Shiraz E-Med J. 2022 March; 23(3):e118125. doi: 10.5812/semj.118125.

Published online 2021 November 16. **Research Article**

Low Vitamin D Level was Associated with Non-alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus

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Received 2021 July 22; **Revised** 2021 September 29; **Accepted** 2021 October 01.

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) are major public health concerns. Besides the known risk factors, other risk factors, such as vitamin D deficiency, have been suggested for NAFLD.
Objectives: This cross-sectional research aimed to investigate the relationship between serum vitamin D levels and NAFLD in a group of patients with T2DM.
Methods: We investigated various clinical and biochemical parameters, including serum vitamin D level, liver function tests, and liver sonography in 1,110 adult patients with T2DM. The mean difference of numerical variables in NAFLD and non-NAFLD groups was analyzed with an independent sample *t*-test. Chi-square test was used to evaluate the association between two categorical variables.
Results: Out of 1,110 patients with T2DM, 837 (75.4%) had NAFLD. The mean vitamin D level in diabetic patients with NAFLD was significantly lower than non-NAFLD group (19.71 ng/mL vs. 27.68 ng/mL, respectively; $P < 0.001$). Furthermore, 410 (49%) patients with NAFLD were found with vitamin D deficiency, while this value was 85 (31.1%) in non-NAFLD group. According to the results of univariate logistic regression analysis, vitamin D deficiency (OR = 3.87) and insufficient vitamin D (OR = 2.83) were the significant variables for NAFLD.
Conclusions: There was a significant association between vitamin D deficiency and NAFLD in patients with T2DM.

Keywords: Type 2 Diabetes Mellitus, Non-alcoholic Fatty Liver Disease, Vitamin D

1. Background

Non-alcoholic fatty liver disease (NAFLD) is defined as the aggregation of triglycerides within hepatocytes exceeding 5% of liver weight. It is not caused by excessive alcohol use or different steatosis sources (1). It encompasses a wide spectrum of liver pathologies, with NAFLD at one end of the spectrum followed by non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma at the other end (2). In some patients, NAFLD progresses to end-stage liver disease, which has made NAFLD a major reason for morbidity and mortality over the last two decades; it is predicted that NAFLD will be the number one etiology of liver transplantation worldwide (3). Evidence suggests that advanced liver fibrosis can be caused by mild degrees of steatosis and inflammation of the liver (4). Today, many experts believe that a lot of patients diagnosed with cryptogenic cirrhosis have NAFLD/NASH as an underlying disease (5).

NAFLD and type 2 diabetes mellitus (T2DM) have been known as major public health concerns. The prevalence of NAFLD in Western countries is 46.2%, and it is the most common liver disease. Its prevalence in some specific groups, such as obese people and patients with T2DM, reaches 75 to 90%. In recent decades, the prevalence of NAFLD has increased along with obesity worldwide, reaching 46.2% in Europe, 33% in North America, and 31.8% in Asia. It is estimated that about one-fourth of the world's population suffers from NAFLD. Also, the global prevalence of diabetes in 2019 was 9.3% (463 million people), which is expected to increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. Notably, patients with NAFLD experience T2DM and vice versa. About 25% of patients with NAFLD and 50% of patients with NASH have T2DM, while NAFLD is reported in about 70% of patients with T2DM. These two conditions have mutual effects on each other (6-11). In patients with T2DM, NAFLD increases the risk of mortality. Also, the presence of T2DM causes a three-fold increase in the risk of progressive liver fibrosis and a two-fold increase in the risk of hepatocellular carcinoma. It is also an independent predictor of liver disease mortality and all-cause mortality (12).

