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Hepatic steatosis indices as predictors of vitamin D_3 deficiency in patients with NAFLD associated with type 2 diabetes

ABSTRACT

Background. Recently, vitamin D_3 deficiency is considered one of the factors associated with the development of non-alcoholic fatty liver disease (NAFLD). The aim was to evaluate steatosis indices and metabolic parameters in NAFLD depending on vitamin D_3 status.

Methods. According to the recommendations of the European Society of Endocrinology, all patients were divided into 3 groups: group 1 — with an optimal level of vitamin D_3 (30 ng/mL); group 2 — vitamin D_3 insufficiency (21–29 ng/mL) and group 3 — vitamin D_3 deficiency (< 20 ng/mL).

Results. The study included 126 T2D patients with NAFLD diagnosed with ultrasound. The highest hepatic steatosis (HSI) and fatty liver (FLI) index values were diagnosed in vitamin D_3 deficiency as compared to optimal group (HSI — 43.34 ± 6.59 *vs.* 39.67 ± 4.37; P = 0.032 and FLI — 79.21 ± 19.61 *vs.* 64.96 ± 17.72; P = 0.007). Triglyceride and glucose index (TyG) also were insignificantly elevated parallel to vitamin D_3 status worsened (P = 0.175). In multivariate logistic regression analysis all steatosis indices were independent from transaminases activity, body mass index (BMI) and T2D duration associated with vitamin D_3 deficiency.

Conclusions. Hepatic steatosis indices (HSI, FLI and TyG) independently from anthropometric parameters and transaminase activity associated with D_3 deficiency in NAFLD patients. (Clin Diabetol 2020; 9; 5: 313–320)

Key words: vitamin D_3, vitamin D_3 deficiency, non-alcoholic fatty liver disease, fatty liver index, hepatic steatosis index, triglyceride and glucose index

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a non-specific, integral and multifactorial liver injury in patients with type 2 diabetes (T2D), obesity, insulin resistance (IR), metabolic syndrome, dyslipidemia and atherosclerosis [1, 2]. NAFLD is characterized by the accumulation of lipids within the hepatocytes exceeding 5% according to histological examination [3]. NAFLD is the most common chronic liver disease in western countries and it has rised up to 60–95% [4]. Its frequency among adults ranges from 17 to 46% depending on the method of diagnosis, age, sex and ethnicity [5]. Current guidelines for the management of patients with NAFLD include the prescription of both hepatoprotective drugs and pharmacological correction of concomitant metabolic disorders such as obesity, hyperlipidemia, IR and T2D [6]. Therapy is mainly based on lifestyle changes to reduce body weight [7], and in some cases the use of metformin, glitazones, hypolipidemic drugs and TNF-α antagonists required to treat concomitant conditions [7, 8]. In addition, recent studies have shown that the use of omega-3 polyunsaturated fatty acids (PUFA) can reduce liver fat content [9, 10], while antioxidants [11–13] and probiotics [14–17] may have anti-inflammatory properties.

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There has been a growing body of research in recent years describing the relationship between vitamin D_3 and NAFLD. However, there is still controversy over the existence of a cause and effect relationship. For example, a recent large-scale study using MRI spectroscopy and liver biopsy for the NAFLD diagnosis did not reveal an associative link between liver disease and vitamin D_3 [18, 19]. On the other hand, it is proved that there is an inverse correlation between vitamin D_3 level and anthropometric parameters. This means that vitamin D_3 can accumulate in adipose tissue in patients with obesity, leading to its extensive dilution, with subsequent formation of its plasma deficiency [20]. Recent meta-analysis involving 17 studies found a significant relationship between vitamin D_3 deficiency and NAFLD [21]. Patients with NAFLD had significantly lower levels of 25(OH)D_3 and 1.26 times more commonly as compared to control were diagnosed with vitamin D_3 deficiency (OR [odds ratio] 1.26; 95% CI [confidence interval]: 1.17, 1.35) [21]. However, it remains unclear what exactly leads to vitamin D_3 deficiency in patients with NAFLD. There is still no evidence as to whether the decrease in D_3 level is primary due to own liver disease or due to a decrease in consumption, exposure to UV, and as a consequence of a decrease in its synthesis.

