

Effect of Vitamin D Replacement on Liver Enzymes in Patients with Non-alcoholic Fatty Liver Disease

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ABSTRACT

BACKGROUND AND AIMS: The relationship between vitamin D-deficiency and pathogenesis of non-alcoholic fatty liver diseases (NAFLD) has been always a controversial subject. The present study is aimed to investigate the effects of vitamin D on the reduction of liver enzymes and, then, compare its effects with those of diet and physical activities individually.

PATIENTS AND METHODS: The present double-blinded randomized clinical trial was conducted atUniversity of Medical Sciences in 2017. For this purpose, 80 patients with NAFLD and vi-

itamin D-deficiency were randomly divided into two groups, namely *intervention* and *control*. Subsequently, the patients in both groups were provided with programs of diets and physical activities. The patients in the intervention group received, in addition to the given treatment, the vitamin at the dosage of (VIT-D/50000 unit) once per week for ten weeks. Afterwards, the data related to the clinical and demographic characteristics of both groups were collected and, then, compared with each other using statistical methods.

RESULTS: The applied therapeutic interventions led to a significant reduction in the variables including AST, ALT, total cholesterol, and LDL-C in both groups ($p < 0.05$). As for the two indices of blood sugar and triglyceride, the control group exhibited significantly less variations ($p < 0.05$).

CONCLUSION: The obtained results showed that the administration of vitamin D had no considerable superiority over the diet and physical activities in terms of reducing the liver enzymes as well as other investigated parameters.

Key words: Vitamin D; Liver Enzymes; Non-alcoholic Fatty Liver Disease

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a range of clinical and pathological states, which can range, in case of no alcoholic drinking, from simple steatosis to steatohepatitis, fibrosis, and cirrhosis, leading finally to the emergence of hepatocellular cancer^[1,2]. The incidence of this disease is originated from the excessive deposition of fat inside the cytoplasm of hepatocytes, most of which is triglyceride^[3].

There is still no affirmed pharmacological treatment for treating patients with NAFLD. Lifestyle modification interventions, including physical activities and diet, constitute the most important non-pharmacological treatments for this disease. Notwithstanding

that such a strategy is the most effective and safest approach for controlling NAFLD, more than 50% of people fail to achieve their intended weight loss^[4-6].

The presence of a relationship among NAFLD, obesity, and metabolic syndrome has been the major subject matter of various studies in recent years^[7,8]. The outstanding role of vitamin D in NAFLD has attracted and guided a considerable deal of attention toward the effects of vitamin D. In this regard, vitamin D may play such a role and affect the metabolic profile and oxidative stress through influencing the cell cycle adjustment, antioxidant enzyme activation, and PTH (*parathyroid hormone*) suppression^[8-10].

Various studies on this subject matter have yielded contradictory results for the effectiveness of this approach. For further elaboration on the details, on the one hand, the obtained results have demonstrated a strong relationship between vitamin D-deficiency and NAFLD^[11,12]; on the other hand, in a clinical trial investigating the effect of vitamin D on the patients with NAFLD and type-II diabetes at *Diabetes Center, University of Rome, Italy*, the findings indicated no significant changes in the steatosis and concentration of liver enzymes, FBS, and other metabolic parameters in the intervention group compared to the control one^[13]. Similarly, according to the results of another clinical trial addressing the effect of compensation for vitamin D-deficiency on hepatic steatosis, the concentration of the liver enzymes showed no significant change. Nonetheless, the hepatic steatosis grade was reduced significantly^[14].

On this basis, regarding the contradictory results, it is necessary to conduct further clinical trials in this field. Accordingly, the present work was conducted to investigate the effect of vitamin D on the reduction of liver enzymes and compare it with those of diet and physical activities.

PATIENTS AND METHODS

Study design

The present double-blinded randomized clinical trial was conducted at University of Medical Sciences in 2017, in which the patients were divided into two groups, namely *intervention* and *control*, with the allocation ratio of 1:1 using randomized blocking method. The patients did not know about the treatment assignment method. Furthermore, this work was approved by *Committee of Ethics* of University of Medical Sciences and the participants completed a written consent form (Ethics Code: IR.QUMS.REC.1396.5). IRCT registration number: IRCT2012071810333N1.

