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Original Scientific Paper

The frequency variation of pupils with lactose intolerance and the variety of the symptoms in Macedonia

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Abstract

Lactose intolerance - LI is a condition in which people have symptoms due to the decreased ability to digest lactose, a sugar found in dairy product. Those affected vary for lactose they can tolerate before symptoms develop. Symptoms may include abdominal pain, bloating, diarrhea, gas and nausea. Lactose intolerance is due to the lack of enzyme lactase in the small intestines to break lactose down into glucose and lactose. The research method is LTT (Lactose Tolerance Test). The purpose of the study consisted in calculating the variation of the frequency of the students with LI and the variation of the symptoms shown by the age at the interval of 12-17 years in Macedonia. For the testing of the analysis has been used the "Glukometer - II" device with the "Dextrostis" indicator strip, making blood glucose measurement before and 40 minutes after lactose drinking. Raising the glucose level by less than 1.1 mmol / l is considered as a sign that the student suffers from LI. Of the 109 students included in the study, the relative observed frequency of students with LI resulted 86.2% and the standard error of 3.3%. Concerning the calculation of the observed relative frequency, the students with symptoms among those with LI, resulted 51.1% and the standard error associated with 1.9%. We have come to the conclusion that the relative frequency of the students with LI in this age interval is not dependent on age.

Keywords: Lactose intolerance, symptoms, pupils, variation, enzyme.

1. Introduction

Lactose is a disaccharide consisting of galactose bound to glucose and is of key importance in animal life as the main source of calories from milk of all mammals, all except the sea lion. Intestinal absorption of lactose requires hydrolysis to its component monosaccharides by the brush-border enzyme lactase. From week 8 of gestation, lactase activity can be detected at the mucosal surface in the human intestine. Activity increases until week 34 and lactase expression is at its peak by birth. The ability to digest lactose during the period of breast-feeding is essential to the health of the infant as demonstrated by congenital lactase deficiency that is fatal if not recognized very early after birth. However, following the first few months of life, lactase activity starts to decrease (lactase non-persistence). In most humans, this activity declines following weaning to undetectable levels as a consequence of the normal maturational down-regulation of lactase expression (Vesa, T.H. *et al*, 2000). The exceptions to this rule are the descendants of populations that traditionally practice cattle domestication maintain the ability to digest milk and other dairy products into adulthood. The frequency of this "lactase persistence trait" is high in northern European populations (>90% in Scandinavia and Holland), decreases in frequency across southern Europe and the Middle East (~50% in Spain, Italy and pastoralist Arab populations) and is low in Asia and most of Africa (~1% in Chinese, ~5%–20% in West African agriculturalists); although it is common in pastoralist populations from Africa (~90% in Tutsi, ~50% in Fulani) (Swallow, D.M., 2003)

Lactase persistence is thought to be related to the domestication of dairy cattle during the last 10,000 years. Lactase persistence is inherited as a dominant Mendelian trait (Enattah, N.S. *et al*, 2002). Adult expression of the gene encoding lactase (LCT), located on 2q21 appears to be regulated by cis-acting elements (Wang, Y. *et al* 1995) A

linkage disequilibrium (LD) and haplotype analysis of Finnish pedigrees identifies two single single nucleotide polymorphisms (SNPs) associated with the lactase persistence trait: C/T-13910 and G/A-22018, located ~14 kb and ~22 kb upstream of LCT, respectively, within introns 9 and 13 of the adjacent minichromosome maintenance 6 (MCM6) gene (Enattah, N.S. *et al*, 2002). The T-13910 and A-22018 alleles are 100% and 97% associated with lactase persistence, respectively, in the Finnish study, and the T-13910 allele is ~86%–98% associated with lactase persistence in other European populations. The genotype in China is C/C-13910, and no SNP associated with lactase persistence has been identified in the lactase gene Poulter, M. *et al*, 2003) regulatory sequence (Sun, H.M. *et al*, 2007). However, there are several lactase gene single nucleotide polymorphisms of this kind in other populations. Lactase persistence is mediated by G-13915 in Saudi Arabia (Imtiaz, F. *et al*, 2007), in African tribes by the G-14010, G-13915, and G-13907 polymorphism (Ingram, C.J. *et al*, 2007). Thus, lactase persistence developed several times independently in human evolution in different areas of the world (Imtiaz, F. *et al*, 2007). Multiple independent variants have allowed various human populations to quickly modify LCT expression and have been strongly conserved in adult milk-consuming populations, emphasizing the importance of regulatory mutations in recent human evolution (Wray, G.A. *et al*, 2003). In adult patients with homozygous lactase persistence, enzyme levels at the jejunal brush border are 10-times higher than for patients with homozygous non-persistence, and heterozygous individuals (Enattah, N.S. *et al*, 2007).