Insulin resistance is a common hallmark of NAFLD and

T2DM, and NAFLD is a hepatic component of metabolic syndrome (13). Metabolic diseases, such as hypertension, visceral obesity, and dyslipidemia are known risk factors for NAFLD (14). Besides the known risk factors, other risk factors, such as vitamin D deficiency, have been recently suggested for NAFLD. Previous research shows that vitamin D deficiency can enhance the risk of insulin resistance and metabolic syndrome (15). Vitamin D is involved in NAFLD by exerting anti-inflammatory and anti-fibrotic effects on liver cells. It exerts these effects through inflammatory cytokines, like tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β, as well as adipokines, like leptin and adiponectin (16). In addition, it has been shown that vitamin D can decrease the cytokeratin 18 apoptotic fragment M30 concentration as an indicator for liver damage (17). In patients with T2DM, vitamin D deficiency can reduce the expression of glucose transporters on the cell surface, reduce glucose export from the liver, and stimulate intrahepatic lipid synthesis, thereby contributing to the pathogenesis of NAFLD in these patients (18).

Low serum vitamin D levels are associated with NAFLD and are involved in its pathogenesis (19-22). However, this association has not been confirmed in all previous studies, and no relationship has been suggested between the serum vitamin D level and NAFLD in some studies (23, 24).

2. Objectives

This cross-sectional research aimed to investigate the relationship between serum vitamin D levels and NAFLD in a group of patients with T2DM.

3. Methods

The current cross-sectional research was performed on 1,110 patients with T2DM (age range: 31 - 75 years) referring to endocrine clinics of Zahedan, Iran, from March 2018 to August 2020. T2DM was diagnosed based on American Diabetes Association (ADA) criteria (24). A physician completed an information form, including the patient's age, sex, duration of diabetes, co-morbidities, smoking, alcohol consumption, and drug history.

Participants with evidence of any chronic liver disease, such as autoimmune hepatitis, hemochromatosis, viral hepatitis, primary biliary cirrhosis, Wilson's disease, and any evidence of liver cirrhosis were excluded from the study. Also, people with other types of diabetes, such as gestational DM, latent autoimmune diabetes of adults, and type 1 DM, were excluded from the study. We also excluded all individuals with acute infection, decreased renal function (eGFR $<$ 60 ml/min/1.73 m^2 or plasma creatinine $>$ 2 mg/dL), malignancy, thyroid dysfunction, or alcohol consumption of any volume. Patients with a history of taking any supplements, including vitamin D, as well as pregnant and lactating women were also excluded from the study.

The patients' height, weight, and blood pressure were evaluated. The weight was measured with minimal clothing by a digital scale, and height was measured while standing without shoes by a stadiometer. Body mass index (BMI) was determined using this formula: Weight (kg) divided by height (m^2). Patients' blood pressure was measured after 15 minutes of rest and before blood sampling using a manual sphygmomanometer.

For all subjects, liver ultrasonography was performed by a sonologist after 12 hours of fasting. Fatty liver was determined based on standard criteria, including liver illumination, variation between the liver and kidneys echogenicity, and the degree of ambiguity of blood vessels. Grading of fatty liver based on the amount of fat deposition in the liver was determined as follows: (1) Grade I – observable periportal and diaphragmatic echogenicity in association with increased liver echogenicity; (2) Grade II – non-observable periportal echogenicity in association with increased liver echogenicity without diaphragmatic ambiguity; and (3) Grade III - non-observable periportal echogenicity in association with increased liver echogenicity with diaphragmatic ambiguity (25). NAFLD was diagnosed according to the American Gastroenterological Association criteria as follows: (1) presence of hepatic steatosis on imaging or histology; (2) no excessive use of alcohol; (3) no other reasons for hepatic steatosis, and (4) no other synchronic reason for chronic liver disease (26).