The aim of this study was to evaluate the hepatic steatosis indices, liver functional state and metabolic profile parameters in NAFLD patients according to vitamin D_3 status.

Methods

Ethical statement

The study protocol was approved by the local ethics committees of Kyiv City Clinical Endocrinology Center and was conducted according to the guidelines of the Declaration of Helsinki from the year 1975. Prior to the study, purpose and methodology of the study were fully explained to the participants by the researchers, and all patients gave written informed consent before any study procedures were initiated.

Study design

After receiving consent, patients from Kyiv City Clinical Endocrinology Center were recruited for one-center cross-sectional study. The cohort was composed with T2D patients over 18 years of age with concomitant NAFLD.

NAFLD diagnosis was concluded according to the recommendations of the American Gastroenterology Association (AGA) and American Association for the Study of Liver Disease (AASLD) on the basis of clinical examination, laboratory values of lipid and carbohydrate metabolism, liver enzyme activities (ALT [alanine aminotransferase], AST [aspartate aminotransferase]), ALT/AST ratio, and ultrasonography (US) examination [7]. The diagnosis of fatty liver was based on the results of abdominal ultrasonography, which was done by trained technicians with Ultima PA (Radmir Co., Kharkiv, Ukraine). Of 4 known criteria (hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring) [22] participants were required to have hepatorenal contrast and liver brightness to be given a diagnosis of NAFLD.

The study did not include patients with chronic diffuse liver disease of another etiology, such as chronic viral hepatitis (associated with HBV, HCV, HDV infection), autoimmune or drug-induced liver disease. Alcohol history was evaluated in all patients and patients were not included in the study when abuse was detected (> 210 grams of alcohol per week in men and > 140 grams of alcohol per week in women over a 2-year period). Wilson-Konovalov's disease, congenital α1-antitrypsin deficiency and idiopathic hemochromatosis were also exclusion criteria. The study also did not include patients who reported that 3 months prior to baseline had taken anticonvulsant drugs or biological supplements with calcium or vitamin D_3.

Data collection and measurement

Following informed consent, serum samples were collected and immediately frozen at –20°C. Appropriate clinical and demographic data were obtained for each patient. Anthropometric parameters included measurements of weight and height to the nearest 100 g and 0.5 cm, respectively. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (weight/height^2). The waist circumference (WC) of the patient was measured without clothing, 1 cm above the upper anterior crest of the iliac, exhaled in a standing position.

The serum 25(OH)D concentration was assessed using an electrochemiluminescence protein binding assay intended for the quantitative determination (Roche Diagnostics, Mannheim, Germany) with an intra-assay precision of < 5.5% and inter-assay precision of < 7.0%. The assay employs a vitamin D binding protein (VDBP) as capture protein, which binds to both 25-OH D_3 and 25-OH D_2. The assay utilizes a 3-step incubation process, which has a duration of 27 minutes. In step 1, the sample is incubated with pretreatment reagent, which releases bound 25-OH vitamin D from the VDBP. In step 2, the pretreated sample is incubated with ruthenium labeled VDBP creating a complex between the 25-(OH) vitamin D and the ruthenylated VDBP [23]. The third incubation step consists in the addition of streptavidin-coated microparticles and 25-OH vitamin D labeled with biotin. The free sites of the ruthenium

labeled VDBP become occupied, forming a complex consisting of the ruthenium labeled vitamin D binding protein and the biotinylated 25-OH vitamin D. The entire complex is bound to the solid phase via interaction of biotin and streptavidin [24]. Initially, the standards are measured, after which a calibration curve is simulated, which determines the concentration of 25-(OH) D_3 in samples in ng/mL.

Deficiency of vitamin 25(OH)D_3 was established, according to the European Society of Endocrinology (ESE) guidelines [25], when its concentration is less than 20 ng/mL, insufficiency — 21–29 ng/mL.

Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations were measured using enzymatic kits and standardized reagents (BioVendor, Czech Republic). Low (LDL-C) and very-low density lipoprotein cholesterol (VLDL-C) levels were calculated applying the Friedewald formula [26] only if TG were below 5,5 mmol/L; if they were above the mentioned cut-off, LDL-C concentrations were measured through lipoprotein electrophoresis and densitometry (BioVendor, Czech Republic).

