ORIGINAL ARTICLE

Efficacy of vitamin D supplementation on adult patients with non-alcoholic fatty liver disease: a single-center experience

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ABSTRACT

Aim: The aim of this study was to determine the efficacy of a 6-month intramuscular vitamin D supplementation in improving the liver parameters in adult patients with non-alcoholic fatty liver disease (NAFLD).

Background: The association between vitamin D level and NAFLD has not been determined.

Methods: A single-blinded non-randomized controlled trial was conducted in 80 NAFLD patients assigned to receive a monthly single intramuscular dose of 200,000 IU cholecalciferol/vitamin D3 (n= 40), or placebo (n= 40) for six months. Transient elastography for the measurement of controlled attenuation parameter (CAP) and liver stiffness measurements (LSM), as well as fibrosis 4 score (FIB4) and NAFLD fibrosis score (NFS) were performed.

Results: The mean serum 25(OH)D was significantly increased after six months of vitamin D treatment (16.31±10.23 Vs 39.37±11.99 ng/ml). In the vitamin D group, most vitamin D deficiency patients (70% deficiency, 10% insufficiency, and 20% sufficiency) had changed to be sufficient (7.5% deficiency, 5% insufficiency, and 87.5% sufficiency). On the other hand, the values of CAP (311.9±42.2 dB/m) and LSM (6.8±2 kPa) had significantly reduced after six months of supplementation (287.0 ±44.3dB/m and 6.1 ±1.1 kPa, respectively) with significantly higher mean CAP and LSM change from baseline in vitamin D group compared to the placebo group. Furthermore, the ALT and AST levels were significantly improved in Vitamin D group compared to the placebo group (P<0.05). Multivariate regression analysis showed that lower serum 25(OH)D level was the only significant predictor for NAFLD (OR=0.89, p=0.001) in this study.

Conclusion: A monthly single intramuscular dose of 200,000 IU cholecalciferol is effective in improving the laboratory and fibroscan parameters of the liver disease in NAFLD patients, which confirm a significant relationship between vitamin D deficiency and the risk of NAFLD.

Keywords: Non-alcoholic fatty liver disease (NAFLD), vitamin D, Controlled attenuation parameter (CAP), Liver stiffness measurements (LSM).

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common metabolic progressive disorder. NAFLD affects about 30% of the adults in developed and developing countries (1). It starts with simple fatty liver and progressed to steatohepatitis, and fibrosis, followed by cirrhosis (2). Patients with NAFLD may have hepatic steatosis, with or without inflammation and fibrosis (3). NAFLD is classified into non-alcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). In NASH, hepatic steatosis is associated with hepatic inflammation, while in NAFL, hepatic steatosis is present without evidence of inflammation (4).

Preclinical studies have indicated that vitamin D and its receptor (VDR) has a role in suppressing fibrogenic signaling in the body (5). However, no clinical evidence exists regarding the effects of vitamin D on the liver fibrosis in NAFLD patients. he multiple-hit

hypothesis is better framing to implicate the variable circumstances in the development and progression of NAFLD (6). However, recent studies show that the progression of NAFLD is not always linear and it is not clear which cases are is more likely to transform into more advanced stages (7). The hepatic manifestation of NAFLD is in most cases associated with type 2 diabetes and dyslipidemia (8).

Previous clinical trials investigated the effects of vitamin D supplementation on the concentrations of inflammatory markers and lipid profiles in NAFLD patients (9)–(12). The pathogenesis of the association between NAFLD and reduced vitamin D levels is still undetermined, yet protective anti-fibrotic and anti-inflammatory function of vitamin D on the hepatic stellate cells have been suggested (13). Vitamin D reduces free fatty acid-induced insulin resistance in peripheral tissues and in hepatocytes (14). Therefore, low vitamin D level may lead to intrahepatic lipid accumulation which is responsible for NAFLD pathogenesis(15). Abramovitch et al. confirmed the antifibrotic effects of vitamin D through inhibition of hepatic stellate cells proliferation in an *in vivo* murine model (16), (17).

About 6000 patients were reviewed in the NHANES III database. Of them, about 300 patients showed an unexplained elevation in alanine aminotransferase (ALT) and lower vitamin D levels than the control group (18).

Given that vitamin D deficiency and NAFLD have direct and indirect associations with obesity and a sedentary lifestyle, it is not unexpected that vitamin D deficiency would be a co-factor in the pathogenesis of NAFLD. Therefore, the aim of this clinical trial was to determine the efficacy of 6-months intramuscular vitamin D supplementation in improving the liver parameters in adult patients with NAFLD.