Fasting venous blood was collected for measurement of the glycemic profile, thyroid function tests, and other biochemical tests. Blood sampling was done between 8, and 9 am following 12 hours of fasting. Plasma glucose was measured with the glucose oxidase method. Measurement of glycated hemoglobin (HbA1c) was carried out using high-performance liquid chromatography (HPLC). Lipids were measured using enzymatic colorimetric tests. Blood urea nitrogen (BUN), creatinine, and liver function tests were assessed by enzymatic colorimetric assays. Serological tests for hepatitis B and C rejection were performed in patients with elevated liver enzymes. The normal AST and ALT were defined as less than 40 u/L. The 25-hydroxyvitamin D (25(OH)D) test was performed using the enzyme immunoassay method. Values less than 20 ng/mL were considered as vitamin D deficiency and values of 20 to 30 ng/mL were regarded as insufficiency (27).

All experiments were performed according to the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the Ethics Committee for Human Studies at Zahedan University of Medical Sciences. Informed consent was obtained from all participants.

3.1. Statistical Analysis

Continuous and categorical data are presented as mean ± standard deviation (SD) and frequency (percentage), respectively. Also, we presented the data with histogram and box plot, as appropriate. The normality of variables was assessed with Shapiro–Wilk test and graphical approaches. The mean difference of numerical variable in NAFLD and non-NAFLD groups was analyzed using the independent sample *t*-test. Chi-square test was used to evaluate the association between two categorical variables. The association between independent factors and NAFLD was assessed using univariate and multivariate logistic regression models. The multivariate logistic regression model was conducted based on the backward stepwise method. A P-value less than 0.05 was considered a significant difference. Data analysis was conducted using Stata software (Release 14. College Station, TX: StataCorp LP).

4. Results

In this study, out of 1,110 patients with T2DM, 837 (75.4%) subjects had NAFLD. Among the patients, 64.9% were females, and gender distribution was not significantly different in patients with and without NAFLD (P = 0.375). The mean age in NAFLD group was significantly higher than non-NAFLD group (54.12 vs. 49.41 years, respectively; P < 0.001). The mean duration of diabetes in patients with NAFLD (10.63 years) was almost twice that of patients without NAFLD (5.84 years), indicating a statistically significant difference (P < 0.001). A comparison of other clinical and laboratory characteristics between the two groups is shown in Table 1.

The mean vitamin D level in NAFLD group was significantly lower than non-NAFLD group (19.71 ng/mL vs. 27.68 ng/mL, respectively; P < 0.001). According to the 25-OH vitamin D status classification, 410 (49%) patients with NAFLD had vitamin D deficiency, while this value was 85 (31.1%) for patients without NAFLD (Figure 1). There was no statistically significant difference in the blood sampling seasonal distribution between the two groups (P = 0.934).

In univariate logistic regression analysis, HbA1c with an odds ratio (OR) of 8.51 and history of insulin use (OR = 5.35) showed the highest OR for NAFLD. Also, duration of diabetes (OR = 2.24), family history of diabetes (OR = 2.84), history of taking the antihypertensive drug (OR = 2.15), vitamin D deficiency compared to normal vitamin D (OR = 3.87), and insufficient vitamin D compared with normal vitamin D (OR = 2.83) were the significant variables with OR > 2 for NAFLD (Table 2).

According to the multivariate logistic model, after eliminating the confounding effect of other variables, the chance of developing NAFLD in patients with vitamin D deficiency was 3.15 times higher than patients with normal vitamin D levels. In the multivariate model, the history of insulin consumption (OR = 20.3), HbA1c (OR = 11.76), and the duration of diabetes (OR = 2.92) were the most important variables for NAFLD (Table 3).

5. Discussion

According to our results, the serum vitamin D level was lower in diabetic patients with NAFLD compared to those in non-NAFLD group. Also, vitamin D deficiency was associated with NAFLD in these patients.

These findings are consistent with some previous studies, indicating that the serum vitamin D level was lower in diabetic cases with NAFLD (28, 29). In this regard, a study by Rhee et al. showed that the serum vitamin D levels were lower in NAFLD patients compared with the control group (30). Another study showed that the serum vitamin D levels were significantly lower in patients diagnosed with NAFLD considering liver biopsy than in the control group (31). However, some studies have reported different results regarding the relationship between vitamin D levels and NAFLD. Two different studies carried out in China (32) and Korea (33) reported no significant difference between patients with and without NAFLD regarding the serum vitamin D level.