To assess the functional state of the liver and pancreas the standard biochemical methods were used (ERBA Lachema, Czech Republic).

The HOMA2-IR model, which is a modernized version of the structural mathematical model based on the determination of fasting plasma glucose (FPG) and fasting insulin — HOMA (homeostasis model assessment), was used to estimate the IR, using the equation (FPG × fasting insulin [FPI]/22.5), first proposed by Matthews et al. [27]. This model can be calculated using software provided by the Oxford Center for Endocrinology, Diabetes and Metabolism and available at http://www.dtu.ox.ac.uk/homacalculator/index.php.

Fatty liver index (FLI) a validated prediction score for hepatic steatosis severity designed by Bedogni et al., as an algorithm in the Dionysos Nutrition & Liver Study [28]. The index varies from 0 to 100 and was calculated based on laboratory and anthropometric measures, including TG, gamma glutamyl transferase (GGT), BMI, and WC, by using the following formula:

$$FLI = [e^{0.953*\log e(\text{triglycerides}) + 0.139*BMI + 0.718*\log e(ggt) + 0.053*\text{waist circumference} - 15.745}/(1 + e^{0.953*\log e(\text{triglycerides}) + 0.139*BMI + 0.718*\log e(ggt) + 0.053*\text{waist circumference} - 15.745})] \times 100$$

The triglyceride and glucose index (TyG) is a new method of screening for IR because it is easy to use and requires only two laboratory definitions [29]:

ln [fasting triglyceride (mg/dL) × fasting glucose (mg/dL)]/2.

Hepatic steatosis index (HSI) was calculated by the following formula:

$$HSI = 8 \times (\text{ALT/AST ratio}) + BMI + 2 \text{ (if female)} + 2 \text{ (if DM)}$$

A threshold of HIS > 36.0 confirms the presence of liver steatosis in a patient with a sensitivity of 93.1%, a specificity of 92.4% and a diagnostic accuracy of AUROC 0.812 [30].

Statistical analysis

The SPSS statistical package, version 21.0 (SPSS, Inc., Chicago, Illinois), was used for all statistical analyses and a P value < 0.05 was considered statistically significant. All continuous values were expressed as mean ± standard deviation (SD) and categorical variables were presented as %. Data distribution was analyzed using the Kolmogorov-Smirnov normality test. Continuous variables with parametric distribution were then analyzed using one-way analysis of variance (ANOVA) and if the results were significant, a Tuckey Post Hoc test was performed. Data with non-parametric distribution were analyzed using the Kruskall-Wallis test. For comparisons of categorical variables, we conducted χ^2 test. Association between vitamin D_3 levels and metabolic changes was assessed with univariate Pearson's and partial with adjustment on sex, BMI and age, correlation analysis.

Univariate and multivariate logistic regression analyses were used to identify the risk factors associated with vitamin D_3 deficiency. The odds ratios (OR) are given with the 95% confidence intervals (95% CI). Variables statistically significant in univariate analysis were included in the multivariate logistic regression analysis. Backward stepwise selection was used at a significance level of $P < 0.10$ to detect the independent risk factors for vitamin D_3 deficiency.

Results

The study included 126 patients with T2D and concomitant NAFLD. Depending on the baseline level of vitamin D_3 (Fig. 1), patients were allocated to one of 3 groups based on current ESE guidelines [25].

The groups of patients included in the study were representative in terms of age ($P = 0.187$), T2D duration ($P = 0.258$) and sex. The group 1 ($n = 23$) included patients with optimal vitamin D_3 levels (> 30.0 ng/ml). The other two groups included patients with low baseline vitamin D_3 levels: group 2 ($n = 31$) with vitamin D_3 insufficiency (20–29.9 ng/mL) and group 3 ($n = 73$) with vitamin D_3 deficiency, which was diagnosed at < 20 ng/mL.