Participants

The participants included the adult patients who referred to two digestive tract super-specialty clinics in Qazvin while complaining about their elevated serum aminotransferase levels in 2017; among the participants, those with diagnosed NAFLD were included in the study. The diagnosis was based on the liver sonography findings as well as the elevated ALT and AST levels (above 40 mg/dL). Therefore, the inclusion criteria in this study included elevated liver enzymes, sonographic findings for fatty liver, and age of > 18 years old. On the other hand, the exclusion criteria included alcohol intake for at least 3 successive months over 5 years (above 20 g/day for females and > 30 g/day for males), having vitamin D blood level of normal range (above 30 ng/ml), suffering from chronic liver diseases, intake of steatohepatitis-inducing drugs such as fibrates, statins, and anti-epileptic drugs, diagnosis of diabetes mellitus under drug therapy, having severe renal diseases, cardiovascular diseases, and thyroid diseases, history of GI tract bypass surgery, multivitamin

intake, and vitamin ampules intake within the last 6 months or intake of tablets such as corticosteroid, metformin, vitamin C, zinc, selenium, or other antioxidant factors within the last 3 months, and being pregnant or breastfeeding women. In addition, the patients with psychologic disorders or intake of psychedelic drugs were excluded from the study.

Intervention

Lifestyle modification was the principal treatment factor, which was provided for all the participants. It included a calorie-limited diet as well as planned physical activities to achieve the ideal weight. The protocol applied for the diet and exercises in the present work was based on the *NAFLD Diagnosis and Management Guidelines Issues*^[15]. A nutritionist, who was unaware of the study protocol, implemented the treatment protocol and evaluated the participants' daily calorie intake over the research period. The patients in the study group, in addition to the recommendations on their diet and physical activities, received vitamin D at the dosage of (Pearl VIT D /50000unit d) once per week for 10 weeks.

Measurements

A radiologist, who was unaware of the patients' clinical data and their allocation manner, performed the liver sonography. The fatty liver was detected through hepatic echo with a 3.5 MHz probe. After 12-h fasting, the participants' serum was analyzed for fasting serum glucose, FSG (fasting serum glucose), TG (triglyceride), CHOL (cholesterol), LDL (low-density lipoprotein), HDL (high-density lipoprotein), ALT (alanine transaminase), AST (aspartate transaminase), and ALP (alkaline phosphatase) were measured via enzymatic methods^[16]. The laboratory researchers did not know about the status of the control and intervention groups.

Statistical Analyses

The two groups (control and intervention) were compared for all the analyzed tests (including LDL, HDL, TG, CHOL, FBS, AST, ALT, and 25-OHVIT-D) both in base conditions and after 10 weeks. All of the quantitative variables and qualitative data were described as *Mean ± SD* and *frequency (%)*, respectively. The quantitative variables in both groups were compared using t-test and Chi-squared test was employed to analyze the qualitative variables. Furthermore, $p < 0.05$ was considered as the significance level.

RESULTS

Table 1 represents the demographic and clinical characteristics of the participants in both groups, indicating no significant difference between the two groups in terms of gender, age, BMI, and weight (Table 1, $p > 0.05$).

Table 2 compares the two groups in terms of fatty liver grade, 25 (OH)D, AST, ALT, TG, LDL-C, HDL-C, total cholesterol, and blood sugar. Regarding these indices, the two groups exhibited no significant difference at the research onset (Table 2, $p > 0.05$).

Table 3 compares the status of each group before and after the intervention (week 10). According to the findings, the "*exercise and diet*" group (control group) exhibited a significant reduction in FBS when compared with "*vitamin-D + exercise and diet*" (intervention group) (93.07 ± 10.26 and 0.2 ± 8.50 , $p = 0.005.91$ vs 92.07 ± 10.04 and 91.62 ± 7.65 , $p = 0.54$). In addition, the control group showed a significant reduction in TG after 10 weeks (TG: 224.65 ± 48.44 and 213.65 ± 40.37 , $p = 0.003$).

