VITAMIN D AS A MARKER OF NON-ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Ninety patients with type 2 diabetes mellitus (DM) aged 39 to 76 years were examined. Women were 71 (78.9 %), men – 19 (21.1 %). The diagnosis of non-alcoholic fatty liver disease (NAFLD) was made based on an ultrasound examination of the abdominal cavity. The levels of vitamin D in the blood were determined by enzyme immunoassay. NAFLD was detected in 66.7 % of patients with type 2 DM. Vitamin D levels in the blood of patients with NAFLD on the background of type 2 DM were lower, and its deficiency was more common than in cases of DM without NAFLD. Serum vitamin D levels in patients with NAFLD and cholestatic syndrome were lower than in cases of NAFLD without signs of cholestasis. According to regression analysis, the development of NAFLD in type 2 DM is affected by reduced vitamin D levels in the blood and increased values of glucose, alanine aminotransferase, and low-density lipoproteins.

Thus, the development of NAFLD against the background of type 2 DM is associated with a decrease in vitamin D concentration in the blood. Serum level of vitamin D less than 16.18 ng/ml can be used as a predictor of NAFLD in patients with type 2 DM.

Keywords: non-alcoholic fatty liver disease, type 2 diabetes mellitus, vitamin D


Ac – accuracy
ALT – alanine aminotransferase
AST – aspartate aminotransferase
BMI – body mass index
CI – confidence interval
DM – diabetes mellitus
GGT – gamma-glutamyltranspeptidase
HDL – high-density lipoproteins
LDL – low-density lipoproteins
NAFLD – non-alcoholic fatty liver disease
NPV – negative predictive value
OR – odds ratio
PPV – positive predictive value
Se – sensitivity
Sp – specificity
TLR – Toll-like receptor
NAFLD is one of the most common chronic liver diseases and includes a wide range of conditions such as steatosis, non-alcoholic steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma, which are based on the accumulation of triglycerides in hepatocytes without alcohol abuse [1, 2].

The prevalence of NAFLD in the world averages 25%, and the main proportion of patients is detected in the cohort of the working population [3]. In patients with type 2 DM, the prevalence of NAFLD doubles, reaching 60% or more [4].

Vitamin D is essential in maintaining calcium homeostasis in the body through a receptor belonging to the nuclear hormone receptor superfamily. In recent decades, particular attention has been paid to the non-classical effects of vitamin D, such as immune modulation, effects on hormonal secretion, and participation in cell differentiation and proliferation, which provide antioxidant protection and reduction of inflammation and fibrosis [5].

It is hypothesized that vitamin D deficiency coexists with NAFLD, given their associations with obesity and type 2 DM. The point of view that there is a causal relationship between hypovitaminosis D and metabolic pathology of the liver is gaining more recognition [6].

Thus, in patients with NAFLD, a reduced content of vitamin D in the blood was determined, and its deficiency (less than 20 ng/ml) occurred in 70% of the cases [7]. Decreased blood levels of vitamin D (less than 30 ng/ml) were detected in 70.1% of patients with steatosis, 89.7% with non-alcoholic steatohepatitis, and 84.6% with liver cirrhosis [8]. A decrease in serum vitamin D values was noticed in NAFLD patients with type 2 DM [9–11], especially in cases of severe liver fibrosis [11].

However, there is an opinion that the concentration of vitamin D in the blood, as well as the frequency of its deficiency in patients with NAFLD, does not differ from the total population [12, 13], although the results of some meta-analyses refute this data [14]. The role of vitamin D in predicting NAFLD in patients with type 2 DM has not yet been determined.

The study aimed to evaluate the predictive value of blood vitamin D levels in patients with NAFLD and type 2 DM.

Material and Methods. The study included 90 patients with type 2 DM aged 39 to 76 (mean age 58.79±8.59 years). Women were 71 (78.9%), men – 19 (21.1%). Inclusion criteria: type 2 DM; age over 18; signed informed consent to participate in the study.