The analysis of the obtained data showed that more than 90% of all examined patients revealed over-

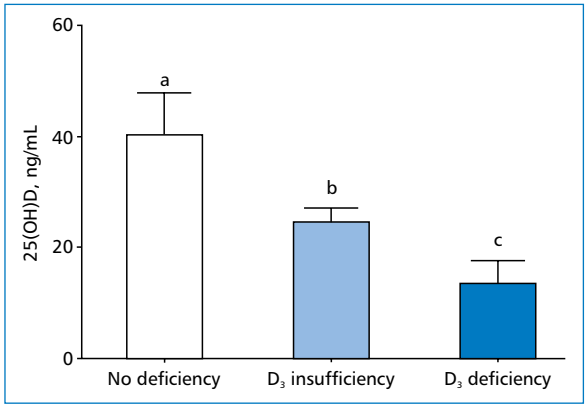

Figure 1. Serum vitamin D_3 level in patients of different studies groups. Data presented as M ± SD. [a, b, c]Values at the same row with different superscript letters show significant differences at $P < 0.05$

weight or obesity. Morbid obesity (BMI > 40.0 kg/m^2) was diagnosed only in patients with low vitamin D_3 concentrations. In the D_3 deficiency group, morbid obesity was observed in 10 (13.9%) patients, which was not significantly different from the D_3 insufficiency (group 2) in which only 2 (6.5%) participants suffered from this pathology (P = 0.113). At the same time as the higher prevalence of obesity, we diagnosed a gradient increase in BMI and WC in parallel with a decrease of serum vitamin D_3 level (Table 1).

According to our results, there was a tendency to increase the transaminases activity parallel with a vitamin D_3 decrease. However, the mean values did not exceed the reference ranges and no significant difference was observed between all studied groups. The activity of GGT, another indicator of the functional state of the liver, was also highest in the D_3 deficiency group — 51.17 ± 19.4 U/L (P = 0.078). Alkaline phosphatase, total bilirubin and markers of pancreas exocrine function did not differ significantly across all study groups (Table 1).

The values of the HOMA2-IR index was at the same level in optimal D_3 and its insufficiency groups (2.41 ± 1.36 *vs.* 2.56 ± 1.44, P = 0.902). In D_3 deficiency patients, the HOMA2-IR index was 3.07 ± 1.68 which was significantly higher as compared to the optimal D_3 group (P = 0.048). Our study analyzed changes in the hepatic steatosis indices (HSI and FLI) in the context of vitamin D_3 status. The maximum value for both indices was diagnosed in patients with D_3 deficiency, which was significantly higher as compared to optimal D_3 (HSI — 43.34 ± 6.59 *vs.* 39.67 ± 4.37; P = 0.032 and, for FLI — 79.21 ± 19.61 *vs.* 64.96 ± 17.72; P = 0.007 respectively). In the D_3 insufficiency group, the mean value for the HSI was 41.46 ± 5.55 and for the FLI — 75.89 ± 19.02, which was not significant as compared to both other groups (Table 2). Elevation of TyG index was also observed in parallel with the worsening of D_3 status (P = 0.175).

In the vast majority of examined patients, disorders of lipid metabolism were detected. Overall, we observed an increase in pro-atherogenic particles (TC, TG, VLDL-C and LDL-C), parallel with the decreasing of HDL-C. The most pronounced changes were observed in the D_3 deficiency group, but as can be seen from the table 1 changes of lipid profile parameters, according to vitamin D_3 status, were not significant between the groups.

Univariate Pearson's analysis in the D_3 insufficiency group found a significant correlation only for LDL and vitamin D_3 level (r = –0.479; P = 0.038), which lost significance after adjusting for BMI, age, and gender (r = –0.440; P = 0.052). However, major changes were observed in patients with D_3 deficiency. According to our data, the reverse, different in strength, but mostly weak, correlation is established between vitamin D_3 level and anthropometric parameters, transaminase activity, liver steatosis indices and lipids (Table 2). However, after adjustment for BMI, age and gender which are predictors that can affect D_3 status, a significant relationship but less powerful remained only for WC (r = –0.342; P = 0.004), AST (r = –0.285; P = 0.018), GGT (r = –0.243; P = 0.044), TyG index (r = –0.294; P = 0.014), FLI (r = –0.255; P = 0.036) and TC (r = –0.280; P = 0.020) respectively (Table 2).

In the univariate logistic regression analysis, the following independent predictors were associated with vitamin D_3 deficiency in NAFLD patients — WC (OR 1.055; 95% CI 1.006–1.106; P = 0.004), HSI (OR 1.108; 95% CI 1.010–1.217; P = 0.031), FLI (OR 1.034; 95% CI 1.010–1.058; P = 0.005), TG (OR 1.716; 95% CI 1.016–2.897; P = 0.043) and BMI (OR 1.181; 95% CI 1.051–1.327; P = 0.005).