Lactase deficiency results in unabsorbed lactose being present in the intestinal tract, which has effects that can lead to symptoms of lactose intolerance in susceptible individuals (Gasbarrini, A. *et al*, 2009). First, the increased osmotic load increases the intestinal water content. Second, lactose is readily fermented by the colonic microbiome leading to production of short chain fatty acids and gas (mainly hydrogen (H2), carbon dioxide (CO2), and methane (CH4)). These biological processes are present also for other poorly absorbed, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) that are ubiquitous in the diet (Magge, S. *et al*, 2012).

Malabsorption is a necessary precondition for lactose. The threshold for dietary lactose tolerance is dependent on several factors including the dose consumed, residual lactase expression (Swallow, D.M., 2003), ingestion with other dietary components (Shaukat, A. *et al*, 2010), gut-transit time, small bowel bacterial overgrowth (Zhao, J. *et al*, 2010), and also composition of the enteric microbiome (e.g., high vs. low fermenters, hydrogen vs. methane producers) (Misselwitz, B. *et al*, 20013). In addition to these environmental and physiological factors, it has been shown that patients with irritable bowel syndrome are at particular risk of both self-reporting dairy intolerance (Bohn, L. *et al*, 2013) and experiencing symptoms after lactose ingestion (Yang, J. *et al*, 2013).

Symptoms of lactose intolerance generally do not occur until there is less than 50% of lactase activity. Regular lactose intake may also have an effect. Although lactase expression is not up-regulated by lactose ingestion, tolerance could be induced by adaptation of the intestinal flora. Further, most people with lactase non-persistence can tolerate small amounts of lactose (less than 12 g, equivalent to one cup), especially when it is combined with other foods or spread throughout the day (Shaukat, A. *et al*, 2010).

Problems with lactose absorption have been described, detected and diagnosed in several ways and this can lead to confusion among doctors and patients (Shaukat, A. *et al*, 2010). Lactase deficiency is defined as markedly reduced brush-border lactase activity relative to the activity observed in infants. Lactose malabsorption occurs when a substantial amount of lactose is not absorbed in the intestine. Because lactose malabsorption is nearly always attributable to lactase deficiency, the presence of this condition can be inferred from measurements of lactose malabsorption such as an increase of glucose in the blood or an increase of hydrogen in the breath. The term lactose intolerance is defined by patient reports of abdominal pain, bloating, borborygmi, and diarrhea induced by lactose. Less often it can present with nausea or constipation and a range of systemic symptoms, including headaches, fatigue, loss of concentration, muscle and joint pain, mouth ulcers, and urinary difficulties (Campbell, A.K. *et al*, 2004); however, it is unclear whether these atypical symptoms are directly due to lactose ingestion, or related to the presence of so-called “functional diseases”, such as irritable bowel syndrome (IBS), which is often accompanied by multiple somatic complaints. Certainly, it is not possible to make a definitive diagnosis on clinical presentation alone because double-blind trials have shown that the association of self-reported lactose intolerance and the occurrence of symptoms after lactose ingestion are very poor (Suarez, F.L. *et al*, 1995), even in patients with lactase deficiency (Zheng, X. *et al*, 2015).

There are various methods for diagnosing lactose malabsorption and intolerance (Misselwitz, B. *et al*, 2013).

Lactose tolerance test. The lactose tolerance test gauges your body's reaction to a liquid that contains high levels of lactose. Two hours after drinking the liquid, you'll undergo blood tests to measure the amount of glucose in your bloodstream. If your glucose level doesn't rise, it means your body isn't properly digesting and absorbing the lactose-filled drink (Arola, H., 1994).

Hydrogen breath test. This test also requires you to drink a liquid that contains high levels of lactose. Then your doctor measures the amount of hydrogen in your breath at regular intervals. Normally, very little hydrogen is detectable. However, if your body doesn't digest the lactose, it will ferment in the colon, releasing hydrogen and other gases, which are absorbed by your intestines and eventually exhaled. Larger than normal amounts of exhaled hydrogen measured during a breath test indicate that you aren't fully digesting and absorbing lactose (Metz, G. *et al*, 1975).

Stool acidity test. For infants and children who can't undergo other tests, a stool acidity test may be used. The fermenting of undigested lactose creates lactic acid and other acids that can be detected in a stool sample (Newcomer, A.D. *et al,* 1975).