The discrepancy between the results of different studies can be attributed to factors such as different methods and designs, different criteria for NAFLD diagnosis, different definitions for vitamin D deficiency, lack of matched study groups for interfering factors (such as BMI), and selection bias in cross-sectional studies. Also, genetic factors, such as polymorphisms in vitamin D receptor genes, may be involved. Therefore, vitamin D may affect the evolution and advancement of NAFLD only in subjects with specific genotypes (34).

Previous studies have shown that vitamin D considerably affects immune system regulation, cell differentiation, regulation of cell proliferation, and inflammatory processes. Vitamin D can improve insulin secretion and reduce insulin resistance and liver fibrosis. Through these mechanisms, which are mediated by cytokines and adipokines, vitamin D may contribute to the evolution and advancement of NAFLD (35). Numerous studies have shown that markers of inflammation, such as CRP, TNF-α, and IL-6 are possibly associated with the pathogenesis of NAFLD (36). Elevated serum TNF-α levels have been associated with the increased risk of NAFLD in healthy non-diabetic individuals (36). Moreover, a direct relationship has been found between the increased serum levels of inflammatory markers and NAFLD severity (37). Overall, vitamin D can reduce inflammation in various ways (38). Therefore, it can be proposed that vitamin D reduces the severity of NAFLD, and its deficiency is associated with the exacerbation of NAFLD.

Table 1. Clinical and Laboratory Characteristics of Patients with T2DM in NAFLD and Non-NAFLD Groups [a, b]

