test by Karl Pearson was used, as it is often applied to accept or reject a hypothesis using a given starting point [22]. At the beginning of the study, the statements from the questionnaire study were considered to be correct, i.e., people themselves had correctly determined their condition. Consequently, 93.3% of the Bulgarian population was considered to be lactose tolerant. That was set as the null hypothesis in the test (H0). The results of the test, used as comparison, confirm or reject the statement with a significance level of $\alpha = 0.05$.

Questionnaire study and manifested symptoms

While studying the data from the reported symptoms during the oral lactose tolerance test, it was determined that 40% of the volunteers were probably lactose intolerant and another 40% were without such symptoms. The remaining 20% had inconclusive results. However, as people were likely to be poorly informed about the symptoms of lactose intolerance, those 20% with inconclusive results were counted as asymptomatic volunteers. Thus, for the purpose of the chi-square test, according to their symptoms, 40% were positive for lactose intolerance and 60% were negative for this condition (Table 5).

Table 5. Comparison of the results from the questionnaire study and the reported symptoms for lactose intolerance

	Questionnaire study (Expected result)	Symptoms (Observed result)
Have lactose intolerance	6.7	40
Do not have lactose intolerance	93.3	60

Table 4. Results of the genetic testing for lactose intolerance

Characteristic	Lactose intolerant (C/C genotype)	Lactose tolerant (T/T genotype)	Heterozygous gene of lactose intolerance (C/T genotype)
Number of volunteers	23 (74%)	1 (3%)	7 (23%)
Male	5 (16%)	1 (3%)	4 (13%)
Female	18 (58%)	0 (0%)	3 (10%)
Average age	30 (21-43)	41 (41-41)	28 (22-34)
Median age	22 (21-43)	41 (41-41)	28 (22-34)

*Only 7 of the volunteers shared their age

The chi-square test results showed 177.39 and P-value less than 0.0001. This demonstrates that the results were highly statistically different and there is no overlap of the questionnaire study and the reported symptoms. According to this result, it is suggested that there were more people with lactose intolerance than expected.

Questionnaire study and oral lactose tolerance self-test

During the oral lactose tolerance self-test, it was found that 15% of the volunteers did not have the ability to break down lactose, 40% can break down lactose and 45% had inconclusive results. For the purpose of the chi-square test, the people with inconclusive results were included to those with positive oral lactose tolerance self-test (Table 6).

Table 6. Comparison of the results from the questionnaire study and the oral lactose tolerance self-test

	Questionnaire study (Expected result)	Oral lactose tolerance self-test (Observed result)
Have lactose intolerance	6.7	15
Do not have lactose intolerance	93.3	85

The chi-square test results show 11.02 and P-value 0.0009. Therefore, those results were highly statistically different and there was no overlap between the questionnaire study and the oral lactose tolerance self-test. According to these results, it was suggested that there are more people with lactose intolerance than expected.

Questionnaire study and genetic test GenoType SugarTol

Due to the inconsistencies between the expected low percentage of people with lactose intolerance and the obtained higher percentage, the use of another test was necessary. In this study the genetic test GenoType SugarTol was used. The genetic results have shown that 97% had the gene for lactose intolerance (C13910), of which 23% are heterozygotes (C/T13910). For the purpose of the chi-square test, the results were divided into people with the lactose-intolerant gene and people without the lactose-intolerant gene (Table 7).

Obviously the test results diverge and could not be compared. If it was assumed that the volunteers with a heterozygous gene belong to the group of “do not have lactose intolerance”, then 26% of the tested volunteers had a gene encoding lactase and were able to break down lactose. However, the difference between the questionnaire study and the genetic test differ greatly and could not be compared.

Table 7. Comparison of the results from the questionnaire study and genetic test GenoType SugarTol

	Questionnaire study (Expected result)	Genetic test (Observed result)
Have lactose intolerance	6.7	97
Do not have lactose intolerance	93.3	3

Genetic test GenoType SugarTol, oral lactose tolerance self-test and manifested symptoms

Due to the insufficient awareness of the volunteers (shown by the results presented in the questionnaire study) we could assume that the genetic test was more reliable and it was considered as the null hypothesis (H0). Therefore, the results of the genetic test were taken as “Expected result” while the results from the oral lactose tolerance self-test and the manifested symptoms would be the “Observed result”.