All variables that were significant in the univariate analysis were included in a stepwise multiple logistic regression analysis. As can be seen from Table 3, we constructed several regression models. According to the results obtained, regardless of the transaminases activity HSI (Nagelkerke $R^2 = 0.215$) and FLI (Nagelkerke $R^2 = 0.163$) were associated with vitamin D_3 deficiency. According to other logistic models, HSI and TyG indices (Nagelkerke $R^2 = 0.358$) as well as BMI and T2D duration (Nagelkerke $R^2 = 0.328$) were independent predictors associated with vitamin D_3 deficiency in this cohort of patients (Table 3).

Table 1. Anthropometric, clinical and laboratory parameters in examined patients (M ± SD or %)

Parameters	No deficiency (n = 23)	Vit. D3 insufficiency (21–29 ng/mL) (n = 31)	Vit. D_3 deficiency (less 20 ng/mL) (n = 72)	P
Age (years)	64.83 ± 7.26	60.68 ± 10.08	60.72 ± 10.15	0.187
Duration of T2D (years)	14.3 ± 8.11	11.42 ± 5.92	13.42 ± 6.78	0.258
BMI [kg/m^2]	28.46 ± 3.64^a	31.88 ± 5.28^b	32.65 ± 6.45^b	0.011
WC [cm]	96.83 ± 7.94^a	100.32 ± 8.37^{ab}	105.23 ± 16.06^b	0.020
ALT [IU/L]	25.76 ± 8.0	31.08 ± 21.95	32.00 ± 21.36	0.420
AST [IU/L]	26.54 ± 13.63	22.64 ± 14.27	27.63 ± 17.68	0.360
Total bilirubin [μmol/L]	11.9 ± 3.81	10.42 ± 4.12	9.62 ± 4.23	0.071
Alkaline phosphatase [IU/L]	81.33 ± 23.2	81.15 ± 23.1	79.3 ± 20.29	0.960
GGT [IU/L]	44.24 ± 17.27	43.06 ± 17.58	51.17 ± 19.4	0.078
Lipase [IU/L]	25.5 ± 12.59	45.32 ± 38.97	43.87 ± 45.63	0.577
Amylase [IU/L]	45.8 ± 14.02	44.5 ± 19.33	46.86 ± 21.45	0.963
HOMA2-IR	2.41 ± 1.36^a	2,56 ± 1.44^{ab}	3.07 ± 1.68^b	0.038
HSI	39.67 ± 4.37^a	41.46 ± 5.55^{ab}	43.34 ± 6.59^b	0.031
FLI	64.96 ± 17.72^a	75.89 ± 19.02^{ab}	79.21 ± 19.61^b	0.010
TyG index	5.02 ± 0.26	5,13 ± 0.34	5.18 ± 0.38	0,175
TC [mmol/L]	5.43 ± 1.0	5.15 ± 1.09	5.68 ± 1.29	0.123
TG [mmol/L]	1.73 ± 0.89	2.15 ± 1.28	2,55 ± 1.75	0,052
VLDL-C [mmol/L]	0.76 ± 0.41	0.98 ± 0.62	1.13 ± 0.89	0.176
HDL-C [mmol/L]	1.6 ± 0.28	1.24 ± 0.32	1.33 ± 0.43	0.105
LDL-C [mmol/L]	2.88 ± 0.97	2.94 ± 0.65	2.93 ± 0.95	0.988

P: the difference between all study groups calculated using one-way ANOVA with Tukey Posthoc test
$^{a, b, c}$Values at the same row with different superscript letters show significant differences at $P < 0.05$

Table 2. Univariate Pearson's correlation analysis between vitamin D_3 amount and different parameters in patients according to baseline vitamin D_3-status