Exclusion criteria: Type 1 DM; gestational diabetes; pregnancy and lactation; malignant neoplasms; internal diseases (n=30) was determined by ELISA. The diagnosis of NAFLD was based on abdominal ultrasonography, which is highly sensitive and specific and is a sufficient diagnostic criterion when excluding other causes of liver pathology [15].

The content of vitamin D in blood serum (SCVital Development Corporation, Russia) was determined by ELISA.

The patients signed an informed consent to participate in the research, confirmed by the university’s ethics committee.

The results were statistically processed using StatTech v. 3.0.9 (Stattech Pvt. Ltd., Russia). Quantitative parameters with normal distribution were described using the mean (M) and standard deviation (SD). Without a normal distribution, parameters are presented as a median (Me) and the lower and upper quartiles (Q1; Q3). To identify differences, the Student’s t-test and Mann–Whitney test were used. The odds ratio (OR) and its 95% confidence interval (CI) were calculated. ROC analysis was performed to determine the diagnostic value of the indicators, and the information content of the test was assessed using the area under the ROC curve. The value of several parameters in the NAFLD prediction was estimated by logistic regression. Sensitivity, specificity, positive and negative predictive values, and accuracy were calculated. The discrepancies were considered statistically significant at p<0.05.

Results and Discussion. NAFLD was detected in 60 (66.7%) patients with type 2 DM; in 30 (33.3%) cases, there were no signs of liver pathology. Patients with NAFLD on the background of type 2 DM had higher glucose levels, glycosylated hemoglobin, ALT, AST, GGT, total cholesterol, LDL, and very low levels of HDL in the blood (Table 1). Triglycerides in this cohort of patients tended to increase.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NAFLD with DM (n=60)</th>
<th>DM without NAFLD (n=30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender abs. (%)</td>
<td>Male 10 (16.7 %)</td>
<td>Female 50 (83.3 %)</td>
<td>0.144</td>
</tr>
<tr>
<td>Age, years</td>
<td>57.87±8.91</td>
<td>60.63±7.73</td>
<td>0.151</td>
</tr>
<tr>
<td>Duration of DM, years</td>
<td>10.50 (6.00; 15.00)</td>
<td>8.00 (4.25; 16.50)</td>
<td>0.504</td>
</tr>
<tr>
<td>Glucosylated hemoglobin, %</td>
<td>9.35 (8.17; 10.81)</td>
<td>7.46 (6.10; 8.43)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>10.74 (8.69; 15.20)</td>
<td>7.28 (5.53; 9.90)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>35.10 (21.75; 49.80)</td>
<td>20.50 (15.70; 27.75)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>25.30 (17.65; 42.33)</td>
<td>20.50 (17.48; 28.00)</td>
<td>0.039**</td>
</tr>
<tr>
<td>GGT, U/l</td>
<td>42.00 (28.75; 71.25)</td>
<td>23.50 (18.00; 33.75)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Total bilirubin, µmol/l</td>
<td>11.32 (7.66; 14.85)</td>
<td>10.05 (8.30; 11.20)</td>
<td>0.262</td>
</tr>
<tr>
<td>BM, kg/m²</td>
<td>33.06±3.92</td>
<td>32.22±5.41</td>
<td>0.402</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>6.17±1.70</td>
<td>5.12±0.82</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.23±0.32</td>
<td>1.41±0.37</td>
<td>0.019*</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>3.87±1.39</td>
<td>3.02±0.84</td>
<td>0.003*</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.9 (1.46; 2.98)</td>
<td>1.69 (1.10; 2.39)</td>
<td>0.062</td>
</tr>
<tr>
<td>Arterial hypertension abs. (%)</td>
<td>52 (86.7 %)</td>
<td>24 (80 %)</td>
<td>0.538</td>
</tr>
</tbody>
</table>

Note: * – Student’s t-test; ** – Mann–Whitney test.
The blood concentration of vitamin D in patients with type 2 DM was decreased and amounted to 15.4 (10.94; 29.98) ng/ml. Serum levels of vitamin D in type 2 DM patients with NAFLD were statistically significantly lower (12.00 [8.53; 16.20] ng/ml) than in diabetic patients without NAFLD (32.33 (25.28; 41.05) ng/ml; p<0.001).