Regarding the comparison of the results of the genetic test and those of the reported symptoms, the volunteers with a hybrid gene were assumed to be into the group without lactose intolerance due to the presence of the dominant T 13910 allele. The inconclusive results of reported symptoms were generalized to those with lactose intolerance, due to people’s low awareness and inability to self-monitor. Thus, it was assumed that 60% of the volunteers had lactose intolerance according to their symptoms, and 40% were without such symptoms (Table 8).

Table 8. Comparison of the results from the genetic test GenoType SugarTol and the manifested symptoms

	Genetic test (Expected result)	Symptoms (Observed result)
Have lactose intolerance	74	60
Do not have lactose intolerance	26	40

Thus, the chi-square test shows 10.187 and the P-value is 0.0014. This shows that the genetic test and the manifested symptoms have a statistically significant difference, i.e., there was no overlap of the results (Figures 7 and 8).

When comparing the results of the manifested symptoms and the oral lactose tolerance self-test, a complete correspondence was observed. That was pos-

sible if it was assumed that those with inconclusive results were assigned to the lactose intolerant group. Thus, in both cases, 60% have lactose intolerance and 40% have no such manifestation (Figure 9).

DISCUSSION

It was confirmed that the Bulgarian population had low awareness of the symptoms of lactose intolerance, which in turn suggests not entirely accurate percent of self-reported lactose intolerant individuals of our previous test [23]. Probably, the low percentage of testing of the Bulgarians was most likely due to the unawareness of the people about that condition, and also the high cost of the tests. In Bulgaria, only genetic tests are offered and their price is approximately BGN 100-120, equal to EUR 50-60 (up to the date of the study 2022-2023).

There are different methods of testing for lactose intolerance. Some of them are based on blood indicators, and others determine the concentration of hydrogen in the patient‘s breath. In this study, the amount of ingested lactose was equal to the common volume in everyday life. One glass of milk (250 ml with 5 g lactose) is more often consumed compared to traditional test dose of 50 g lactose [16]. Consequently, the symptoms associated with lactose malabsorption in that trail were reduced.

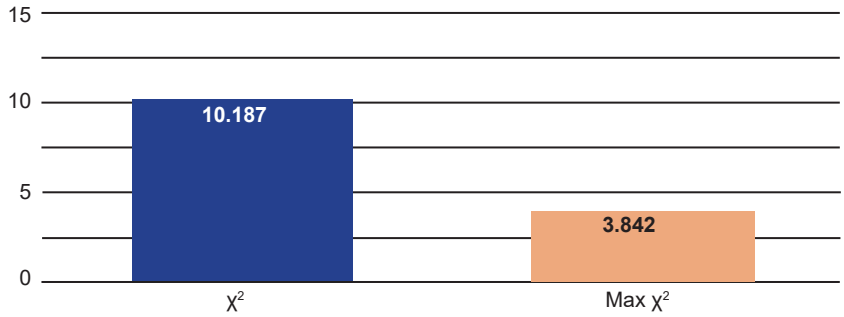

Fig. 7. Chi-square value (χ^2) and the maximum acceptable value for accepting that the results of the genetic test and manifested symptoms correspond

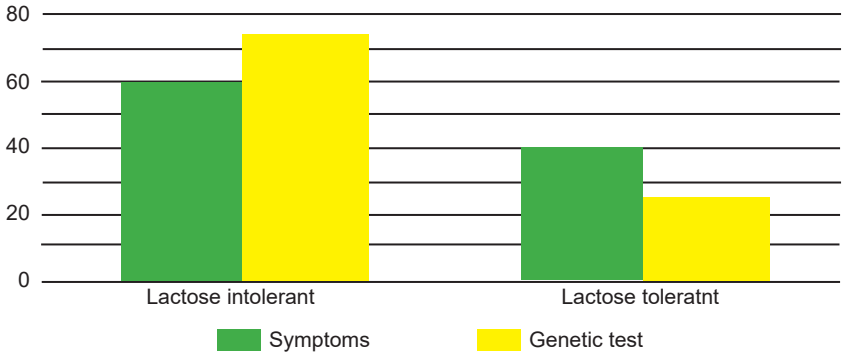

Fig. 8. Comparison of the results from the genetic test GenoType SugarTol and the manifested symptoms