Methods

Study design

A hospital-based prospective single-blinded non-randomized control trial was carried out in the Internal Medicine Department at the Faculty of Medicine, Zagazig University Hospitals. The institutional review board approved the study (ZU-IRB#3776-30-5-2017). Our study was comprised of two arms: the first arm received a monthly single intramuscular dose of 200,000 IU cholecalciferol/vitamin D3 (Devarol-S ampoule®) for 6 months (Vitamin D Group), and the second arm received a monthly single intramuscular dose of the placebo (ampoule containing 2 ml of normal saline 0.9% that was obtained from our colleagues in pharmacology department, Zagazig University) for 6 months (Placebo Group). Patients were instructed to avoid any other nutritional drugs such as those containing vitamin D, A, C, E, calcium, zinc, omega 3 fatty acids and herbals during the six months of the study. Written informed consent was obtained from all individual participants in the study.

Patients selection and data collection

To be eligible for this study, the patient had to fulfill the following inclusion criteria: (1) age 18 to 60 years, (2) having bright hepatic texture proven with abdominal ultrasound and quantified with Controlled Attenuation Parameter (CAP) in transient elastography (Fibroscan), (3) having no history of current or past excessive alcohol drinking as defined by an average daily consumption of alcohol < 30 g/day in men and < 20 g/day in women, and (4) Being tested negative for the presence of hepatitis B surface antigen and antibody to hepatitis C virus.

We excluded patients with cirrhosis and other chronic liver diseases, primary biliary cirrhosis, primary sclerosing cholangitis, as well as those on calcium or vitamin D supplementation, pregnant women, and patients with renal diseases. We also excluded any patients with prior vitamin D treatment in the previous two months.

Laboratory determinations and clinical assessments

The following data were collected for each patient eligible for the study: age, gender, body mass index (BMI), residency, smoking status, hemoglobin A1C (HbA1C) hemoglobin, fasting blood glucose (FBG), complete blood count (CBC), international normalized ratio (INR), total bilirubin, direct bilirubin, aspartate transferase (AST), alanine transferase (ALT), albumin, total plasma protein, alkaline phosphatase, creatinine, blood urea nitrogen (BUN), lipid profile (Total cholesterol, triglyceride, low density lipoprotein (LDL), and high density lipoprotein (HDL)). In addition, serum 25(OH)D level was measured for both groups before and after treatment. The level of vitamin D was classified into: vitamin D deficiency (25(OH)D level

<20 ng/ml), vitamin D insufficiency (25(OH)D equal 20-30 ng/Ml), and vitamin D sufficiency (25(OH)D equal 30-100 ng/ml) (19)–(21).

Abdominal Ultrasonography (US) was performed to study liver echogenicity, size, cirrhotic changes or other abnormalities, as well as Liver US scanning to assess the degree of fatty liver (steatosis). Also, an experienced physician who was blinded to the clinical data of the patients carried out Transient elastography (Fibroscan) for measurement of controlled attenuation parameter (CAP) and liver stiffness measurements (LSM) (22), (23). Fibrosis 4 score (FIB4) and NAFLD fibrosis score (NFS) were also calculated.

Assessment procedures

Serum 25(OH)D level was measured by DBC`s immunoassay of 25(OH)D enzyme-linked immunosorbent assay (ELISA, DBC Diagnostics Biochem Canada: CAN-VD-510) at the Immunology Research Lab in Microbiology and Immunology Department, Zagazig University Hospitals (24). All other laboratory tests, including liver and renal function tests, and coagulation tests, were run using the routine laboratory testing methods.

Statistical analysis

All statistical analyses carried out using the statistical software program, SPSS, for Windows version 25.0 (SPSS; Chicago, IL, USA). Categorical variables were presented in frequency and percentage, and numerical variables in mean ± standard deviation (SD). Comparative analysis and inferential statistics were