Variables	All (n = 1110)	NAFLD Status		P-Value
		NAFLD (n = 837)	Non-NAFLD (n = 273)	
Age (y)	52.96 ± 10.46	54.12 ± 11.22	49.41 ± 6.54	< 0.001
Sex, female	720 (64.9)	549 (65.6)	171 (62.6)	0.375
Diabetes duration (y)	9.45 ± 4.24	10.63 ± 4.15	5.84 ± 1.66	< 0.001
Positive family history of DM	780 (70.3)	636 (76.0)	144 (52.7)	< 0.001
Use of antihypertensive drug	601 (54.1)	492 (58.8)	109 (39.9)	< 0.001
Use of statin	939 (84.6)	711 (84.9)	228 (83.5)	0.570
Use of OHA	644 (58.0)	449 (53.6)	195 (71.4)	< 0.001
Use of insulin	364 (32.8)	334 (39.9)	30 (11.0)	< 0.001
BMI (kg/m^2)	27.07 ± 2.93	27.39 ± 2.93	26.06 ± 2.71	< 0.001
Systolic blood pressure (mmHg)	133.26 ± 10.90	133.52 ± 11.29	132.46 ± 9.57	0.131
Diastolic blood pressure (mmHg)	81.58 ± 9.43	81.43 ± 9.68	82.05 ± 8.63	0.314
Hypertension, BP ≥ 140/90 (%)	507 (45.7)	379 (45.3)	128 (46.9)	0.644
Fasting plasma glucose (mg/dL)	163.68 ± 27.50	167.72 ± 28.05	151.29 ± 21.49	< 0.001
HbA1c (%)	8.37 ± 0.94	8.63 ± 0.89	7.56 ± 0.53	< 0.001
Total cholesterol (mg/dL)	185.24 ± 46.96	188.48 ± 49.0	175.29 ± 38.47	< 0.001
Triglycerides (mg/dL)	119.07 ± 62.98	119.47 ± 63.99	117.84 ± 59.85	0.702
LDL cholesterol (mg/dL)	119.44 ± 44.34	122.28 ± 46.41	110.75 ± 35.98	< 0.001
HDL cholesterol (mg/dL)	45.05 ± 10.08	45.67 ± 10.11	43.13 ± 9.74	< 0.001
VLDL (mg/dL)	23.68 ± 12.69	23.81 ± 12.94	23.30 ± 11.91	0.559
Blood urea nitrogen (mg/dL)	14.62 ± 3.51	14.60 ± 3.55	14.70 ± 3.40	0.698
Creatinine (mg/dL)	1.11 ± 0.17	1.11 ± 0.17	1.10 ± 0.17	0.625
ALT (IU/L)	36.06 ± 13.20	38.81 ± 12.99	27.63 ± 9.86	< 0.001
AST (IU/L)	32.49 ± 12.72	35.06 ± 12.55	24.59 ± 9.60	< 0.001
Alk.ph (IU/L)	108.46 ± 21.59	108.91 ± 21.43	107.07 ± 22.05	0.221
Vit-D (ng/mL)	21.67 ± 12.78	19.71 ± 11.97	27.68 ± 13.36	< 0.001
25-OH vitamin D status				< 0.001
Vit-D < 20 (deficiency)	495 (44.6)	410 (49.0)	85 (31.1)	
Vit-D: 20 - 30 (insufficiency)	377 (34.0)	294 (35.1)	83 (30.4)	
Vit-D ≥ 30 (normal)	236 (21.3)	131 (15.7)	105 (38.5)	
Season of blood sampling				0.934
Spring	306 (27.6)	232 (27.7)	74 (27.1)	
Summer	257 (23.2)	191 (22.8)	66 (24.2)	
Autumn	224 (20.2)	172 (20.5)	52 (19.0)	
Winter	321 (28.9)	241 (28.8)	80 (29.3)	
Vit-D by season of blood sampling				
Spring	22.99 ± 14.84	21.01 ± 13.57	29.19 ± 16.89	< 0.001
Summer	21.59 ± 11.55	19.26 ± 10.62	28.33 ± 11.55	< 0.001
Autumn	21.57 ± 13.22	19.15 ± 12.57	29.56 ± 12.23	< 0.001
Winter	20.49 ± 11.20	19.15 ± 10.83	24.51 ± 11.37	< 0.001

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; Alk.ph, alkaline phosphatase; BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; OHA, oral hypoglycemic agents.
[a] Values are expressed as No. (%) or mean ± SD.
[b] P-values are obtained using independent *t*-test or Pearson χ^2 test.

In patients with NAFLD, insulin sensitivity is reduced in the muscles, fat, and liver (39). Vitamin D increases insulin sensitivity by increasing the number of insulin receptors in myocytes, increasing insulin sensitivity in insulin receptors, and affecting peroxisome proliferator-activated receptor delta (PPAR-δ) (40). On the other hand, during oxidative stress, an elevation in reactive oxygen species (ROS) and lipid peroxidation occurs, which ultimately leads to intracellular damage (41). The concentrations of lipid peroxidation biomarkers are correlated with the severity of liver disease (42). According to previous research, vitamin D deficiency increases the concentration of oxidative

