The role of cholecalciferol deficiency in the development of latent autoimmune diabetes in adults

Abstract. Background. Recently, a lot of information has appeared on the role of cholecalciferol deficiency in the development of classical types of diabetes mellitus (DM) and its complications. However, there are currently almost no data regarding latent autoimmune diabetes in adults (LADA). The purpose of this study was to determine the effect of vitamin D deficiency on the compensation of carbohydrate metabolism in LADA. Materials and methods. The study included 56 patients with DM: 34 with LADA and 22 with classical type 1 DM (DM1), as well as 20 practically healthy individuals of the control group. According to the main phenotypes, patients with LADA were divided into 2 groups: LADA1 and LADA2. Cholecalciferol status was determined by the immunochemiluminescence method. Results. The fasting blood glucose level in LADA and DM1 group was significantly higher than in the controls, by 63.9 and 91.1 % (p < 0.001), respectively, and was also 16.6 % higher when comparing DM1/ LADA groups (p < 0.05). The level of HbA1c in 66.1 % of patients of the experimental groups was more than 7 %, which indicates insufficient compensation of the disease. The content of vitamin D was significantly lower in the experimental groups compared to the controls; when comparing LADA/DM1 — by 43.7 % lower in case of classical DM1 (p < 0.05). Compensation of carbohydrate metabolism is worse in patients with LADA1 than in LADA2, and the lowest level of vitamin D was recorded in LADA1. According to the linear regression analysis of correlations in patients with LADA, negative correlations of medium strength were recorded between the level of cholecalciferol and fasting blood glucose (r = 0.487; p < 0.05), HbA1c (r = –0.593; p < 0.05); positive — between cholecalciferol and C-peptide (r = 0.412; p < 0.05). Conclusions. In patients with autoimmune diabetes, there is an insufficient supply of cholecalciferol. Low cholecalciferol content is observed in both groups of patients with LADA regardless of the disease phenotype and is associated with worse compensation of DM. Keywords: type 1 diabetes mellitus; latent autoimmune diabetes in adults; cholecalciferol, phenotypes

Introduction

Latent autoimmune diabetes in adults (LADA) is a heterogeneous type of diabetes mellitus (DM) that combines features of type 1 diabetes mellitus (DM1) and type 2 (DM2) [1] and is diagnosed by detecting antibodies to islet antigens in the blood with manifestation of the disease in adults over 35 years [2].

As shown by the results of epidemiological studies conducted in different countries, LADA is a fairly common type of diabetes.

Today, it is believed that the prevalence of LADA is up to 14 % among all cases of DM in the adult population, it varies in different countries and, apparently, depends on ethnicity, lifestyle and methodological approaches used by different researchers [3].

The complex and specific mechanism of the development of LADA is evidenced by its marked heterogeneity in terms of genetic, phenotypic, and immunological features.

Research into the genetic nature of LADA has shown that it is more similar to autoimmune DM1, but the disease also has determinants associated with DM2. Given that LADA is characterized by greater body weight than DM1, moderate systemic inflammation due to excess visceral adipose tissue may cause a latent autoimmune process characterized by IA-2 autoantibody positivity with loss of β-cell function and decreased insulin secretion [3, 4].

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Therefore, patients with LADA may share genetic features of both DM1 and DM2, which is associated with high variability in the rate of pancreatic β-cell destruction, insulin resistance and autoimmunity. The latter is characterized by the positivity of autoantibodies to pancreatic islet antigens. At the same time, lymphoid infiltration of islets (insulitis) causes progressive death of β-cells, which occurs more slowly than in DM1 [3, 5].

Recently, a lot of information has appeared on the role of vitamin D deficiency in the development of insulin resistance, diabetes and its complications [6–9]. Cholecalciferol is a fat-soluble steroid hormone that promotes absorption of calcium and phosphorus from food through the vitamin D receptor (VDR) [10]. VDRs are present in every organ in the human body, including the heart, liver, kidneys, blood vessels, and central nervous system. In addition, pancreatic β-cells also have VDR, which plays important role in the occurrence of dysfunction of these cells [11].