Table 1. Demographic and baseline characteristics of the two study groups

Variables		Vitamin D Group(N=40)	Placebo Group (N=40)	P-value
Age (Year), Mean ±SD		47 ±9	46 ±10	0.301
Sex, N (%)	Female	27 (67.5%)	27 (67.5%)	1.0
	Male	13 (32.5%)	13 (32.5%)	
Residence, N (%)	Rural	22 (55%)	23 (57.5%)	1.0
	Urban	18 (45%)	17 (42.5%)	
Comorbidities, N (%)	Diabetes Mellitus	10 (25%)	9 (22.5%)	0.52
	Diabetes/Hypertension	7 (17.5%)	5 (12.5%)	
	Gout	1 (2.5%)	1 (2.5%)	
	Hypertension	7 (17.5%)	6 (15%)	
	Hypothyroidism	1 (2.5%)	0 (0.0%)	
Smoking, N (%)	No	35 (87.5%)	34 (85%)	1.0
	Yes	5 (12.5%)	6 (15%)	
Fatty liver (Ultrasonograhic grading)	Mild	16 (40%)	18 (45%)	0.31
	Moderate	21 (52.5%)	20 (50%)	
	Severe	3 (7.5%)	2 (5%)	
BMI (kg/m^2), Mean ±SD		30.6 ±4.3	29.8 ±6.3	0.51
FBG (mg/dl), Mean ±SD		108.7 ±25.4	110.2 ±32.9	0.82
HbA1C (%), Mean ±SD		7.99 ±0.44	7.67 ±0.95	0.06
White Blood Cells (10^9/L)		7.4 ±1.9	6.8 ±1.8	0.23
Hemoglobin (g/dL)		12.2 ±1.3	12.3 ±1.4	0.49
Platelets (10^9/L)		258 ±57	246 ±51	0.32
Total Bilirubin (mg/dL)		0.75 ±0.20	0.75 ±0.20	0.96
Direct Bilirubin (mg/dL)		0.20 ±0.13	0.20 ±0.14	0.94
ALT (U/L)		43 ±18	42 ±13	0.77
AST (U/L)		36 ±28	37 ±21	0.85
Albumin (g/dL)		4.1 ±0.3	4.1 ±0.4	0.71
Total Plasma Protein (g/dL)		7.37 ±0.37	7.36 ±0.38	0.93
Alkaline Phosphatase (U/L)		90 ±20	86 ±20	0.35
INR		1 ±0.08	0.99 ±0.19	0.71
Creatinine (mg/dL)		0.78 ±0.16	0.83 ±0.20	0.26
BUN (mg/dL)		14.1 ±2.3	13.7 ±22	0.91
Cholesterol (mg/dL)		213.2 ±37.6	210.3 ±12.6	0.46
Triglyceride (mg/dL)		167.1 ±69.6	163.2 ±65.6	0.79
LDL (mg/dL)		130 ±36	128 ±16	0.75
HDL (mg/dL)		45.6 ±6.6	44.9 ±9.6	0.71

HCV; Hepatitis C Virus, HBV; Hepatitis D Virus, BMI; Body Mass Index, HbA1C; Hemoglobin A1C, FBG; Fasting Blood Glucose, INR; International Normalized Ratio, BUN; Blood Urea Nitrogen, ALT; Alanine Transferase, AST; Aspartate Transferase, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein

performed using parametric independent t-test, and for Gaussian distribution of the variables we ran Mann-Whitney U-test . For categorical variables, the Chi-Square test (or Fisher's exact test if appropriate) was employed. For all statistical tests, P-value ≤ 0.05 was considered statistically significant. Multivariate logistic regression analysis was carried out to determine predictor variables for the NAFLD. The primary endpoint was the evaluation of a 6-month vitamin D supplementation on the biochemical and sonographic parameters of fatty liver.

Results

Eighty NAFLD patients participated in the present study, of whom 40 subjects received vitamin D, whereas 40 patients received placebo and served as control group. The demographic and baseline characteristics of the subjects based on the groups are presented in Table 1. The two groups were comparable in all demographics, baseline characteristics, and laboratory data. The mean serum 25(OH)D in the Vitamin D group was 16.31±10.23 ng/ml at baseline, while in the placebo group, the mean serum 25(OH)D was 17.35 ±10.58 ng/ml. After six months of a monthly single intramuscular dose of 200,000 IU cholecalciferol, the mean serum 25(OH)D increased to 39.37 ±11.99 ng/ml, p<0.001. About 70% of patients in

Table 2. Comparison of the vitamin D status (Serum 25(OH)D) between the study groups at baseline and after 6 months of vitamin D supplementation and placebo.