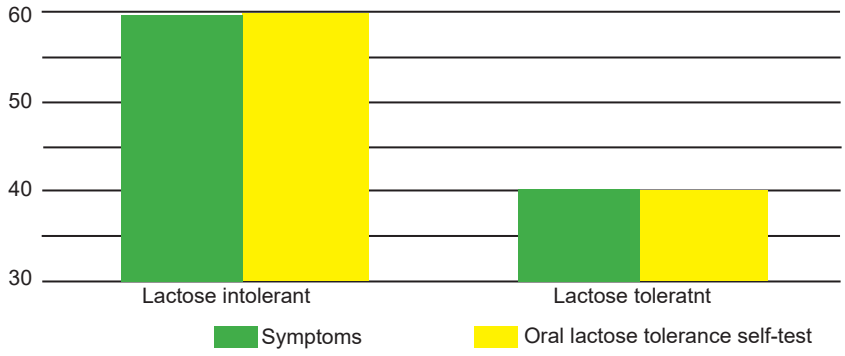

Fig. 9. Comparison of the results from the oral lactose tolerance self-test and the manifested symptoms

Based on the symptoms described by the volunteers, no significant relationship was established between the described symptoms after lactose-containing food consumption and the presence or absence of lactose intolerance according to the oral self-test, although a similar lactose amount was used in the test. Those varying results showed the need or more detailed study of the volunteers and also the whole population.

Genetic tests are very accurate and have a low error rate but they are expensive and do not show the current status of the individual, especially in the case of a heterozygous allele. The offered in this study oral lactose tolerance test gave information about the current status but was not accurate enough and often the results were inconclusive. However, there were several health disorders, including stress, that could affect the absorption of lactose and the release of glucose into the bloodstream [3]. A lot of the examined volunteers, according to the genetic test, were homozygous for the recessive allele (lactose intolerant) but did not report any symptom manifestation associated with lactose intolerance. That could be explained by the traditions in our society and in particular, the frequent and long-standing consumption of fermented dairy products, such as yogurt. Those products are rich in Lactobacillus bulgaricus and Streptococcus thermophilus, which are probiotics that are able to use lactose (not only glucose) as a carbon source [24]. According to Ibrahim et al., lactose is a prebiotic that stimulates the growth and development of lactic acid bacteria [25]. With their help, the lactose that enters the organism is broken down into glucose and galactose, where the microorganisms themselves metabolize the glucose for their own needs, and the galactose is released into the extracellular space. Thus, the human body successfully breaks down galactose and uses it as an energy source, instead of creating byproducts, mainly gases, which cause the unpleasant symptoms of lactose intolerance [4].

The best choice appears to be a genetic test for lactose intolerance, at an early age, to clarify the genotype. In the presence of a homozygous allele characteristic of a lactose tolerant patient, lactose intolerance should be rejected in the future when similar symptoms appear. Unless the patient is elderly, because it is possible for the enzyme to decrease significantly. Also, exceptions are the cases where, due to illness or surgery, the integrity and function of the intestine is impaired.

In the case of a homozygous individual with the mutation responsible for the lactose intolerance, a diet excluding large amounts of lactose should be established. Manifestation of symptoms from small amounts (less than 1 glass of milk) are rare [26], and this is most often detected in infancy and does not require re-examination later. Such patients should be recommended to consume lactose-free preparations in the pharmacies, and also foods like sausages, cakes, salad dressing and others, which are the so-called „hidden lactose“ [27].