Thus, despite the extensive scientific work on the impact of vitamin D deficiency on the occurrence and course of classical types of diabetes, there is currently almost no data on LADA.

**The purpose of the study was to determine of the effect of cholecalciferol deficiency on the state of carbohydrate metabolism in latent autoimmune diabetes in adults.**

**Materials and methods**

The study included 56 patients with DM, who were divided into two groups: 34 patients with LADA and 22 patients with classical DM1. The control group included 20 practically healthy individuals.

The diagnosis of DM was made according to the Standards of Medical Care for Diabetes Mellitus of the American Diabetes Association (ADA) [12], the consensus report of the ADA and the European Association for the Study of Diabetes (EASD) on the management of DM1 in adults [13], the diagnosis of LADA — according to the recommendations of the Immunology of Diabetes Society (IDS, 2005) [14] and the consensus of the International Expert Group (2020) [15].

Patients with LADA were divided into 2 groups according to the main phenotypes: LAD1 (18 patients) with high antiGAD titers ≥ 180 U/ml and LAD2 (16 patients) with low antibody titers, from 18 to 180 U/ml [16].

At the time of the study, the duration of the disease in patients with LADA was 7 [4.0; 10.0] years, in patients with DM1 — 13 [8.0; 19.0] years.

All patients underwent clinical, laboratory and instrumental examination. The patient’s complaints, medical history, life history, hereditary history were collected in detail. The patients underwent a number of general clinical laboratory examinations: general blood count, biochemical blood analysis, lipidogram.

The following research methods were used in the work: questionnaires (to determine levels of anxiety and depression, assessment of quality of life), general clinical, instrumental (ophthalmoscopy), laboratory (clinical blood and urine analysis, biochemical analyses), spectrophotometric (blood lipid spectrum), immunoenzymatic (determination antiGAD and IA-2 ab, C-peptide levels), immunochrominecent (cholecalciferol). According to the Central and Eastern European Expert Consensus Statement 2022 on the prevention, diagnosis and treatment of vitamin D deficiency, cholecalciferol concentrations < 20 ng/ml are defined as deficiency, concentrations between 21 and 29 ng/ml as insufficiency, and serum levels > 30 ng/ml as normal [17].

**Statistical methods.** Mathematical processing of the obtained data was carried out using Statistica 13.3 StatSoft Inc., Microsoft Excel 2016 programs. Quantitative data are presented in the form of median (Me) and interquartile range (Q25-Q75). Before testing the statistical hypotheses, coefficients of asymmetry and kurtosis were determined using the Hahn-Shapiro-Wilk test to analyze the normality of the distribution of values in randomized samples. Non-normal distribution of data was established in most groups, so the nonparametric Mann-Whitney U test was used for further calculations. Analysis of variance using the Kruskal-Wallis test was used to assess the probable difference between 3 or more groups. Analysis of qualitative features was performed using one- and two-sided Fisher’s exact test. Differences were considered reliable at a significance level of p < 0.05 (taking into account the Bonferroni correction).

To conduct a correlation analysis with a non-normal distribution of the sample, we used the non-parametric Spearman’s rank correlation coefficient in the Statistica 13.3 StatSoft Inc. program.

**Results**

The fasting blood glucose level in the LADA group was 63.9 % higher than in the control group (p < 0.001) (Table 1). In DM1 it exceeded the value in practically healthy individuals by 91.1 % (p < 0.001) and was registered higher by 16.6 % compared to the LADA group (p < 0.05).

The HbA1c increased by 64.7 % in the LADA group (p < 0.001) and by 95.9 % in DM1 (p < 0.001), respectively, when compared with the control group. In patients with DM1, this indicator exceeded that of LADA by 19 % (p < 0.001). It is worth noting that in 66.1 % of patients of the research groups, the level of HbA1c was more than 7 %, which indicates insufficient compensation of the disease.

Thus, patients with LADA have unsatisfactory diabetes control, just like those with classical DM1. At the same time, indicators of compensation of carbohydrate metabolism in LADA do not differ from those in DM1.