Vitamin D status	Vitamin D Group			Placebo Group		
	Baseline	After 6 M.	P-value	Baseline	After 6 M.	P-value
Serum 25(OH)D (ng/ml) Mean ±SD	16.31±10.23	39.37 ±11.99	<0.001	17.35 ±10.58	18.76 ±8.33	0.51
Vitamin D Deficiency, No (%)	28 (70%)	3 (7.5%)		24 (60%)	22 (55%)	0.31
Vitamin D Insufficiency, No (%)	4 (10%)	2 (5.0%)	0.03	6 (15%)	5 (12.5%)	
Vitamin D Sufficiency, No (%)	8 (20%)	35 (87.5%)		10 (25%)	13 (32.5%)	

Vitamin D Deficiency: Serum 25(OH)D <20 ng/ml, Vitamin D Insufficiency: Serum 25(OH)D= 20-30 ng/ml, Vitamin D Sufficiency: Serum 25(OH)D= 30-100 ng/m

Table 3. Comparison of the liver parameters and lipid profile between the baseline and after 6 months in the two study groups.

Parameters	Vitamin D Group			Placebo Group			P value**
	Baseline (Mean ±SD)	After 6 M (Mean ±SD)	P-value*	Baseline (Mean ±SD)	After 6 M (Mean ±SD)	P-value*	
FIB4	1.4 ±0.5	1.3 ±0.5	0.944	1.37± 0.3	1.35± 0.4	0.81	0.62
NFS	1.03 ±1.19	1.12 ±1.24	0.085	1.02 ±1.26	1.19 ±1.29	0.79	0.55
CAP (dB/m)	311.9 ±42.2	287.0 ±44.3	<0.001	308±53.3	308±23.4	0.89	0.001
LSM (kPa)	6.8 ±2.0	6.1 ±1.1	0.05	6.62±1.5	6.59±1.1	0.88	0.04
ALT (U/L)	43 ±18	36 ±12	0.04	42 ±18	44±19	0.42	0.02
AST (U/L)	37 ±15	30 ±12	0.02	36 ±21	38 ±14	0.69	0.007
Cholesterol (mg/dL)	213.2 ±37.6	210.2 ±53.6	0.75	210.3 ±12.6	211.5 ±30.1	0.79	0.33
Triglyceride (mg/dL)	167.1 ±69.6	165.5 ±23.7	0.89	163.2 ±65.6	167.1±48.5	0.52	0.85
LDL (mg/dL)	130 ±36	124.6 ±6.7	0.21	128 ±16	135 ±28	0.07	0.004
HDL (mg/dL)	44.6 ±6.6	49.7 ±4.2	0.01	44.9 ±9.6	43.1 ±4.2	0.16	0.04

FIB4: Fibrosis 4 score; NFS: NAFLD Fibrosis Score; CAP: Controlled Attenuation Parameter; LSM: Liver Stiffness. Measurement; ALT: Alanine Transferase; AST: Aspartate Transferase; BMI: Body Mass Index, ALT; Alanine Transferase, AST; Aspartate Transferase, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein. *P-value of comparison between before and after treatment. **P-value of comparison between the two groups after treatment

Table 4. Follow-up of patients in Vitamin D Group after 6 months of vitamin D supplementation.

Serum 25(OH)D level at baseline			Serum 25(OH)D level after 6 months			
	No (%)	Mean ±SD		No. (%)	Mean ±SD	P value
Vitamin D Deficiency	28 (70%)	10.23±4.31	Vit D Deficiency	3 (10.71%)	19.13 ±0.81	<0.001*
			Vit D Insufficiency	2(7.14%)	28.41 ±0.07	
			Vit D Sufficiency	23(82.14%)	36.07 ±5.59	
Vitamin D Insufficiency	4 (10%)	23.06 ±3.01	Vit D Deficiency	-	-	<0.001*
			Vit D Insufficiency	-	-	
			Vit D Sufficiency	4 (100%)	47.92 ±6.96	
Vitamin D Sufficiency	8(20%)	33.3 ±5.6	Vit D Deficiency	-	-	<0.001*
			Vit D Insufficiency	-	-	
			Vit D Sufficiency	8(100%)	54.92±10.86	

