Effect of Vitamin D Supplementation on C-reactive Protein in Patients with Nonalcoholic Fatty Liver

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Original Article

ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the worldwide. It is associated with chronic disorders such as diabetes and heart diseases. Inflammation is one of the basic causes of metabolic diseases. Several studies have shown that Vitamin D can reduce inflammation. The purpose of this study was to investigate the effect of Vitamin D supplementation on inflammation in patients with NAFLD.

Methods: This study involved 60 NAFLD patients, divided equally into two intervention and placebo groups. During 10 weeks, patients in the intervention group receive Vitamin D (capsules containing 50,000 IU vitamin D), weekly. Vitamin D levels, C-reactive protein (CRP), triglyceride (TG), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured at the beginning and end of the study. Data were analyzed using analysis of covariance tests. Analyses were done using SPSS software (version 16) (SPSS Inc., Chicago, USA). $P < 0.05$ set as significant level.

Results: Vitamin D supplementation resulted in an increase of serum 25(OH) D concentrations in inter group ($P < 0.05$) and intra-group ($P < 0.05$) in intervention group. At the end of the study, in the intervention group, TG and CRP reduced significantly compare with baseline ($P < 0.05$). A significant increase was seen in calcium serum in the intervention group in comparison with baseline ($P < 0.05$) and compared with the placebo group ($P < 0.05$). After adjusting for baseline values, level of ALT, AST, TG, and CRP were reduced in the intervention group compared with the placebo group, but this decrease was not significant between the two groups.

Conclusions: Vitamin D supplementation had no effect on CRP and other variables in the intervention group compared with the placebo group. Further studies with strong design and more sample must conduct to demonstrate the effect of Vitamin D supplementation on inflammation in patients with NAFLD.

Keywords: Inflammation, nonalcoholic fatty liver, vitamin D

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases in the worldwide and it is associated with chronic diseases such as diabetes and heart disease. NAFLD encompasses a wide spectrum of liver damage.[1] This range is include from simple esteatosis to esteatohepatitis and ultimately cirrhosis.[2] NAFLD is associated with insulin resistance and is defined as fat accumulation in liver, exceeding 5% of liver weight, in the absence of ethanol intake >10 g/day.[3,4] Prevalence of NAFLD is estimated at around 34% in the adult population. Two theories exist about the development of NAFLD. The first one is about the development of insulin resistance that explains the effect of insulin resistance in NAFLD. The second theory is increased inflammation which can lead to NAFLD. In a study conducted in Japan, a significant relationship was observed between the level of serum C-reactive protein (CRP) and risk of NAFLD.[5] However, in another study, no significant correlation was seen between serum CRP and NAFLD.[3]

Vitamin D is a fat-soluble vitamin that exists in a number of food products such as oils and dairy products. Vitamin D is traditionally known as a regulator of calcium and phosphorus metabolism, but in recent years a significant relationship has been observed between serum levels of Vitamin D and risk of chronic diseases such as diabetes and cardiovascular diseases.[6] Vitamin D receptors are present in more than 38 tissues and its receptors affect genes controlling oxidative stress and inflammation.[7] Moreover, macrophages and dendrite cells have Vitamin D receptors.[8,9] There are several lines of evidence demonstrate an association between Vitamin D and liver disease.[10,11] However, in several studies, there were no association between Vitamin D concentration and severity of fatty liver.[12,13] The effect of Vitamin D on its receptor in these cells regulates and balances the inflammation and oxidative stress.[14,15] Moreover, several studies have confirmed the relationship between serum Vitamin D levels and inflammation.[16-18] Due to the role of inflammation in NAFLD, therefore probably Vitamin D supplementation can reduce inflammation. Hence, the purpose of this study was to investigate the effect of Vitamin D supplementation on inflammation in patients with NAFLD.

METHODS

Participants' characteristics

A randomized double-blind placebo-controlled clinical trial was conducted on 60 patients (30-70 years) with NAFLD. Samples were calculated with a confidence interval of 95% and 80% power of the test by below formula[19] (S = 1.7, d = 0.7).

$$n = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \left(S_1^2 + S_2^2\right)}{(d)^2}$$

This study was conducted in Metabolic Liver Disease Research Center in Isfahan University of Medical Sciences, Isfahan, Iran. The Study was performed with the approval of Isfahan University of Medical Sciences Local Ethics Committee. Informed consent was obtained from participants. Inclusion criteria were that NAFLD was confirmed by ultrasound test. Normal range alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was <31 IU/L in this study. Exclusion criteria in our study were defined as acute illnesses, chronic kidney disease, hyperparathyroidism, hypoparathyroidism, chronic heart failure, hepatitis C or hepatitis B, Wilson syndrome, history of chronic liver diseases, or disorders that affect gallbladder and bile ducts, pregnancy or history of taking any drugs affecting levels of ALT including (valproic acid, tamoxifen, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, metformin, angiotensin-converting enzyme 1 and angiotensin-converting enzyme-related 1). Furthermore, subjects should not follow any especial diet. Subjects should not intake oral Vitamin D/calcium/multivitamin supplementation in the previous study.

