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The effect of vitamin D status on non-alcoholic fatty liver disease: a population-based observational study

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Abstract

Introduction: The effect of vitamin D status on steatosis has not been fully elucidated. In this study, we planned to investigate this interaction using a large-scale population-based cohort.

Material and methods: Patients diagnosed with simple steatosis (K76.0) and non-alcoholic steatohepatitis (NASH) (K75.8) by using the International Classification of Diseases 10th Revision (ICD-10) coding system, and who had 25-hydroxyvitamin D (25OHD) measurements at the diagnosis, were included in the study. Control group comprised subjects without liver diseases. Age, gender, alanine aminotransferase (ALT) and 25OHD levels, and the date of the measurements were recorded.

Results: We compared ALT and 25OHD measurements between the patient and control groups, and between the simple steatosis and NASH subgroups. 25OHD levels were lower and ALT levels were higher in the patient group ($p < 0.001$, effect size = 0.028, and $p < 0.001$, effect size = 0.442, respectively). Logistic regression analysis showed that when 25OHD levels decrease by 1 ng/dl, it increases the risk of being in the patient group by 3.7%.

Conclusion: Our results suggest that vitamin D status may be related to the development of non-alcoholic fatty liver disease (NAFLD). Although this relationship is weak, it may be important in the pathogenesis of steatosis. **(Endokrynol Pol 2023; 74 (1): 63–66)**

Key words: *vitamin D; NAFLD; steatohepatitis*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease, and it may transform from simple steatosis to non-alcoholic steatohepatitis (NASH) and into cirrhosis. NAFLD is considered as a hepatic mirror image of the metabolic syndrome and is characterized by lipid infiltration in hepatocytes. NAFLD is also related to insulin resistance, type 2 diabetes (T2D), and cardiovascular diseases [1]. It has been estimated that almost one out of 4 subjects has NAFLD worldwide [2]. Most of the patients remain undiagnosed, although it is a well-known risk factor for cardiovascular diseases. Although several studies have shown beneficial therapeutic effects of several medications on NAFLD progression, no effective treatment has been found to eliminate steatohepatitis [3].

Vitamin D is not only considered as a vitamin, but it is also a pleiotropic hormone, which has functions beyond calcium homeostasis such as influencing im-

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munity and metabolic diseases [4, 5]. Vitamin D deficiency has been related to insulin resistance, metabolic syndrome, T2D, and finally NAFLD. Vitamin D has anti-inflammatory properties in liver, and low levels of vitamin D may be a triggering factor for hepatic inflammation. Furthermore, hepatic vitamin D receptor (VDR) expression was found to be related to steatosis severity and lobular inflammation. These findings led to a hypothesis that vitamin D treatment might prevent NAFLD progression to NASH. However, there are controversial findings from interventional clinical trials investigating the efficacy of vitamin D supplementation to treat NAFLD. A recent meta-analysis found that supplemental vitamin D had significant beneficial effects in NAFLD patients [6]. Therefore, we can conclude from the present knowledge that the strength of the interaction between vitamin D status and hepatosteatosis has not been fully elucidated. Vitamin D fortification is currently in use in many countries, which is a serious confounding factor in population-based studies [7]. In Turkey there is no such vitamin D enrichment program yet; therefore, vitamin D levels are not influenced by vitamin D supplements in nutrients.

In this population-based study, we aimed to investigate the association between vitamin D status and the severity of hepatosteatosis. We also aimed to investigate the strength of this association.

Material and methods

Study design and participants

In this observational nationwide study was conducted using the International Classification of Diseases 10th Revision (ICD-10) registry of the Turkish Ministry of Health National Electronic Database, which covers the public health insurance of more than 95% of the Turkish population, under the supervision of the Ministry of Health. We included all the patients diagnosed with simple steatosis (ICD: K76.0) and non-alcoholic steatohepatitis (ICD: K75.8) during the period 2014 to 2021 by using the ICD10 coding system. We excluded patients diagnosed with other liver diseases (ICD: K70–77) and viral hepatitis (ICD: B15–19). Individuals who were not diagnosed with the above-mentioned liver pathologies were selected for the control group. Patients and controls who were prescribed vitamin D products during the last year before the diagnosis date were excluded from the study. Demographic characteristics (gender, age), standardized 25-hydroxyvitamin D (25OHD), and alanine aminotransferase (ALT) measurements at the time of the diagnosis, and the dates of the measurements were recorded. The design and procedures in the study are in accordance with the Declaration of Helsinki, and the study protocol was approved by the Investigation Review Board under the General Directorate of Health Services Bioethics Committee (IRB number: 95741342-020). We obtained a total of 48,387 patients diagnosed with NAFLD according to ICD10 codes. After removing patients without either 25OHD or ALT measurements, we had 25,208 patients and 84,291 control subjects. We further selected the subjects according to age (18–75 years), 25OHD measurements (1–200 ng/dL), and ALT levels (1–35 IU/L for the control group, and simple steatosis groups, and 35–175 IU/L for the NASH group). After cleaning the data, propensity scoring with the nearest neighbour method and a 1:1 allocation ratio was used. While calculating the propensity score, gender, age, and seasonality (because 25OHD levels are affected by the seasons of the year) were taken into consideration.