Parameters	No deficiency (n = 23)		Vit. D_3 insufficiency (21–29 ng/mL) (n = 31)		Vit. D_3 deficiency (less 20 ng/mL) (n = 72)	
Age (years)	–0.038 (0.863)	–	–0.114 (0.541)	–	–0.285 (0.015)*	–
Sex	–0.260 (0.231)	–	0.113 (0.544)	–	–0.105 (0.381)	–
BMI [kg/m^2]	0.059 (0.789)	–	0.138 (0.460)	–	–0.286 (0.015)*	–
Duration of T2D (years)	0.019 (0.933)	–0.102 (0.669)	0.130 (0.485)	0.328 (0.158)	–0.074 (0.540)	–0.009 (0.949)
WC [cm]	0.127 (0.564)	0.236 (0.317)	0.065 (0.727)	–0.058 (0.809)	–0.364 (0.002)*	–0.342 (0.004)*
ALT [IU/L]	–0.064 (0.772)	–0.011 (0.964)	0.225 (0.223)	–0.256 (0.276)	–0.283 (0.016)*	–0.166 (0.173)
AST [IU/L]	–0.331 (0.122)	–0.251 (0.286)	0.265 (0.150)	–0.085 (0.723)	–0.371 (0.001)*	–0.285 (0.018)*
GGT [IU/L]	–0.238 (0.274)	–0.259 (0.270)	0.077 (0.682)	–0.016 (0.948)	–0.346 (0.003)*	–0.243 (0.044)*
HOMA2-IR	0.223 (0.319)	0.230 (0.343)	–0.031 (0.869)	0.250 (0.287)	–0.185 (0.122)	–0.093 (0.450)
HIS	–0.212 (0.332)	–0.272 (0.260)	0.141 (0.451)	0.329 (0.157)	–0.303 (0.010)*	–0.128 (0.299)
FLI	–0.047 (0.830)	0.150 (0.541)	0.244 (0.186)	–0.314 (0.178)	–0.381 (0.001)*	–0.255 (0.036)*
TyG index	–0.147 (0.504)	–0.004 (0.988)	0.118 (0.529)	–0.209 (0.376)	–0.341 (0.003)*	–0.294 (0.014)*
TC [mmol/L]	0.183 (0.404)	0.180 (0.447)	–0.331 (0.069)	–0.402 (0.079)	–0.376 (0.001)*	–0.280 (0.020)*
TG [mmol/L]	–0.261 (0.230)	0.207 (0.308)	–0.083 (0.658)	–0.110 (0.643)	–0.303 (0.010)*	–0.230 (0.057)
VLDL-C [mmol/L]	–0.242 (0.291)	0.149 (0.554)	–0.002 (0.998)	–0.126 (0.597)	–0.290 (0.043)*	–0.229 (0.126)
HDL-C [mmol/L]	0.258 (0.238)	0.154 (0.804)	0.240 (0.323)	0.012 (0.960)	0.029 (0.851)	0.107 (0.496)
LDL-C [mmol/L]	0.011 (0.094)	0.685 (0.520)	–0.479 (0.038)*	–0.440 (0.052)	–0.430 (0.004)*	–0.228 (0.151)

The data is presented as r (P)
*Marked statistically significant correlation

Table 3. Multiple stepwise logistic regression analysis using vitamin D-deficiency as a dependent variable and as an independent predictors for all factors that were significantly associated in univariate analysis

Models	Regression coefficient ± SE	OR (95% CI)	P
Model 1			
(Nagelkerke R2 = 0.215)			
Constant	–5.671 ± 2.43		
HSI	0.164 ± 0.062	1.178 (1.043–1.331)	0.008
ALT	0.083 ± 0.036	1.087 (1.013–1.166)	0.021
AST	–0.086 ± 0.036	0.918 (0.855–0.985)	0.017
Model 2			
(Nagelkerke R2 = 0.328)			
Constant	–5.442 ± 3.784		
BMI	0.324 ± 0.141	1.381 (1.049–1.823)	0.022
T2D duration	–0.109 ± 0.058	0.896 (0.800–1.005)	0.060
Model 3			
(Nagelkerke R2 = 0.358)			
Constant	–22.063 ± 8.811		
HSI	0.256 ± 0.121	1.292 (1.020–1.637)	0.034
TyG index	2.912 ± 1.284	18.386 (1.484–227.818)	0.023
Model 4			
(Nagelkerke R2 = 0.163)			
Constant	–0.635 ± 0.880		
FLI	0.030 ± 0.013	1.030 (1.005–1.057)	0.021
AST	–0.061 ± 0.029	0.940 (0.889–0.995)	0.032
ALT	0.057 ± 0.030	1.058 (0.997–1.123)	0.064