Participants were divided into two groups using permuted block randomization: (a) 30 patients in Vitamin D group (capsules containing 50,000 IU Vitamin D), (b) 30 patient in the placebo group. Placebo capsules were in the same color, odor and taste appearance. Intervention period followed for 10 weeks and patients received vitamin supplements or placebo capsules every week. To evaluate the acceptability of Vitamin D supplementation, serum 25-hydroxy Vitamin D level was measured at the beginning and end of the study. Dietary records were collected every 2 weeks, and intakes

were determined based on estimated values in household measurements. To obtain nutrient intakes of participants on the basis of these 5-d food diaries, Nutritionist IV software (version 7.0; N-squared Computing, Salam, OR, USA), which was modified for Iranian food items were used. 5-day physical activity records taken (one per 2 weeks). Physical activity levels estimated as metabolic equivalent of task hours per week (MET-hr/wk). MET-hr/wk for each exercise program calculated (days per week × hours of exercise each time × MET equivalent of exercise) and summed of all MET-min/wk values for each patient evaluated.

Anthropometric measurements

Height and body weight were measured while the subjects were in a standing position at baseline and 10th week. Body mass index was measured by the formula (weight $_{(kg)}$/height^2 $_{(meter)}$).

Biochemical measurements

Fasting blood samples were taken at the beginning and end of the study. 25-hydroxy Vitamin D was assessed by using a commercial ELISA kit (Immuno Diagnostic Systems). at the beginning and the end of the study. Serum calcium was measured by commeriacal kit (Pars Azmun). Triglycerides (TGs) were measured by enzymatic methods. AST and ALT serum levels were measured through enzymatic photometric method (IFCC) with a sensitivity of 2 U/L and a coefficient of variation of 14% (ALT, AST, and alkaline phosphatase (ALP) were measured by commercially available enzymatic reagents (Pars Azmoon) on a BT-3000 (Biotechinica) autoanalyzer. CRP serum was measured by high-sensitivity enzyme (test Pars Tehran, Tehran, Iran).

Degree of fat accumulation in liver

Level of liver esteatosis was assessed by using ultrasonography with Esaote Medical ultrasound machine (convex 3.5 MHz) at the beginning and the end of the study. US performed by the same expert radiologist with the same instrument in the begging and the end study. Hepatic ultrasonography was conducted by someone who is unaware of the objectives of the study. Patients for ultrasound should be fasting for 8 h. Ultrasonography is performed in the supine position. Right and left lobes of the upper and lower surfaces are studied. Echogenicity the liver, the presence or absence of bulky tumors cystic or solid and calcification was assessed. Intrahepatic bile ducts, portal vein and hepatic artery were evaluated.

Statistical analysis

Normality of studied variables was evaluated using Kolmogorov–Smirnovtest or probability-probability plot. For nonnormal variables, log transformation was used. Independent-samples student's *t*-test was used to detect differences in general characteristics and dietary intakes between two groups. In this analysis, Vitamin D and placebo treatment was regarded as between-subjects factor. Further analyses were conducted to investigate between group comparisons by using analysis of covariance. Chi-square test for comparison the grades of fatty liver between two groups of placebo and intervention used and paired *t*-test used to assess the within group comparison of quantitative variables. P <0.05 was considered to be significant level. All statistical analyses conducted using Statistical Package for the Social Sciences (SPSS), version 16 (SPSS Inc., Chicago, USA).

RESULTS

The study flow chart shows the screening, randomization and follow-up of the participants [Figure 1]. In this study, 29 men and 31 women participated. Mean age of participants was 48.5 years. Baseline subclinical characteristics of 60 patients are given in Tables 1 and 2. Data shows that 23.6% and 10.9% of our subjects have abnormal ALT and AST levels at baseline, respectively. Compliance with the treatments was good in both groups and no side-effects were presented. On the basis of 5-d dietary intake and physical activity records, no significant differences were seen between the two groups [Table 3].