Vitamin D deficiency in patients with NAFLD was more common (81.7 and 16.7 % of cases, respectively), and normal values of vitamin D were rare (6.6 and 56.6 % of cases, respectively) than in diabetic patients without NAFLD. Vitamin D deficiency in both groups was determined in 11.7 % and 26.7 % of cases, respectively (Fig. 1).

Blood levels of vitamin D in patients with NAFLD in combination with cytolysis were lower (11.63 [6.86; 15.03] ng/ml) than in patients without increased aminotransferase activity (13.07 [9.77; 22.64] ng/ml), but the differences did not reach statistical significance (p>0.05).

Serum values of vitamin D in patients with NAFLD and cholestatic syndrome were lower (11.27 [6.86; 13.50] ng/ml) than in cases of NAFLD without biochemical signs of cholestasis (13.50 [10.30; 21.35] ng/ml, p=0.038).

Threshold levels of vitamin D in the blood less than 16.18 ng/ml were associated with an increased risk of NAFLD in patients with type 2 DM (OR = 87.0; 95 % CI (10.89; 694.55)) and were highly sensitive and specific (Table 2). The area under the ROC curve was 0.895±0.041 (p<0.001).

Using multiple logistic regression, the significance of 16 parameters was assessed (age, gender, duration of DM, presence of arterial hypertension, body mass index values, activity of AST, ALT, GGT, total bilirubin, glucose, glycosylated haemoglobin, total cholesterol, triglycerides, HDL, LDL, vitamin D in the blood) in the prediction of NAFLD in patients with type 2 DM. According to regression analysis, the development of NAFLD in this population of patients is prognostically significantly affected by serum levels of vitamin D, glucose, ALT, and LDL. The chances of having NAFLD decrease by 1.094 times with an increase in vitamin D by one ng/ml and, on the contrary, increase with an elevation in glucose by one mmol/l (1.377 times), ALT by one U/l (1.067 times), LDL per 1 mmol/l (2.208 times) (Fig. 2).

The study showed that in NAFLD patients with type 2 DM, there is a decrease in the blood content of vitamin D and an increase in the frequency of its deficiency (81.7 %), which coincides with previously obtained data [9–11].

Vitamin D deficiency is thought to be unrelated to NAFLD but is associated with increased vitamin D content in fatty tissues during obesity, as well as a sedentary lifestyle and less contact with sunlight in patients, and high-level calorie food with low content of minerals and vitamins [8, 16]. However, obesity does not explain vitamin D deficiency in NAFLD, as some studies have shown that vitamin D levels in the blood have also been reduced in non-obese patients [16].

The high risk of NAFLD associated with hypovitaminosis D persisted even after excluding such a factor as visceral obesity [13].

Several ways exist to understand the pathogenetic relationship between vitamin D deficiency and NAFLD. First, vitamin D reduces insulin resistance of peripheral tissues and hepatocytes, so its deficiency leads to hepatic steatosis. In the NAFLD model, adding vitamin D reduces glucose and insulin levels, and triglycerides in the liver. The protective effect was associated with the activation of the vitamin D receptor, which increased the expression of hepatocyte nuclear factor 4α (controls the expression of triglyceride transport genes) [17].

Secondly, vitamin D influences hormone secretion (increases adiponectin production, inhibits resistin and renin activity), immune response (reduces the release of pro-inflammatory mediators), and cell proliferation. In the model of NAFLD, vitamin D deficiency led to an increase in hepatic expression of the resistin gene, genes of acquired (interleukins 1, 4, 6) and innate immunity (TLR-2, -4, -9), which was accompanied by an increase of fat in the liver, parameters of lobular inflammation and NAFLD activity score [18].