Vitamin D Deficiency: Serum 25(OH)D <20 ng/ml, Vitamin D Insufficiency: Serum 25(OH)D= 20-30 ng/ml, Vitamin D Sufficiency: Serum 25(OH)D= 30-100 ng/ml
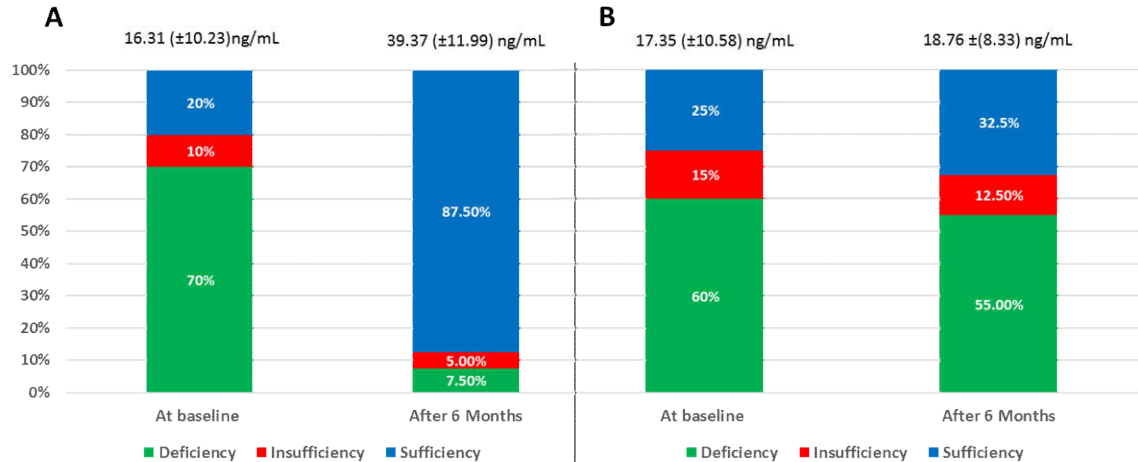
Figure 1. Comparison of the Vitamin D status at baseline and after 6 month of Vitamin D supplementation in (A) Vitamin D Group and (B) Placebo Group. Vitamin D Deficiency: Serum 25(OH)D <20 ng/ml, Vitamin D Insufficiency: Serum 25(OH)D= 20-30 ng/ml, Vitamin D Sufficiency: Serum 25(OH)D= 30-100 ng/ml.

Table 5. Multiple logistic regression analysis for factors predicting the NAFLD

	B	Wald	OR	95% C.I for OR		P Value
				Lower	Upper	
Gender (Reference: Male)	-0.067	0.005	0.935	.137	6.387	0.945
Age (Year)	0.076	1.872	1.079	0.968	1.202	0.171
BMI (kg/m2)	-0.223	6.990	0.800	0.678	0.944	0.08
FBG (mg/dl)	0.002	0.013	1.002	0.974	1.030	0.909
Triglyceride (mg/dL)	0.007	0.495	1.007	0.987	1.028	0.482
LDL (mg/dL)	-0.002	0.019	0.998	0.966	1.031	0.889
HDL (mg/dL)	-0.072	1.093	0.931	0.814	1.065	0.296
Serum 25(OH)D	-0.557	11.782	0.89	0.417	0.98	0.001
Vitamin D Deficiency (Reference: Sufficiency)	-8.972	7.907	4.0	1.0	6.06	0.005
Vitamin D Insufficiency (Reference: Sufficiency)	-3.029	2.351	0.048	0.001	2.324	0.125

BMI: Body Mass Index, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein; FBG: Fasting Blood Glucose.

vitamin D group had vitamin D deficiency, which significantly reduced to 7.5%, as presented in Figure 1. However, in the placebo group, there was no significant difference in the vitamin D level (deficiency, insufficiency, and sufficiency) between the baseline and after 6 months of supplementation (Table 2).

In Vitamin D group, the values of CAP (311.9 ±42.2dB/m) and LSM (6.8 ±2 kPa) had significantly reduced after 6 months of supplementation (287.0 ±44.3dB/m and 6.1±1.1 kPa, respectively, Figure 2), while in the placebo group, the results of CAP and LSM did not differ significantly after six months. The mean change from the baseline of CAP and LSM between the two groups was significantly higher in the Vitamin D Group (p=0.001 and 0.04, respectively). However, the results of FIB4 and NFS did not differ significantly between the two groups (Table 3). The liver enzymes (ALT and AST) had significantly improved in Vitamin D group compared to Placebo group. Besides, HDL level in Vitamin D group was significantly increased after cholecalciferol treatment compared to the placebo group (45.6 ±6.6 Vs 49.7 ±4.2, Vitamin D group and 44.9 ±9.6 Vs 43.1 ±4.2 in placebo group, p=0.04). The LDL level after six months of supplementation was significantly lower in the vitamin D Group compared to the placebo group (p=0.04).