Vitamin D supplementation resulted in an increase of serum 25(OH) D concentrations in inter group ($P < 0.05$) and intra-group ($P < 0.05$).

At the end of study, in the intervention group, TG and CRP reduced significantly compare with baseline ($P < 0.05$). A significant increase was seen in calcium serum in the intervention group in comparison with baseline ($P < 0.05$) and compared with the placebo group ($P < 0.05$).

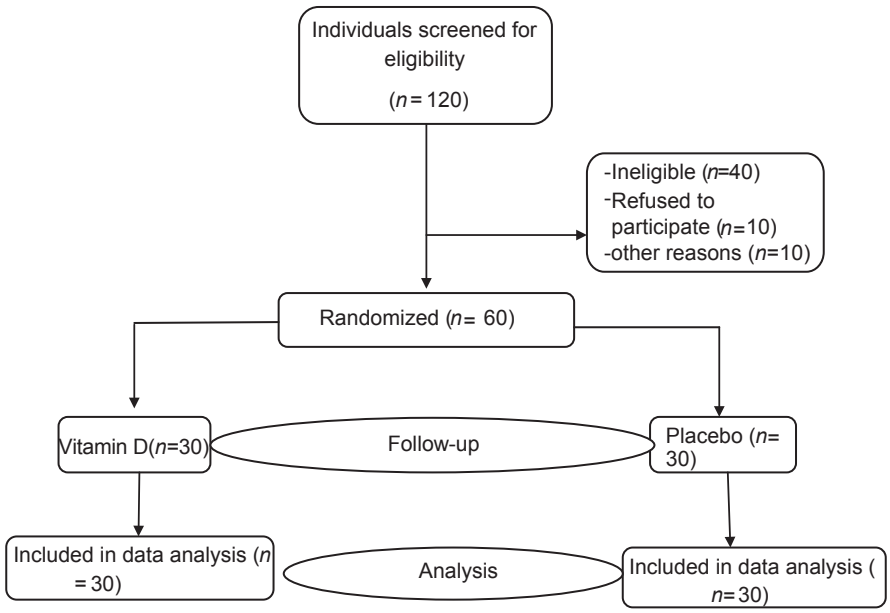

Figure 1: Study diagram

Table 1: The comparison of subclinical characteristics at baseline and after intervention of study[a]

Characteristic	Vitamin D supplementation (*n*=30)	Placebo (*n*=30)	*P* value[c]
BMI (kg/m^2)			
Before	31.2±4	32.4±6	0.14
After	30.78±3.86	32.23±5.78	-
P value[b]	0.07	0.054	-
AST (IU/L)			
Before	25.88±8.74	30.24±19.56	0.16
After	21.48±6.84	27.44±17.3	-
P value[b]	0.06	0.06	-
ALT (IU/L)			
Before	31.93±16.17	37.64±23.56	0.17
After	29.53±14.57	36.84±21.56	-
P value[b]	0.057	0.07	-
Triglyceride (mg/dL)			
Before	195.44±65.87	148.32±50.44	0.19
After	188.01±31.22	145.08±16.86	-
P value[b]	0.04*	0.06	-
CRP (mg/dL)			
Before	0.91±0.18	0.98±0.15	0.44
After	0.8±0.3	1.064±0.09	-
P value[b]	0.04*	0.056	-
Calcium (mg/dL)			
Before	9.5±3	12.9±2	0.76
After	13±1	9.7±1	-
P value[b]	0.03*	0.055	-
Vitamin D serum (ng/dL)			
Before	49±1	47±2	0.32
After	117±13	45.8±0.44	-
P value[b]	0.001*	0.065	-

[a]All values are means±SDs, [a]All values are means±SEs adjusted for baseline values. [b]Obtained from independent-sample *t*-test. [c]Significant levels at <0.05. ALT=Alanine amino transferees, AST=Aspartat amino transferees, CRP=C-reactive protein

After adjusting for baseline values, levels of ALT, AST, TG and CRP were reduced in the intervention group in comparison with the placebo group, but these changes were not significant between two groups after intervention.

DISCUSSION

This study was the first one that investigated the effect of Vitamin D supplementation on inflammation in patients with NAFLD. In this study, Vitamin D supplementation did not reduce inflammation in patients with NAFLD in the intervention group compared with the control group. In addition, Vitamin D supplementation did not significant effect on serum TG levels and liver enzymes.