Moreover, the two groups demonstrated significantly reduced mean

values in some parameters, including AST, ALT, total cholesterol, and LDL-C, the details of which can be found in table 3. As for HDL-C, the variations in both groups over 10 months were not significant ($p > 0.05$).

A comparison of the laboratory data of both groups at the end of the treatment course (after 10 weeks) is shown in Table 4, which indicates that the LDL-C and total cholesterol variations in the control groups were significantly less than those of the intervention group ($p < 0.05$).

DISCUSSION

The present study was designed to respond to and resolve the ambiguities in the effectiveness of vitamin D supplementation as a treatment for the patients with NAFLD. As indicated by the obtained results, the administration of vitamin D (50000 units) once a week along with lifestyle modification for 10 weeks proved to have significant superiority over the application of mere lifestyle modification by itself. Meanwhile, the two indices, namely blood sugar (FBS) and triglyceride (TG), showed a further reduction.

Comparison of the status of the two groups before and after the intervention indicated improvement in both groups in terms of some

variables, including AST, ALT, total cholesterol, and LDL-C, while no significant difference was observed in HDL-C levels in both groups. Since no difference was observed among all the variables in the two groups in base conditions, the variables were compared again after 10 weeks in both groups. The obtained results indicated a trend of improvement in total cholesterol and LDL-C levels in the control group compared to the intervention one, which could be attributed to better compliance of the participants with the given diet and physical activities in the control group. Some of the studies in this regard have shown that the prescription of medication would lead to less consideration of pharmacological recommendations among the patients^[17]. As lifestyle modification was applied in both groups, it was to some extent expectable to achieve such results, since the use of this approach for controlling the daily calorie intake is considered as a *gold standard of care* for the patients with NAFLD^[18]. Various studies in this regard have represented that achieving the ideal body weight would result in stable biochemical improvement (serum liver enzymes and insulin level) in the patients with NAFLD^[19,20]. Based on the results of the present study, it can be probably argued that the administration of vitamin D had no additional effect on the above-mentioned indices and the resulted improvements in both groups could be attributed to the given exercises and diet modifications. Albeit, such findings were not tremendously unexpected. Consistently, in the study conducted by *Papapostoli et al.*, the administration of vitamin D could not reduce the liver fat level compared to the base levels^[21].

The review of the literature in this field led to somehow contradictory results. For instance, *Sakpal et al* showed that the administration of vitamin D combined with lifestyle modification could significantly improve the serum ALT level compared to mere lifestyle modification^[14].

In contrast, in another work by *Dabbaghmanesh et al.*, the administration of vitamin D and calcitriol failed to exhibit positive effects on the treatment of the patients with NAFLD^[18], which was to some extent consistent with the results of the present work. They supposed that the presence of liver disorders might disturb the

Table 1 Demographic characteristics of the participants in both groups.

Variable	Intervention group (n=40)	Control group (n=40)	p-value
Male N (%)	13(32.5%)	15(37.5%)	0.63
Female N (%)	27(67.5%)	25(62.5%)	
Age (year)	34.9 ± 13	39.7 ± 11.3	0.09
BMI (kg/m ²)	29.8 ± 2.4	29.9 ± 2.2	0.84
Weight	89.62 ± 10.55	88.61 ± 11.11	0.67

Table 2 Laboratory data at the research onset for both groups

Variable	Intervention group (n = 40)	Control group (= 40)	p-value
Fatty liver grade			
Grade-1	26 (%65)	27 (%67.5)	0.971
Grade-2	13 (%32.5)	12 (%30)	
Grade-3	1 (%2.5)	1 (%2.5)	
25(OH)D (nmol/L)	15.23 ± 4.73	14.34 ± 4.65	0.4
Aspartate transaminase (SGPT or ALT)	47.85 ± 22.19	43.77 ± 12.48	0.315
Alanine transaminase (ALT or SGPT)	63.12 ± 20.71	60.62 ± 20.34	0.588
Triglyceride	215.17 ± 51.32	224.65 ± 48.44	0.39
LDL-C (mg/dL)	137.45 ± 19.80	147.97 ± 34.80	0.1
HDL-C (mg/dL)	41.58 ± 7.12	40.55 ± 13.11	0.69
Total cholesterol (mg/dL)	199.03 ± 20.05	208.62 ± 35.51	0.14
Blood sugar (FBS)	92.07 ± 10.04	93.07 ± 10.26	0.661

Table 4 Laboratory data for both groups at the end of treatment.