The status of vitamin D level in 28 (70%) patients in Vitamin D group, who had vitamin D deficiency, had changed to vitamin D sufficiency 23 (82.14%) patients, vitamin D insufficiency 2 (7.14%) patients, and vitamin D deficiency 3 (10.71%). All other patients, who had vitamin D insufficiency or vitamin D sufficiency had changed to vitamin D sufficiency with higher serum 25(OH)D levels, as presented in Table 4.

In the multivariate logistic regression analysis model, by considering the presence of NAFLD as the dependent variable, lower serum 25(OH)D (mainly vitamin D deficiency) was the only significant predictor for NAFLD (OR=0.89 (95%CI 0.417:0.98, p=0.001) independent from age, gender, BMI, lipid profile, and FBG (Table 5).
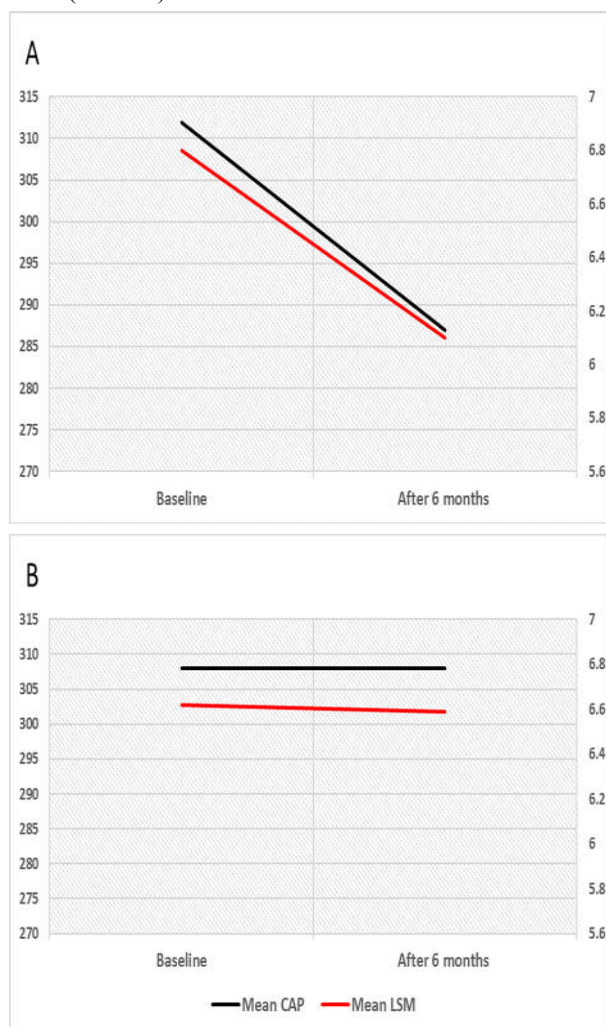

Figure 2. Comparison of Fibroscan results at baseline and after 6 months of vitamin D supplementation in (A) Vitamin D Group and (B) Placebo Group.

Discussion

Vitamin D deficiency is common in chronic liver disease patients, especially those with cirrhosis (25). Low serum levels of vitamin D have been observed in chronic liver diseases, especially with liver cirrhosis (26), (27); however, in patients with NAFLD, the available data about the association between the vitamin D deficiency and NAFLD are still scanty.

The initial diagnosis of NAFLD in clinical practice depends on the laboratory findings and radiological imaging techniques in the absence of other causes of fatty liver (28). Recently, attention has been focused on transient elastography, which is a non-invasive ultrasound-based method that uses shear wave velocity to assess the stiffness of liver tissue. Depending on the physical characteristics such as the velocity and intensity attenuation of the shear wave, the acquired data are processed as LSM and CAP.

On the other hand, simple blood-based scores can be easily obtained as NAFLD fibrosis score (NFS) (22), which has shown high sensitivity for detecting advanced fibrosis (29). Additionally, FIB-4 that simple, inexpensive, and noninvasive test can be easily obtained to determine the degree of hepatic fibrosis (30). In the present study, Transient elastography was performed for the measurement of CAP and LSM.FIB4 and NFS were also calculated.

About 70% of NAFLD patients in Vitamin D group and 60% of Placebo group had vitamin D deficiency. The recent systematic review (31) included 45 studies exploring the association between vitamin D status and NAFLD/NASH. Of them, 29 studies reported an inverse association between vitamin D status and NAFLD, while 16 studies did not support this association.