The level of C-peptide was lower in the group of patients with LADA by 2 times compared to the control (p < 0.05). In DM1, this indicator decreased by 29.4 times compared to control and by 14.8 times compared to LADA (p < 0.001).

When determining the titers of antibodies against islet antigens, we obtained the following data: the content of antiGAD in LADA was 95.2 times higher compared to the control (p < 0.001). In DM1, it increased by 128.5 times compared to control and by 35 % compared to the corresponding indicator in LADA patients (p < 0.001).

The IA-2 ab titer was 19.1 times higher in LADA and 31.5 times higher in DM1 compared to the control group, respectively (p < 0.001). In DM1, IA-2 ab titers were 65 % higher compared to the rate in LADA patients (p < 0.05).
Thus, in patients with LADA, antibody titers are high, but antiGAD and IA-2 ab are lower than in classical DM1, indicating a lower degree of diabetes-associated autoimmunity in this category of patients.

As the results of the study showed, the content of vitamin D was probably lower in the experimental groups compared to the control group: by 37.2 % in LADA (p < 0.001) and by 2.8 times in DM1 (p < 0.001). When comparing the data of patients with LADA and DM1, the content of vitamin D was lower by 43.7 % in the case of classical DM1 (p < 0.05).

Next, we analyzed changes in the above parameters in patients depending on the LADA phenotype (Table 2). As can be seen from the above data, there is a significant increase in fasting glycemia by 90.9 % in patients with LADA compared to practically healthy individuals (p < 0.001), but there are no likely changes found between LADA1 and LADA2 groups.

The value of HbA1c was also significantly different when comparing LADA1—2/control indicators (79.8 and 63.7 % excess, respectively; p < 0.001), while the comparison of different LADA phenotypes revealed a 9 % lower value of this parameter in the LADA2 group relative to LADA1 (p < 0.05). This indicator exceeded the level of 7 % in 88.9 % of patients with LADA1 and in 43.8 % of patients with LADA2.

Thus, the compensation of carbohydrate metabolism according to the HbA1c indicator is worse in patients with LADA1 than in LADA2.

Changes in the level of C-peptide in patients with different phenotypes of LADA were as follows: a probable decrease of 9 times when comparing LADA1/control (p < 0.001), by 46.4 % when comparing LADA2/control (p < 0.05) and in 4.8 times when comparing LADA1 and LADA2 groups with a high degree of statistical significance (p < 0.001).

When comparing the obtained indicators of autoimmunity in LADA phenotypes, it was found that in patients with LADA1, antiGAD titers were 124.6 times higher compared to LADA2.

Table 1. Characteristics of indicators of carbohydrate metabolism, β-cell function and cholecalciferol status in patients with autoimmune diabetes

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Control group, n = 20</th>
<th>LADA, n = 34</th>
<th>DM1, n = 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c, %</td>
<td>4.16 [4.03; 5.01]</td>
<td>6.85 [5.98; 8.58]</td>
<td>8.15 [6.78; 10.32]</td>
</tr>
<tr>
<td>C-peptide, ng/ml</td>
<td>2.35 [1.81; 3.41]</td>
<td>1.18 [0.86; 1.76]</td>
<td>0.80 [0.05; 0.19]</td>
</tr>
<tr>
<td>antiGAD, U/ml</td>
<td>2.06 [0.73; 3.12]</td>
<td>196.08 [86.35; 269.80]</td>
<td>264.70 [118.00; 398.00]</td>
</tr>
<tr>
<td>IA-2 ab, U/ml</td>
<td>1.35 [0.68; 2.32]</td>
<td>25.80 [18.90; 29.42]</td>
<td>42.56 [27.17; 68.32]</td>
</tr>
</tbody>
</table>

Notes: p1 — probability of changes compared to the control group; p2 — probability of changes compared to LADA.