The genetic test result could show the presence of a heterozygous allele, i.e., presence of both alleles – for lactose intolerance and for lactose tolerance. When those alleles are established in early childhood, it is recommended to perform an oral lactose tolerance test periodically or equal to that test (like breath test) in the individual's life to monitor the current condition. It is also recommended to watch out for the typical symptoms of the lactose intolerance, the most common being bloating and meteorism after consuming a lactose-containing food. The unnecessary exclusion of dairy products from the daily meal might lead to a number of disorders associated with an unbalanced diet. For example, people who regularly consume dairy products are less likely to suffer from a heart attack. Also, colon cancer is associated with a lack of certain nutrients that are inherent in milk and milk products. Such nutrients are vitamin D, calcium, probiotic lactic acid bacteria, and bioactive peptides from milk protein [28]. Overall, dairy consumption is associated with a reduced risk of hypertonia, coronary heart disease, and stroke [29].

Study Limitations

The method used in this study had some limitations that should be excluded in the subsequent studies. First, the oral lactose self-test measured glucose in the capillary blood which showed higher results, but the offered amount of lactose to the patients was low, so the measured values fluctuated periodically. Also, the test was made by a commercial glucometer. It is convenient that it does not require qualified personnel, complex reagents and shows the results immediately. Much more sensitive would be an enzyme assay (with glucose oxidase and peroxidase) to prove the serum glucose level but this complicates and delays the procedure. Also, qualified personnel with specialized equipment are needed. Consequently, the oral lactose test will not be able to be done easily at home. In the following studies of the Bulgarian population, it is good to perform oral lactose tests with enzyme detection of serum glucose, and also hydrogen breath tests.

The limitations in the used genetic test were the use of only one possible SNP for lactose intolerance. It is good to explore the possibility of other SNPs, both known for other regions, and new ones unique to this region can be proven.

CONCLUSIONS

In our latitudes, the probability of being lactose intolerant is high (93.3% with recessive allele responsible for lactose intolerance). However, following a normal diet, including good amounts of quality fermented

dairy products, will help break down lactose, even at higher doses (more than 1 cup of milk a day). If the dairy products consumption is stopped permanently, then the lactic acid bacteria in the intestinal tract will not have the necessary prebiotics and thus the microbiological composition in the intestine would change.

Based on the obtained results, new horizons are revealed for research aimed at the bacterial composition in the intestinal microflora of the people in Bulgaria and their comparison with the composition of the intestinal microbiome of individuals inhabiting other countries and even continents. Also, more research needs to be done on this, because a huge percentage of people have no complaints and at the same time carry genes for lactose intolerance. It is possible to find a connection not only with the gut microbiome, but also with aspects of everyday life or traditional herbs. These findings would be useful for recommending people with severe symptoms to return to a free lifestyle without restrictions and enjoy this sweet disaccharide – lactose.