Statistical analyses

In the analyses, the normal distribution of data was evaluated using the Kolmogorov-Smirnov test. Continuous variables are shown as medians (quartile deviation). To assess differences between groups the Mann-Whitney U or Kruskal-Wallis H test was applied. Due to the large sample sizes, to avoid any p-hacking problems, r effect sizes are given for Mann-Whitney U tests, and eta-squared effect sizes are given for Kruskal-Wallis H tests. To determine which groups are different from each other in terms of 25OHD and ALT, the Dunn-Bonferroni post-hoc test was conducted for pairwise comparisons. Additionally, univariate and multiple binomial logistic regressions were applied to the control and patient groups to find odds ratios. Finally, receiver operating characteristic (ROC) analysis was applied to determine the optimal cut-off value of the 25OHD associated with ALT in control-patient groups with the Youden J index. All statistical analyses were conducted using R software (R Core Team, 2020) and SPSS 23 for Windows (SPSS Inc., Chicago, IL, United States). Two-sided $p < 0.05$ was considered statistically significant.

Results

There were 2 main groups in the study: the patient (NAFLD) group, which comprised simple steatosis and NASH subgroups, and the control group. After the propensity score match, 22,824 control subjects and 22,824 patients were allocated equally to the control and patient groups. The median age of the control group was 50 with 10 quartile deviation, and the median age of the patient group was 49 years with 10 quartile deviation. The male proportion was 44% in both groups (n = 9957).

We compared ALT and 25OHD measurements between the patient and control groups, and among the control, simple steatosis, and NASH groups. Table 1 and Table 2 show the results of the 2 comparisons. ALT and 25OHD measurements were statistically significant in both comparisons (for both, $p < 0.001$), and as expected the effect of 25OHD had a small effect ($r = 0.028$) on the development of NAFLD. 25OHD levels were not significantly different between the simple steatosis and NASH subgroups.

Logistic regression analysis showed that when 25OHD levels decrease by 1 ng/dL, it increases the risk of being in the patient group by 3.7% (Tab. 3).

ROC analysis revealed that a 25OHD value of 16.435 ng/dl had low sensitivity and specificity for predicting the presence of NAFLD [area under the curve (AUC) = 0.516, $p < 0.001$, 44% sensitivity, 59% specificity].

Discussion

In this study, we evaluated the association between vitamin D levels and the severity of the fatty liver disease. We found a significant but weak relationship between hypovitaminosis D and the whole Turkish NAFLD cohort.

Table 1. *25-hydroxyvitamin D (25OHD) and alanine aminotransferase (ALT) levels between control and patient groups*

Variables	Overall (n = 45648)	Control group (n = 22824)	Patient group (n = 22824)	p-value	Effect size
25OHD [ng/dL] — *qd*	18 (7)	19 (7)	18 (7)	< 0.001*	0.028
ALT [IU/L] — *qd*	21 (9)	17 (5)	28 (17)	< 0.001*	0.442

qd — quartile deviation; *statistically significant

Table 2. *25-hydroxyvitamin D (25OHD) and alanine aminotransferase (ALT) levels among control, simple steatosis, and non-alcoholic steatohepatitis (NASH) groups*

Variables	Control group (I) (n = 22,824)	Simple steatosis (II) (n = 13,571)	NASH (III) (n = 9253)	p-value	Effect size	Pairwise comparisons		
						I *vs*. II	I *vs*. III	II *vs*. III
25OHD [ng/dL] — *qd*	19 (7)	19 (8)	17 (7)	< 0.001*	0.002	< 0.001*	< 0.001*	0.301
ALT [IU/L] — *qd*	17 (5)	20 (6)	59 (18)	< 0.001*	0.496	< 0.001*	< 0.001*	< 0.001*

qd — quartile deviation; *statistically significant

Table 3. *Univariate and multiple logistic regression according to control and patient groups, respectively*

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI of OR	p-value	OR	95% CI of OR	p-value
25OHD [ng/dL]	0.999	0.998–1.000	0.007*	0.963	0.961–0.964	< 0.001*
ALT [IU/L]	1.018	1.017–1.018	< 0.001*	1.041	1.040–1.043	< 0.001*