Table 2. Characteristics of indicators of carbohydrate metabolism, β-cell function and cholecalciferol status in patients with latent autoimmune diabetes in adults depending on the phenotype of the disease

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Control group, n = 20</th>
<th>LADA1, n = 18</th>
<th>LADA2, n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>4.38 [4.01; 5.30]</td>
<td>8.36 [6.87; 11.02]</td>
<td>7.13 [5.95; 9.19]</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>4.16 [4.03; 5.01]</td>
<td>7.48 [6.62; 9.11]</td>
<td>6.81 [5.42; 7.69]</td>
</tr>
<tr>
<td>C-peptide, ng/ml</td>
<td>2.35 [1.81; 3.41]</td>
<td>0.26 [0.15; 1.23]</td>
<td>1.26 [0.89; 1.86]</td>
</tr>
<tr>
<td>antiGAD, U/ml</td>
<td>2.06 [0.73; 3.12]</td>
<td>258.63 [213.88; 296.13]</td>
<td>107.28 [37.89; 132.16]</td>
</tr>
<tr>
<td>IA-2 ab, U/ml</td>
<td>1.35 [0.68; 2.32]</td>
<td>37.16 [23.12; 47.61]</td>
<td>14.08 [11.45; 18.01]</td>
</tr>
</tbody>
</table>

Notes: p1 — probability of changes compared to the control group; p2 — probability of changes compared to LADA1.
to control (p < 0.001). With the LADA2 phenotype, they increased by 52.1 times compared to the control group, but were registered 2.4 times lower than in LADA2 (p < 0.001).

IA-2 ab were 27.5 times higher in LADA1 compared to control, and 10.4 times higher in LADA2 (p < 0.001); in the intergroup comparison, the titers were 2.6 times higher in case of LADA1 phenotype.

Thus, indicators of diabetes-associated autoimmunity are significantly higher in patients with LADA1 phenotype compared to LADA2 group.

Comparing the vitamin D status between LADA phenotypes, the following results were obtained: in patients with LADA1, the level of cholecalciferol was 2.4 times lower compared to control with a high degree of probability (p < 0.001). In LADA2 phenotype it was lower by 37.7 % compared to the control group (p < 0.001), but higher by 47.9 % than in LADA1 (p < 0.05).

According to the linear regression analysis of correlations in patients with LADA between the indicators of vitamin D supply status and carbohydrate metabolism, negative correlations of medium strength were recorded between the level of cholecalciferol and fasting blood glucose levels (r = 0.487; p < 0.05), Hba\(_{1c}\) indicator (r = −0.593; p < 0.05); antiGAD titers and C-peptide level (r = 0.584; p < 0.05); positive correlations of medium strength were registered between the level of cholecalciferol and the C-peptide (r = 0.412; p < 0.05).

In patients with LADA1, negative correlations of medium strength were registered between the content of cholecalciferol and the Hba\(_{1c}\) (r = 0.318; p < 0.05); positive of medium strength — between antiGAD titers and fasting blood glucose (r = 0.444; p < 0.05), Hba\(_{1c}\) (r = 0.473; p < 0.05) in LADA2 phenotype — negative correlations of medium strength were registered between the content of cholecalciferol and fasting blood glucose levels (r = −0.436; p < 0.05), the Hba\(_{1c}\) index (r = −0.487; p < 0.05).

**Discussion**

As the results of the study showed, the content of vitamin D was probably lower in the experimental groups compared to the control: by more than 37 % in LADA and almost three times in DM1 with a high degree of probability.

The obtained results also indicate a pronounced deficiency of this vitamin in LADA, regardless of the disease phenotype, but with a greater degree of deficiency in patients with LADA1.

Linear regression analysis of correlations data showed that a higher degree of cholecalciferol deficiency in LADA patients was associated with worse diabetes compensation and lower insulin-producing function of β-cells. In the case of LADA1 and LADA2 phenotypes, additional associations were found between a decrease of vitamin D with worse compensation indicators, and in LADA2 — also with a deterioration in insulin production.