Our study demonstrates that a monthly single intramuscular dose of 200,000 IU cholecalciferol (vitamin D3) could improve the liver condition in patients with NAFLD proved with a significant reduction in the CAP and LSM after 6 months of supplementation. This result was consistent with Papapostoli et al., who demonstrated that the mean CAP reduction relative to baseline at four weeks and three and six months in 40 NAFLD patients received 20,000 IU vitamin D weekly for six months (32). Furthermore, most vitamin deficiency patients, who

received vitamin D, had their vitamin D status changed to be sufficient.

A recently published meta-analysis included six clinical trials assessing the effect of vitamin D on the metabolic function of patients with NAFLD(33). They revealed that vitamin D supplementation might improve the lipid profile when compared with placebo. Besides, vitamin D supplementation may not improve the glycemic index or the anthropometric measures among patients with NAFLD but might improve NAFLD symptoms. Similar results were observed from another meta-analysis in 2013 (34), which were consistent with our findings regarding the LDL and HDL but not with cholesterol and triglyceride, as presented in table 3. HDL level in Vitamin D group was significantly increased after cholecalciferol treatment compared to the placebo group. The LDL level was significantly lower than the placebo group after six months of supplementation. Nonetheless, no significant difference was observed in cholesterol and triglyceride levels before and after the supplementation in both groups.

Using the NHANES III database, screened in 6,800 patients, they found that 308 patients with unexplained elevation in liver enzymes (elevated ALT mainly) had lower vitamin D levels compared to 979 matched controls (18). In this study, we proved an association between the elevated liver enzymes and lower vitamin D levels because the ALT and AST levels had significantly improved in vitamin D Group compared to Placebo Group. Targher et al. also confirmed an association between NAFLD and vitamin D deficiency. In addition, vitamin D levels were lower in NASH patients when compared to those with isolated fatty liver (35).

In the multivariate logistic regression analysis model, lower serum 25(OH)D concentrations (mainly vitamin D deficiency) were the only significant predictor for NAFLD independent of age, gender, BMI, lipid profile and FBG. This result was consistent with Barchetta and colleagues who performed a multivariate logistic analysis adjusting for BMI demonstrating an association between NAFLD and 25(OH) vitamin D after BMI adjustment (36).

The strength of our study is its prospective interventional nature and regardless of serum vitamin D status, all NAFLD patients received intramuscular injectable fixed dose of vitamin D supplementation, but not all patients had acquired higher serum 25 (OH)D. Additionally, we could frequently and non-invasively monitor hepatic steatosis through measuring CAP by fibroscan.

The main limitation of our study is the relatively small sample size, which might limit the generalizability of the results. This clinical trial was non-randomized and single-blinded, which increases the chance of selection bias. We included only adult patients, although the study of Manco et al. (37) reported that, in children, low levels of 25(OH)D with NAFLD were associated with histological severity of the hepatic steatosis regardless of the metabolic characteristics.

In conclusion, our results showed a significant relationship between vitamin D levels and the risk of NAFLD, and that a monthly single intramuscular dose of 200,000 IU cholecalciferol is effective in improving the laboratory and fibroscan parameters of the liver in NAFLD patients. Further studies with large sample size and higher doses of vitamin D supplementation are recommended to approve Vitamin D as a potential treatment for NAFLD.

Ethics approval

Acknowledgment

Conflict of interests

Funding

References

1. Eliades M, Spyrou E. Vitamin D: a new player in non-alcoholic fatty liver disease? World J Gastroenterol 2015; 21:1718-27.

2. Wang X, Li W, Zhang Y, Yang Y, Qin G. Association between vitamin D and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: results from a meta-analysis. Int J Clin Exp Med 2015;8:17221-34.

3. Younossi Z, Loomba R, Rinella M, Bugianesi E, Marchesini G, Neuschwander B, et al. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2018;68:361-71.

4. Sheth S, Gordon F, Chopra S. Nonalcoholic steatohepatitis. Ann Intern Med 1997;126:137-45.

5. Zúñiga S, Firrincieli D, Housset C, Chignard N. Vitamin D and the vitamin D receptor in liver pathophysiology. Clin Res Hepatol Gastroenterol 2011;35:295-302.

6. Buzzetti E, Pinzani M, and Tsochatzis E. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism 2015;65:1038-48.

7. Younossi Z, Loomba R, Rinella M, Bugianesi E, Marchesini G, Neuschwander B, et al. et al. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. Hepatology 2018;68:349-60.

8. Angulo P. Obesity and Nonalcoholic Fatty Liver Disease. Nutr Rev 2008; 65:57-63.

9. Sharifi N, Amani R, Hajiani E, Cheraghian B. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. Endocrine 2014;47:70-80.