25OHD — 25-hydroxyvitamin D; ALT — alanine aminotransferase; OR — odds ratio; CI — confidence interval; *statistically significant

There are very few population-based studies investigating the role of vitamin D metabolism and the development of NAFLD, with conflicting results. In a recent study from Brazil, 25OHD levels were significantly lower in patients with NAFLD, but low 25OHD levels were not related to severity and comorbidities of steatohepatitis [8]. In another study, in the Portuguese population diagnosed with hepatic steatosis, the serum levels of 25OHD did not differ from those of the control subjects [9]. However, several other studies found a significant association between 25OHD levels and NAFLD. The United States Third National Health and Nutrition Examination Survey (data collected from 1988 to 1994) showed that vitamin D levels were inversely correlated with the degree of hepatosteatosis. Interestingly, vitamin D deficiency was associated with the mortality of diabetes and Alzheimer's disease in NAFLD patients [10]. In a large study including 7514 Korean adults, a significant relationship between vitamin D deficiency and NAFLD was found exclusively in male patients [11]. A recent study on the Chinese population with obesity demonstrated that vitamin D levels in NAFLD patients were lower than those of controls. The researchers also found that the serum 25OHD concentration was negatively correlated with the prevalence of NAFLD only in patients with obesity. They found an odds ratio 95% confidence interval (CI) of 0.987 (0.981–0.993) in patients with obesity, which is slightly higher than the odds ratio of 0.963 (0.961–0.964) in our study [12]. A large study from Finland analysing the data collected from 2 different surveys showed that the risk for advanced hepatosteatosis started to increase significantly at 25OHD levels below 40–50 nmol/l [13]. In our study, we found a significant but weak effect of vitamin D on the development of NAFLD. This is an expected result; considering that most of the population has vitamin D deficiency, if there was a strong relationship, most of the population would have steatohepatitis.

The molecular effects of vitamin D on NAFLD have not been fully elucidated yet. Several experimental studies shed light on the interaction between vitamin D and metabolic disturbances of liver. Vitamin D has anti-inflammatory properties, which were also demonstrated at the hepatic level. Treatment with active vitamin D in a rat NAFLD model reduced liver inflammation and oxidative stress by inhibiting the p53–p21 signalling pathway and associated cell senescence [14]. Vitamin D deficiency exacerbates liver inflammation, and hepatic VDR expression was found to be inversely correlated with steatosis severity and lobular inflammation at the liver histology. VDR activation in hepatic macrophages by vitamin D improved liver

inflammation, steatosis, and insulin resistance [1]. It was also shown that vitamin D ameliorated metabolic disturbances and hepatosteatosis in animal models by the VDR-mediated activation of the hepatocyte nuclear factor 4 [15]. Furthermore, Barchetta et al. have recently shown that liver VDR expression has an important function in modulating intra-hepatic lipid accumulation, by controlling the local levels of angiopoietin-like protein-3 and lipoprotein lipase [16].

Vitamin D supplementation was investigated as a potential treatment modality for NAFLD. However, controversial findings resulted from interventional clinical trials investigating the efficacy of vitamin D supplementation, probably because low doses were used in most of these studies [1]. Higher pharmacological vitamin D doses may be required to be more effective in reducing lipid accumulation in hepatocytes. A recent meta-analysis found that supplemental vitamin D significantly improved glycaemic control and insulin resistance, and marginally reduced the ALT and triglyceride levels in NAFLD patients. However, the pooled data were not able to prove that vitamin D treatment could ameliorate aspartate aminotransferase and cholesterol levels [6]. Until further knowledge becomes available, it would be wise to recommend vitamin D supplementation to NAFLD patients with vitamin D deficiency. Our data showed that 25OHD levels below 16 ng/dl were a statistically significant cut-off level for the development of NAFLD with relatively low sensitivity and specificity.

This study has several limitations. We were not able to examine the ultrasonography and liver biopsy reports of the whole Turkish hepatosteatosis population. We could not match the study and control population according to blood glucose, lipid levels, and blood pressure measurements.

Conclusion

To the best of our knowledge, this is the first population-based study investigating the role of vitamin D status on NAFLD comprising the whole population at the same time. We found a statistically significant 25OHD cut-off value for the development of NAFLD, which may help practitioners in routine daily clinical practice.

Statement of ethics

The study was conducted according to the guidelines of the Declaration of Helsinki and received ethical approval from the Investigation Review Board under the General Directorate of Health Services Bioethics Committee (IRB number: 95741342-020).

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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