In addition, the activation of the vitamin D receptor signifies the pathway of the pressures of oxidative stress soup and the proliferation of hepatic stellation cells, which improves fibrosis in NAFLD. For example, in vitamin D-deficient rats, hepatic expression of the hemoygenase-1 gene, a marker of oxidative stress involved in fibrogenesis, was increased due to increased fibrosis severity in the NAFLD [18] model.
Finally, an important role of the gut microbiota in the pathogenesis of NAFLD may be associated with vitamin D deficiency. The microbiome’s interaction with intestinal epithelial cells is mediated by the TLRs expressed on them. Vitamin D deficiency is responsible for increased expression of TLR-2, -4, and -9, followed by an increase in endotoxin exposure to the liver, which contributes to the development of NAFLD [5].

According to our data, blood levels of vitamin D less than 16.18 ng/ml were associated with an increased (87-fold) risk of NAFLD in patients with type 2 DM and were characterized by high sensitivity and specificity (75.0 and 96.7 %, respectively). Previously, it was noticed that NAFLD is negatively associated with vitamin D levels, and the risk of NAFLD occurrence increased by 20–30 % in cases of vitamin D deficiency [13, 19]. Blood levels of vitamin D less than 11 ng/ml predicted the presence of NAFLD in the general population with a sensitivity of 45 % and a specificity of 98 % [7].

Our regression analysis showed that the development of NAFLD in patients with type 2 DM is affected by serum levels of vitamin D, glucose, ALT, and LDL. In other studies, according to binary logistic regression, NAFLD risk factors in type 2 DM were age, HOMA-IR values, BMI, GGT, cystatin C, HDL, and vitamin D in the blood [7, 10, 11].

Thus, the formation of NAFLD against the background of type 2 DM is associated with a reduced vitamin D content in the blood. The relationship between an increased risk of developing NAFLD with reduced vitamin D levels indicates its pathogenetic significance in the onset of the disease.

Conclusions
1. In patients with NAFLD on the background of type 2 DM, there is a decrease in the levels of vitamin D in the blood, especially in cases of cholestatic syndrome.
2. The risk of developing NAFLD in patients with type 2 DM is increased 87 times, with blood levels of vitamin D less than 16.18 ng/ml.
3. Predictors of the formation of NAFLD in patients with type 2 DM are parameters of vitamin D, ALT, glucose, and LDL.

Disclosure: The authors declare no conflict of interest.

References

ORIGINAL RESEARCH
ВОРИНАЛЬНЫЕ ИССЛЕДОВАНИЯ

Intrernal diseases

ОРИГИНАЛЬНЫЕ

ИССЛЕДОВАНИЯ

Интенни воленези
CHARACTERISTICS OF IRON DEFICIENCY IN CHILDREN WITH CELIAC DISEASE


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The iron deficiency (ID) structure was estimated in 235 children with celiac disease aged eight months to 18 years. The patients included in the study were divided into three groups: group I included 64 (27.2 %) children without ID, group II – 106 (45.1 %) children with latent iron deficiency (LID), and group III – 65 (27.7 %) with iron deficiency anemia (IDA). In the period of manifestation of celiac disease, ID conditions were diagnosed in 72.8 % of cases. At the same time, LID dominated the structure of ID forms in all age groups. It was found that in children with celiac disease complicated by IDA, there was a lag in the pace of physical development, as well as more pronounced gastrointestinal symptoms: vomiting was noted more often (p=0.022), bloating (p=0.027) and diarrhea (p=0.018), than in the group I. Total atrophy of the small intestine mucosa villus predominates in 87.7 % of patients with IDA. Thus, specialized laboratory and instrumental methods of diagnosing celiac disease in children should include a detailed analysis of ID.

Keywords: iron deficiency anemia, atrophy of the small intestine mucosa, celiac disease, children