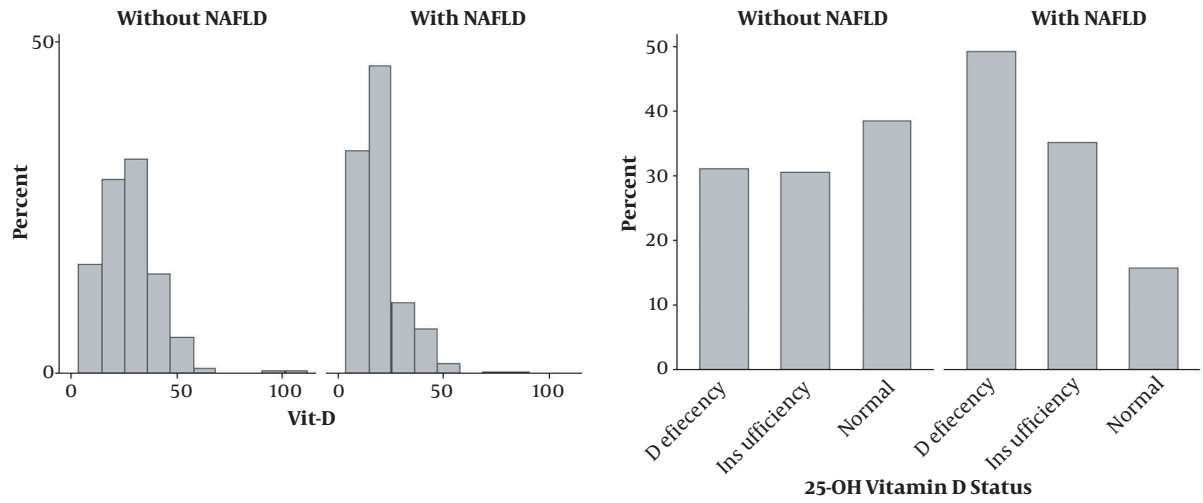

Figure 1. Vit-D distribution in NAFLD and non-NAFLD patients

Table 2. Univariate Logistic Regression Analysis of NAFLD in Participants

Variables	OR (95% CI)	P-Value
Age (y)	1.05 (1.03 to 1.06)	< 0.001
Sex, female	1.14 (0.856 to 1.51)	0.375
Diabetes duration	2.24 (2.0 to 2.51)	< 0.001
Positive family history of DM	2.84 (2.13 to 3.77)	< 0.001
Use of antihypertensive drug	2.15 (1.62 to 2.84)	< 0.001
Use of statin	1.11 (0.768 to 1.62)	0.570
Use of OHA	0.463 (0.344 to 0.622)	< 0.001
Use of insulin	5.38 (3.59 to 8.05)	< 0.001
BMI	1.17 (1.12 to 1.23)	< 0.001
Systolic blood pressure	1.01 (0.996 to 1.02)	0.164
Diastolic blood pressure	0.993 (0.979 to 1.01)	0.342
Hypertension, BP $\geq$ 140/90	0.937 (0.713 to 1.23)	0.644
Fasting plasma glucose	1.02 (1.02 to 1.03)	< 0.001
HbA1c	8.51 (6.40 to 11.33)	< 0.001
Total cholesterol	1.01 (1.0 to 1.01)	< 0.001
Triglycerides	1.0 (0.998 to 1.0)	0.711
LDL cholesterol	1.01 (1.0 to 1.01)	< 0.001
HDL cholesterol	1.03 (1.01 to 1.04)	< 0.001
VLDL	1.0 (0.992 to 1.01)	0.559
Blood urea nitrogen	0.992 (0.954 to 1.03)	0.698
Creatinine	1.22 (0.553 to 2.68)	0.625
ALT	1.11 (1.09 to 1.12)	< 0.001
AST	1.10 (1.08 to 1.12)	< 0.001
ALK.ph	1.0 (0.998 to 1.01)	0.221
Serum vitamin-D level	0.953 (0.942 to 0.964)	< 0.001
Vitamin D deficiency vs. normal	3.87 (2.73 to 5.47)	< 0.001
Vitamin D insufficiency vs. normal	2.83 (1.99 to 4.03)	< 0.001

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; Alk.ph, alkaline phosphatase; BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OHA, oral hypoglycemic agents; VLDL, very-low-density lipoprotein.