Variable	Intervention group	Control group	p-value
Blood sugar (FBS)	91.62 ± 7.65	91.2 ± 8.50	0.84
Aspartate transaminase (AST or SGOT)	39.87 ± 10.39	41.40 ± 9.28	0.49
Alanine transaminase (ALT or SGPT)	54.12 ± 13.52	53.85 ± 14.79	0.93
TG	209.67 ± 35.64	213.65 ± 40.37	0.64
Total cholesterol	201.97 ± 32.83	182.96 ± 19.20	0.002
HDL-C (mg/dL)	41.26 ± 5.19	39.45 ± 6.68	0.18
LDL-C (mg/dL)	142.52 ± 32.64	121.12 ± 17.69	< 0.001
25(OH)D (nmol/L)	41.38 ± 13.38	14.13 ± 5.42	< 0.001

Table 3 Comparing the two groups in terms of laboratory parameters before and after 10 weeks of treatment.

Variable	Intervention			Control		
	Before	After	p-value	Before	After	p-value
Blood sugar (FBS)	92.07 ± 10.04	92.07 ± 10.04	92.07 ± 10.04	93.07 ± 10.26	91.2 ± 8.50	0.005
Aspartate transaminase (AST or SGOT)	47.85 ± 22.19	47.85 ± 22.19	47.85 ± 22.19	43.77 ± 12.48	41.40 ± 9.28	0.004
Alanine transaminase (ALT or SGPT)	63.12 ± 20.71	63.12 ± 20.71	63.12 ± 20.71	60.62 ± 20.34	53.85 ± 14.79	0.003
TG	215.17 ± 51.32	215.17 ± 51.32	215.17 ± 51.32	224.65 ± 48.44	213.65 ± 40.37	0.003
Total cholesterol (mg/dL)	208.62 ± 35.51	208.62 ± 35.51	208.62 ± 35.51	197.64 ± 18.27	182.96 ± 19.20	< 0.001
HDL-C (mg/dL)	41.58 ± 7.12	41.58 ± 7.12	41.58 ± 7.12	40.65 ± 13.11	39.45 ± 6.68	0.69
LDL-C (mg/dL)	147.97 ± 34.85	147.97 ± 34.85	147.97 ± 34.85	137.45 ± 19.80	121.12 ± 17.69	< 0.001
25(OH)D (nmol/L)	15.23 ± 4.73	15.23 ± 4.73	15.23 ± 4.73	14.34 ± 4.65	14.13 ± 5.42	0.7

process of 25(OH)D unit conversion; thus, they used the active form of vitamin D (known as calcitriol) along with vitamin D. Consistent with this study, Sharif *et al* showed that the application of vitamin D supplementation treatment was not effective in reducing the serum aminotransferase level^[22]. In the same way, in another clinical trial, the weekly administration of vitamin D capsules (5000 units) could not reduce the serum aminotransferase level^[23].

As for the study limitations, not using the liver biopsy to determine the histological response can be mentioned. In the present work, the diagnosis of NAFLD was based on sonography, while the liver biopsy is known as the gold standard method for NAFLD detection^[24].

Notwithstanding the significant reduction in AST, ALT, total cholesterol, and LDL-C variables in both groups, the administration of vitamin D showed no considerable superiority over the application of the diet and physical activities treatment by themselves. On this basis, it is recommended to utilize liver biopsy after finishing the study, since the administration of vitamin D might have reduced the hepatic steatosis grade.

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