Table 2a: Adjusted changes in metabolic variables in NAFLD who received either Vitamin D supplements or placebo[a]

	n=30 (%)		*P* value[b]
	Vitamin D group 3	Placebo group	
Vitamin D (ng/mL)	68±12 (138.5)	−1.9±2.44 (−4.04)	0.02
Calcium (mg/dL)	4±0.4 (42)	−3.2±1 (−24)	0.04
BMI (kg/m^2)	−0.42±0.14 (−1.3)	−0.17±0.22 (−0.52)	0.65
Triglyceride (mg/dL)	−7.43±34.65 (−3.7)	−2.92±22.3 (−1.9)	0.44
CRP (mg/dL)	−0.11±0.27 (−12)	0.084±0.14 (8.5)	0.2
ALT (IU/L)	−2.4±1.6 (−7.5)	−0.8±2 (−2.1)	0.18
AST (IU/L)	−4.4±1.9 (−17)	−2.8±2.2 (−9.25)	0.19

[a]All values are means±SEs. [b]Obtained from ANCOVA adjusted for (baseline value, age, sex, BMI and physical activity). ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, SEs=Standard errors, CRP=C-reactive protein, NAFLD=Nonalcoholic fatty liver disease, BMI=Body mass index

Table 2b: Fatty liver grades in NAFLD who received either Vitamin D supplements or placebo

Grade fatty liver	Group (30)		*P* value[a]
	Vitamin D	Placebo	
1	2	5	0.78
2	24	20	
3	4	5	

[a]Obtained from Chi-square test. NAFLD=Nonalcoholic fatty liver disease

In several studies, a significant relationship was seen between NAFLD and inflammation.[3] Inflammation was considered in the pathogenesis of NAFLD. Several studies have shown significant relationship between low serum Vitamin D and increase inflammatory markers. In a study conducted by Grossmann *et al*., 12 weeks of Vitamin D supplementation caused a significant decrease in the levels of inflammatory factors in patients with cystic fibrosis.[20] In another study that conducted by Chandler *et al*. on 328 African-American patients, Vitamin D supplementation significantly decreased systemic inflammation in these patients.[21] In another

Table 3: Dietary intakes and physical activity of NAFLD who received either Vitamin D supplements or placebo throughout the study[a]

	Group (*n*=30)		*P* value[b]
	Placebo	Vitamin D	
Energy (kcal/day)	2045.1±461	2217.2±461	0.76
Carbohydrate (% calorie/day)	58	61	0.52
Protein (% calorie/day)	12	12	0.91
Fat (% calorie/day)	30	27	0.65
Cholesterol (mg/day)	236±111	225±57	0.44
Dietary fiber (g/day)	24±5	19±7	0.34
Vitamin D (mg/day)	4±0.3	3±0.4	0.18
Physical activity score (MET-h/week)	33.2±1.22	32.3±1.44	0.54

[a]All values are means±SDs, [b]Obtained from independent-samples *t*-test. SD=Standard deviation, MET=Metabolic equivalent, NAFLD=Nonalcoholic fatty liver disease

study, the effect of Vitamin D supplementation was investigated on inflammatory markers in patients with type 2 diabetes. In this study, Vitamin D supplementation significantly decreases systemic inflammation and CRP concentration in patients with type 2 diabetes.[22] However, in several studies, Vitamin D supplementation had no effect on systemic inflammation and CRP.[23] Vitamin D may reduce inflammation through several mechanisms.[24] There are Vitamin D receptors in >37 tissues in the body.[4] Vitamin D affects through receptors on these organs and regulates pro-inflammatory and systemic inflammation in the body.[7] Vitamin D receptors are located in the nucleus of the macrophages. Numerous macrophages produce cytokines, particularly tumor necrosis factor-alpha (TNF-α).[25,9,10] TNF-α expression depends on the effect of nuclear factor-κB (NF-κB), significantly. Vitamin D increases expression of the inhibitory protein NF-κB. Vitamin D reduces the expression of NF-κB and through TNF-α level is reduced.[26,27] In addition, Vitamin D with binding to its receptors in monocytes can reduce pro-inflammatory cytokines. It also suppresses expression of NF-κB gene. Thereby Vitamin D reduces the production CRP and systemic inflammation.[28-30]

In this study, we had several limitations. The first one was use of ultrasound method for diagnosis of fatty liver disease, while for accurate diagnosis of fatty liver, liver biopsy should be used. The second

limitation was a small sample size of participants, especially in grades one and three.

Vitamin D supplementation had no effect on CRP and other variables in the intervention group compared with the placebo group. Further studies with strong design and more sample must conduct to demonstrate the effect of Vitamin D supplementation on inflammation in patients with NAFLD.

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