Table 3. The Results of Multivariate Logistic Regression Related to NAFLD in Participants

Variables	OR (95% CI)	P-Value
Model 1: Step 1 of Backward Stepwise		
Age (y)	1.07 (1.03 to 1.11)	< 0.001
Diabetes duration	2.91 (2.35 to 3.61)	< 0.001
Positive family history of DM	1.96 (1.04 to 3.69)	0.038
Use of antihypertensive drug	9.15 (4.44 to 18.86)	< 0.001
Use of insulin	20.18 (8.74 to 46.61)	< 0.001
BMI	1.30 (1.15 to 1.46)	< 0.001
HbA1c	11.79 (6.64 to 20.92)	< 0.001
Total cholesterol	1.0 (0.986 to 1.02)	0.724
LDL cholesterol	1.01 (0.988 to 1.03)	0.521
HDL cholesterol	0.998 (0.996 to 1.03)	0.911
ALT	1.14 (1.05 to 1.23)	0.001
AST	0.990 (0.916 to 1.07)	0.792
Vitamin D deficiency vs. normal	3.12 (1.36 to 7.15)	0.011
Vitamin D insufficiency vs. normal	2.82 (1.26 to 6.27)	0.007
Model 2: Final Step of Backward Stepwise		
Age	1.07 (1.03 to 1.11)	< 0.001
Diabetes duration	2.92 (2.36 to 3.61)	< 0.001
Positive family history of DM	1.96 (1.05 to 3.69)	0.036
Use of insulin	20.30 (8.80 to 46.82)	< 0.001
BMI	1.30 (1.15 to 1.46)	< 0.001
HbA1c	11.76 (6.64 to 20.83)	< 0.001
LDL cholesterol	1.01 (1.0 to 1.02)	0.029
ALT	1.13 (1.09 to 1.17)	< 0.001
Vitamin D deficiency vs normal	3.15 (1.38 to 7.19)	0.011
Vitamin D insufficiency vs normal	2.82 (1.27 to 6.28)	0.006

Abbreviations: OR, odds ratio; CI, confidence interval; AST, aspartate transaminase; ALT, alanine transaminase; BMI, body mass index; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

stress biomarkers, and vitamin D intake reduces the concentration of these biomarkers (43). Also, abnormal lipid metabolism results in fat accumulation in the liver, which in turn increases the production of various adipokines, inflammation, and oxidative stress, all of which play an important role in the NAFLD pathogenesis (44).

This research had some limitations. First, it was a cross-sectional research, in which a cause-and-effect relationship could not be indicated between vitamin D deficiency and NAFLD. Second, no liver biopsy was performed in this study. Generally, liver biopsy is the gold standard technique to diagnose NAFLD and differentiate it from NASH. However, considering the aggressiveness of liver biopsy, ultrasound has been used to diagnose NAFLD in previous studies. Ultrasound sensitivity for the diagnosis of NAFLD ranges from 60 to 94%, depending on the severity of steatosis.

On the other hand, since ultrasound is an operator-dependent method, all liver ultrasounds were performed by an experienced radiologist in this study, which is one of its main strengths. Also, elimination of other reasons for chronic liver disease and relatively acceptable sample size are other strengths of this study.

In summary, vitamin D status is associated with the presence of NAFLD in T2DM patients. However, large-scale prospective studies are needed to demonstrate this association and suggest vitamin D deficiency as a risk factor for NAFLD in diabetic patients. Further investigation is warranted to examine the effect of vitamin D supplementation on liver steatosis status in these cases.

Acknowledgments

The authors wish to thank all the participants.

Footnotes

Authors' Contribution: Vahid Sheikhi, designed the study and wrote the paper; Zahra Heidari, designed the study, performed bioinformatics analyses, and co-wrote the paper.

Conflict of Interests: The authors declared no conflicts of potential interest in conducting this research and its publication.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission or after publication. Otherwise, all consequences of possible withdrawal or future retraction will be with the corresponding author.

Ethical Approval: All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee, as well as the 1964 Helsinki declaration and its later amendments. The Ethics Committee for Human Studies at Zahedan University of Medical Sciences approved the study protocol.

Funding/Support: This study was funded by Zahedan University of Medical Sciences (grant number: 1805003).

Informed Consent: Informed consent was obtained from all participants.

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