There are several mechanisms of the relationship between vitamin D and the onset and progression of insulin resistance. Cholecalciferol stimulates insulin receptors and participates in glucose transport, increasing reactivity to insulin [18]. On the other hand, vitamin D can reduce the effects of systemic chronic inflammation and protect against cytokine-induced β-cell apoptosis by directly modulating cytokine expression and activity [19, 20]. Activation of VDR by a vitamin D analog reduces liver inflammation and reduces insulin resistance [21]. In addition, cholecalciferol improves the hyperactivity of the aldosterone effect through the renin-angiotensin system, activates the function of pancreatic β-cells and reduces insulin resistance [22, 23].

According to the consensus of Central and Eastern European experts on the prevention, diagnosis and treatment of vitamin D deficiency 2022, it is recommended the prescription of calcidiol in a dose of 4,000 to 10,000 IU/day with subsequent transition to standard doses of cholecalciferol upon reaching a serum level of 30–50 ng/ml [17].

**Conclusion**

In patients with autoimmune diabetes there is an insufficient supply of cholecalciferol. Low content of vitamin D is observed in both groups of patients with LADA regardless of the phenotype of the disease. In patients with LADA, low cholecalciferol content is associated with poorer rates of diabetes compensation and decreased insulin-producing function.

**References**


Що вказує на недостатню компенсацію захворювання. Уміст HbА1c на 16,6 % при порівнянні груп ЦД1/LADA (p < 0,05). Рівень глюкози в сироватці крови у 63,9 та 91,1 % (p < 0,001) відповідно, також він був вищим у пацієнтів з LADA та ЦД1 та був вірогідно вищим, ніж у контролі, — на 24,3 % (p < 0,001) методом.

Ці дані відповідають результатам, що були одержані в експериментальних умовах.

Роль дефіциту холекальциферолу в розвитку латентного автоімунного діабету дорослих

Рецензія. Актуальність. Останнім часом з'явилося чимало відомостей щодо ролі дефіциту холекальциферолу в розвитку класичних типів цукрового діабету (ЦД) та його ускладнень, проте даних відносно латентного автоімунного діабету дорослих, як це зазначено в чинному законодавстві, є недостатньо.

Мета: визначити вплив дефіциту холекальциферолу на компенсацію вуглеводного обміну при LADA1. За даними лінійно-регресійного аналізу кореляцій у пацієнтів з LADA, зворотні зв'язки середньої сили відповідають вимогам до змін параметрів, оціниваних кореляційною статистикою.

Матеріали та методи. У дослідження включено 56 пацієнтів із ЦД: 34 з LADA та 22 із класичним ЦД1. Результати аналізу показали, що відповідальність LADA1 та LADA2 статус холекальциферолу визначали імуннокомплексову гістологічну імунологічну реакцію.

Результати. Показник глікемії нації в групах LADA та ЦД1 був вірогідно нижчим (p < 0,05), ніж у контрольній групі пацієнтів. При порівнянні групи LADA1/LADA2 (p < 0,05). Рівень HbА1c у 61,6 % пацієнтів досліджених груп становив більше 7 %, що вказує на недостатню компенсацію захворювання. Уміст холекальциферолу був вірогідно нижчим у дослідних групах порівняно з контрольною; при порівнянні LADA1/ЦД1 — на 43,7 % нижчим у разі класичного ЦД1 (p < 0,05). Компенсація вуглеводного обміну є гіршою в пацієнтів із LADA1, ніж при LADA2, а найнижчий рівень холекальциферолу реєструвався при LADA1. За даними лінійно-регресійного аналізу кореляцій у пацієнтів з LADA1, зворотні зв’язки середньої сили реєструвалися між рівнем холекальциферолу та гілікемією нації (r = –0,487; p < 0,05), показником HbА1c (r = –0,593; p < 0,05); позитивний — між показником холекальциферолу та C-пептиду (r = 0,412; p < 0,05). У пацієнтів з автономним ЦД відзначається недостатній забезпечення холекальциферолом. Низький уміст холекальциферолу спостерігається в обох групах пацієнтів із LADA незалежно від фенотипу захворювання і асоціюється з різкими показниками компенсації ЦД.

Ключові слова: цукровий діабет 1-го типу; латентний автоімунний діабет дорослих; холекальциферол; фенотипи