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REFERENCES

1. Shkembi B, Huppertz T. Glycemic responses of milk and plant-based drinks: food matrix effects. Foods 2023; 12(3), 453.
2. Kowalówka M, Kosewski G, Lipiński D, et al. A Comprehensive Look at the-13910 C> T LCT Gene Polymorphism as a Molecular Marker for Vitamin D and Calcium Levels in Young Adults in Central and Eastern Europe: A Preliminary Study. International Journal of Molecular Sciences 2023; 24(12), 10191.
3. Porzi M, Burton-Pimentel KJ, Walther B, et al. Development of personalized nutrition: applications in lactose intolerance diagnosis and management. Nutrients 2021; 13(5), 1503.
4. Malik TF, Panuganti KK. Lactose intolerance. [Updated 2023 Apr 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing 2024 Jan.
5. Gallo A, Pellegrino S, Lipari A, et al. Lactose malabsorption and intolerance: What is the correct management in older adults? Clinical Nutrition 2023; 42(12), 2540-2545.
6. Pienar C, Pop L, Lăzărescu M, et al. Genetic Predisposition to Primary Lactose Intolerance Does Not Influence Dairy Intake and Health-Related Quality of Life in Romanian Children: A Hospital-Based Cross-Sectional Study. Children 2023; 10(6), 1075.
7. Robles L, Priefer R. Lactose intolerance: What your breath can tell you. Diagnostics 2020; 10(6), 412.
8. De Geyter C, Van de Maele K, Hauser B, et al. Hydrogen and methane breath test in the diagnosis of lactose intolerance. Nutrients 2021; 13(9), 3261.
9. Sendino T, Sandúa A, Calleja S, et al. Lactose tolerance test as an alternative to hydrogen breath test in the study of lactose malabsorption. Advances in Laboratory Medicine/Avances En Medicina De Laboratorio 2020; 1(4), 20200102.
10. Shafi A, Husain Q. Intolerance to milk lactose, diagnostic tests and dietary management: a recent update. Avicenna J Med Biochem 2022; 10(1):71-81.
11. Domínguez Jiménez JL, Fernández Suárez A. Correlation between capillary and venous blood glucose in the lactose tolerance test. Digestive diseases and sciences 2016; 61, 208-214.
12. Tomczonek-Moruś J, Wojtasik A, Zeman K, et al. 13910C>T and 22018G>A LCT gene polymorphisms in diagnosing hypolactasia in children. United European Gastroenterology Journal 2019; 7:210–6.
13. Chengolova Z, Ivanova R, Gabrovska K. Lactose intolerance–single nucleotide polymorphisms and treatment. Journal of the American Nutrition Association 2023; 43(2), 213-220.
14. Catanzaro R, Sciuto M, Marotta F. Lactose intolerance: An update on its pathogenesis, diagnosis, and treatment. Nutrition Research 2021; 89, 23-34.
15. Spahić L, Šehović E, Šećerović A, et al. Lactose intolerance prediction using artificial neural networks. In International Conference on Medical and Biological Engineering 2020; pp. 505-510.
16. Suarez FL, Savaiano DA, Levitt MD. A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance. New England Journal of Medicine 1995; 333(1), 1-4.
17. Bayless TM, Brown E, Paige DM. Lactase non-persistence and lactose intolerance. Current gastroenterology reports 2017; 19, 1-11.
18. Khan MMU, Ali MS, Khan S, et al. Analysis of Lactose Tolerance Significance, Diagnosis, And Therapy. Journal of Namibian Studies: History Politics Culture 2023; 37, 1-24.
19. Scriver CR, et al. eds. The Metabolic and Molecular Bases of Inherited Disease. 7th ed. 1995.
20. Dzialanski Z, Barany M, Engfeldt P, et al. Lactase persistence versus lactose intolerance: is there an intermediate phenotype? Clinical biochemistry 2016; 49(3), 248-252.
21. Qibtia M, Faryal S, Wasim M, et al. Polymorphism in MCM6-Gene Associated with Lactose Non-Persistence in Pakistani Patients. Pakistan Journal of Zoology 2022; 54(5), 2029-2038.
22. Franke TM, Ho T, Christie CA. The chi-square test: Often used and more often misinterpreted. American journal of evaluation 2012; 33(3), 448-458.
23. Syarova R, Shentov P, Chengolova Z. Lactose intolerance and oral health. 60th Annual Scientific Conference – University of Ruse and Union of Scientists 2021; 204-208.
24. Yamamoto E, Watanabe R, Ichimura T, et al. Effect of lactose hydrolysis on the milk-fermenting properties of Lactobacillus delbrueckii ssp. Bulgaricus 2038 and Streptococcus thermophilus 1131. Journal of Dairy Science 2021; 104(2), 1454-1464.
25. Ibrahim SA, Gyawali R, Awaisheh SS, et al. Fermented foods and probiotics: An approach to lactose intolerance. Journal of Dairy Research 2021; 88(3), 357-365.
26. Levitt M, Wilt T, Shaukat A. Clinical implications of lactose malabsorption versus lactose intolerance. Journal of Clinical Gastroenterology 2013; 47(6), 471-480.
27. Facioni MS, Raspini B, Pivari F, et al. Nutritional management of lactose intolerance: the importance of diet and food labelling. Journal of translational medicine 2020; 18, 1-9.
28. Rogeli I. Milk, dairy products, nutrition and health. Food Technology and Biotechnology 2000; 38(2), 143-148.
29. Chen Z, Ahmed M, Ha V, et al. Dairy product consumption and cardiovascular health: A systematic review and meta-analysis of prospective cohort studies. Advances in Nutrition 2022; 13(2), 439-454.