10. Lorvand Amiri H, Agah S, Mousavi S, Hosseini A, Shidfar F. Regression of Non-Alcoholic Fatty Liver by Vitamin D Supplement: A Double-Blind Randomized Controlled Clinical Trial. Arch Iran Med 2016;19:631-8.

11. Barchetta I, Del Ben M, Angelico F, Di Martino M, Fraioli A, La Torre G, et al. No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. BMC Med 2016;14:92.

12. Foroughi M, Maghsoudi Z, Askari G. The effect of vitamin D supplementation on blood sugar and different indices of insulin resistance in patients with non-alcoholic fatty liver disease (NAFLD). Iran. J. Nurs. Midwifery Res 2016;21:100.

13. Abramovitch S, Dahan-Bachar L, Sharvit E, Weisman Y, Tov AB, Brazowski E, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. Gut 2011;60:1728-37.

14. Zhou Q, Hou F, Guo Z, Liang M, Wang G, Zhang X. 1,25-Dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. Diabetes Metab Res Rev 2008;24:459-64.

15. Konstantakis C, Tselekouni P, Kalafateli M, Triantos C. Vitamin D deficiency in patients with liver cirrhosis. Ann Gastroenterol 2016;29:297-306.

16. Abramovitch S, Sharvit E, Weisman Y, Bentov A, Brazowski E, Cohen G, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide induced liver fibrosis in rats. Gut 2014;60:1728-37.

17. Abramovitch S, Dahan-Bachar L, Sharvit E, Weisman Y, Ben Tov A, Brazowski E, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. Gut 2011;60:1728-37.

18. Liangpunsakul S, Chalasani N. Serum Vitamin D Concentrations and Unexplained Elevation in ALT Among US Adults. Dig Dis Sci 2011;56:2124-9.

19. Holick M. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006;81:353-73.

20. Bischoff-Ferrari H, Giovannucci E, Willett W, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28.

21. Dawson-Hughes B, Heaney R, Holick M, Lips P, Meunier P, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005;16:713-6.

22. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846-54.

23. Sterling R, Lissen E, Clumeck N, Sola R, Cassia Correa M, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-25.

24. Yousif M, A. Sadek M, Farrag H, Selim F, Hamed E, Salama R. Associated vitamin D deficiency is a risk factor for the complication of HCV-related liver cirrhosis including hepatic encephalopathy and spontaneous bacterial peritonitis. Intern Emerg Med 2019;14:753-61.

25. Arteh J, Narra S, Nair S. Prevalence of Vitamin D Deficiency in Chronic Liver Disease. Dig Dis Sci 2010;55:624-2628.

26. Kitson M, Roberts S. D-livering the message: The importance of vitamin D status in chronic liver disease. J Hepatol 2012;57:897-909.

27. Lange C, Bojunga J, Ramos-Lopez E, Badenhoop K, Zeuzem S, Sarrazin C, et al. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. J Hepatol 2010;54:887-893.

28. Byrne C, Targher G. NAFLD: A multisystem disease. J Hepatol 2014;62:47-64.

29. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta□analysis. Hepatology 2017;66:1486-501.

30. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: An inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. Hepatology 2007;46:32-6.

31. Pacifico L,. Osborn J, Bonci E, Pierimarchi P, Chiesa C. Association between Vitamin D Levels and Nonalcoholic Fatty Liver Disease: Potential Confounding Variables. Mini-Reviews Med Chem 2019;19:310-32.

32. Papapostoli I, Lammert F, Caroline S. Effect of Short-Term Vitamin D Correction on Hepatic Steatosis as Quantified by Controlled Attenuation Parameter (CAP). J Gastrointestin Liver Dis 2016;25:175181.

33. Hariri M, Zohdi S. Effect of Vitamin D on Non-Alcoholic Fatty Liver Disease: A Systematic Review of Randomized Controlled Clinical Trials. Int J Prev Med 2019;10:14.

34. Eliades M, Spyrou E, Agrawal N, Lazo M, Brancati F, Potter J, et al. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2013;38:246-54.

35. Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, et al. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2007;17:517-24.

36. Barchetta I, Angelico F, Del Ben M, Baroni M, Pozzilli P, Morini S, et al. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC Med 2011;9:85.

37. Manco M, Ciampalini P, Nobili V. Low levels of 25-hydroxyvitamin D3 in children with biopsy-proven nonalcoholic fatty liver disease. Hepatology 2010;51:2229.