There's currently no way to boost your body's production of lactase, but you can usually avoid the discomfort of lactose intolerance by: Avoiding large servings of milk and other dairy products; Including small servings of dairy products in your regular meals; Eating and drinking lactose-reduced ice cream and milk; Drinking regular milk after you add a liquid or powder to it to break down the lactose.

2. 2. Material and methods

The study includes data on determining the percentage of the Lactose intolerance (LI) spreading and the onset of LI symptoms, in a total of 109 healthy schoolchildren aged 12-17, hence with no family history for chronic or genetic diseases (boys and girls).

In this context, each student was given 50 grams of lactose (2 grams per kg of body weight) dissolved in 400 ml of water to be drunk in empty stomach. We had in mind that lactose is not dissolved well in cold water, therefore lactose dissolution was done with warm water and the solution was mixed well before being drunk. The analysis was done 40 minutes after drinking the lactose. For purposes of measuring glucose levels in the blood, the "Glucometer - II" device with "Dextrostis" indicators, produced by "Miles International" was used before and 40 minutes after taking the lactose. The "Glucometer II" device was provided by the "Varus" company from Skopje.

The increase of the glucose level by less than 1.1 mmol/l was evaluated as a sign or symptom pointing to the fact the student in question suffers from primary hypolactasia. The fact that those who suffer from hypolactasia exhibit clinical symptoms several hours after taking lactose has also been helpful during the diagnosis process.

The analysis was conducted on a voluntary basis after the students were shown the possible symptoms and after they were consulted with and were granted permission by their parents. Patients with illnesses in general and with gastrointestinal diseases in particular, as well as those with family history of gastrointestinal or genetic diseases, did not undergo the examinations. During the examinations, we were always accompanied and assisted by medical nurses and the examinations went smoothly in general.

The students were classified into 5 sample units (classes) with 1-year age intervals, thus forming an initial 5-class sample and each group was composed of about 25 students.

During data processing a correlation and regression analysis will be performed, namely the determination of coefficients of correlation between variables; regression analysis; determination of linear regression line coefficients, etc.

3. Results and Discussions

The working sample with both male and female students and the age-dependent frequency variations of students with LI in the 12-17 intervals

Based on the previous data processing acquired from the working sample, we can see that the average of the LI phenotype of the Albanian population in Macedonia that underwent the examination is characterized by a high prevalence with about 86.2%.

The working sample data: class number – I, the interval mean Xmi (y.) of the respective class age-group, the number of students in class Ni, numerical frequency of students with LI, Yoi (num) and the relative frequency in the observed % Yoi (%) – have been given in Table 1.

Table 1. The working sample data and the relative frequency of students with LI from one class to another

I	Xmi (y.)	**Ni**	**Yoi (num)**	**Yoi (%)**	
Class number	**The interval mean of the respective class age-group**	**The number of students with LI in class**	**Numerical frequency of students with LI**	**The relative frequency in the observed %**	**The equation of linear regression line (y) Correlation of coefficient (r) and their significance**
1	12.5	20	17	85%	**y=6.39x+10.50**
2	13.5	23	21	91.3%	
3	14.5	23	19	82.6%	**r=-0.11**
4	15.5	20	17	85%	
5	16.5	23	20	87%	**0.025<p<0.05**
Total		109	94	86.2%	

The relative frequency flow of observed students with LI from one class to another has been illustrated in Figure 1.

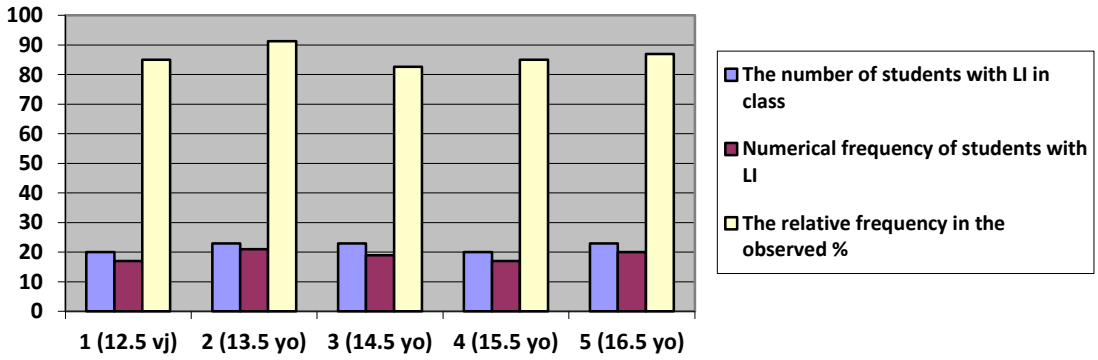

Fig. 1. The relative frequency flow of observed students with LI

Based on the data from Table 1 and Figure 1 we can conclude that the relative frequency of observed students with LI in classes from 1 to 5 is characterized with a "saturating" tendency.