SE: standard error of the regression coefficient; R2: coefficient of determination

Discussion

Vitamin D_3 is a product of photosynthesis from its precursor 7-dehydrocholesterol in human skin under the influence of ultraviolet light. Thereafter, vitamin D_3 undergoes hepatic 25-hydroxylation to form 25-(OH)D_3, a most sensitive clinical marker for vitamin D status in humans. The active metabolite of vitamin D_3 (1, 25-dihydroxyvitamin D_3), is synthesized in kidney by the enzyme 25-(OH)D_3 1-α-hydroxylase [31] and contributes to calcium and phosphate homeostasis, bone mineralization, and regulates cell proliferation, differentiation and apoptosis [32, 33]. Vitamin D_3 exerts its biological effect mainly through VDR (vitamin D receptor), which belongs to the nuclear receptor superfamily and regulates gene expression in a ligand-dependent manner. Their discovery and synthesis in cells of "unconventional" organs and tissues for this vitamin implies a wider range of physiological effects of the vitamin [34, 35].

Data from our study, as well as recent in patients with biopsy proven NAFLD, demonstrated that 25 (OH) D_3 deficiency was significantly associated with histologic features such as hepatic steatosis, necro-inflammatory changes and fibrosis ($P < 0.001$) after adjustment on age, sex, BMI, creatinine, calcium, IR and other clusters of metabolic syndrome [36]. On the other hand, in the retrospective pediatric study (n = 234) with confirmed on biopsy NAFLD, the proportion of patients with significant fibrosis (stage ≥ 2) was significantly higher in patients with D_3 insufficiency (29%) as compared to D_3 deficiency — 15% ($P = 0.040$). Other pathomorphological parameters, such as severity of steatosis, ballooning, lobular/portal inflammation and total histologic score on the NAS scale, changes insignificantly between the groups [37].

In recent decades, less invasive methods of biochemical NAFLD verification become increasingly common as for diagnosis and treatment effectiveness assessment. These methods can be divided into routine — lipid parameters, and the estimated coefficients: steatotest (SteatoTest®), hepatic steatosis indices (HSI and FLI), the NAFLD liver fat score, whose results are calculated on the basis of special biochemical parameters [38]. The external validity of these tests is confirmed in the general population and in persons with morbid obesity, they allow with varying degrees of accuracy to predict the results and mortality associated with metabolic, hepatic and cardiovascular manifestations [39].

The TyG Index was recently proposed as a new simple marker for IR. Further studies have demonstrated

that the TyG index is independently associated with hepatic steatosis [40], and a TyG threshold ≥ 8.5 is a sufficiently effective diagnostic marker of NAFLD with an AUC of 0.782 and has a higher diagnostic value as compared to ALT [41].

Our study first analyzed changes in hepatic steatosis indices with the assessment of associative relationships with other metabolic parameters in the context of vitamin D_3 status. The highest HSI and FLI index values were diagnosed in vitamin D_3 deficiency as compared to optimal group. TyG also increased insignificantly parallel to D_3 status worsened ($P = 0.175$). In multivariate logistic regression analysis all steatosis indices were independent from transaminases activity, body mass index (BMI) and T2D duration associated with vitamin D_3 deficiency.

This study has some possible limitations. The essential limitation is that NAFLD was not assessed by histopathological examination or by elastography. This fact may result in missing some number of patients with steatosis affecting less than 30% of hepatocytes and influence obtained results. The small sample size also may be a limiting factor, particularly in the group with optimal vitamin D_3.

Conclusion

Our study demonstrated that in patients with T2D and concomitant NAFLD, the maximum values of hepatic steatosis indices were diagnosed in patients with D_3 deficiency. There was an inverse correlation between vitamin D_3 level and transaminases (AST, GGT), WC, steatosis indices, and total cholesterol levels. In the regression analysis it was proposed that steatosis indices may be used as independent predictors of D_3 deficiency in NAFLD patients.

Conflict of interest

All authors of this paper have contributed to, read and approved the final version submitted. The contents of this manuscript have not been copyrighted or published previously. The contents of this manuscript are not now under consideration for publication elsewhere. The authors declare that they have no conflict of interest and funding is none.

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