For variable pairs [Xmi (vj), Yoi (%)] of the five classes from 1 to 5, we have calculated the correlation coefficient, which resulted in r = -0.11 (close to 0). Based on this result, we can conclude that the relative frequency of students with LI in the age interval between 12 and 17 is not age-dependent. Therefore, for the stated age interval (i.e. 12 – 17) in the saturation zone of the working sample, all observations have been summarized into one and only, i.e. the zone has dealt with one observation only.

In this context, the relative frequency of the observed students with LI for this characterized zone of saturation and its accompanying standard error, calculated according to (Crawsh *et al*), is as follows:

$$Ys = [94/109,100\%] = 86.2\% \quad (1)$$

$$S.Eys = 3.3\% \quad (2)$$

The variation of frequency of the emergence of symptoms in students with LI in the 12-17 age interval

In Table 2, we can see the data relating to the emergence of symptoms in a section of students with LI in the 12-17 age interval

Table 2. The variation of frequency of the emergence of symptoms in students with LI from one class to another

I	Xmi (year)	Ni	Yoi sim. (num)	Yoi sim. (%)
Class number	**The interval mean of the respective class age-group**	**The number of students with LI in class**	**Numerical frequency of students with symptoms**	**The relative frequency in the observed with symptoms**
1	12.5	17	9	52.9%
2	13.5	21	13	61.9%
3	14.5	19	6	31.6%
4	15.5	17	9	52.9%
5	16.5	20	11	55.0%
Total		94	48	51.0%

The data from Table 2 have been presented in Figure 2.

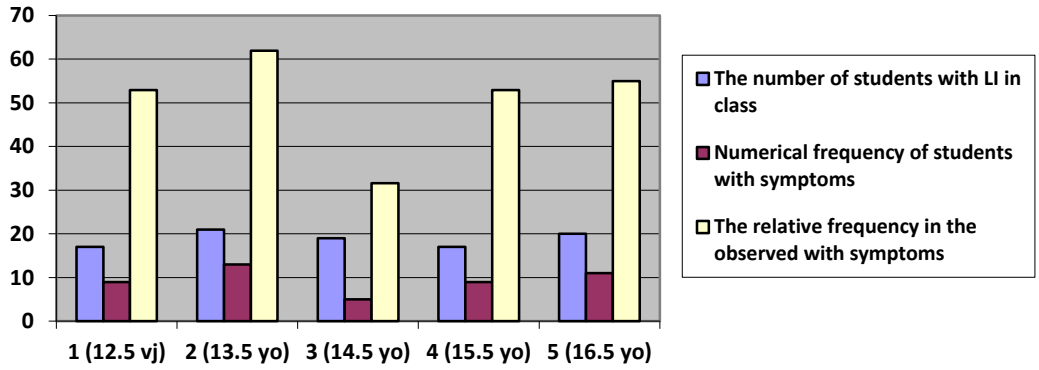

Fig. 2. Graphical representation of the emergence of symptoms in students with LI

With regard to variable pairs [Xmi (y.), Yoi sim (%)] of the five classes, in Table 2, in the 12-17 age interval, we have calculated the correlation coefficient which resulted in having r = 0.067 (close to 0). According to the gathered data in one observation, in Table 2, the relative frequency of observed students with LI in the 12-17 age interval has been calculated, including its accompanying standard error, resulting in the following values:

$$Ysim = [48/94,100\%] = 51.1\% \quad (3)$$

$$S.E = 1.9\% \quad (4)$$

In Case 4 we have a corrected result for the accompanying standard error, compared to the previous Case 2. This is so because the sample community with 94 students with LI comprises a high fraction of the total number of students, which is 109.

4. Conclusions

Based on the data processing of the sample characterized as a saturation zone (109) students aged between 12 and 17, we have come to the following conclusions:

- Variables in consideration do not depend on each other and the assessment of the frequency can be done through a single observation;
- The observed relative frequency (proportion) of students with LI in the interested sample is Ys = 86.2%, with standard error S. Eys = 3.3%.
- The observed relative frequency (proportion) of students with symptoms among those with LI is Ysim = 51.1%, with standard error SE. ysim = 1.9%.

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