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Original Article

Vitamin D supplementation for the treatment of non-alcoholic fatty liver disease: A randomized double blind placebo controlled trial

Mohammad Hossein Dabbaghmanesh[a,*], Farideh Danafar[a], Ahad Eshraghian[b], Gholamhossein Ranjbar Omrani[a]

[a] Endocrine and Metabolism Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

Background: Low serum vitamin D has been associated with metabolic syndrome and Non-alcoholic fatty liver disease (NAFLD). This study aimed to investigate the impact of vitamin D supplementation in treatment of patients with NAFLD.
Methods: In a double blind, randomized, placebo controlled trial patients with NAFLD were randomized to receive one weekly pearl of placebo, 50,000 U vitamin D3 (cholecalciferol) pearl per week and 0.25 mg calcitriol (1,25 dihydroxycholecalciferol) pearl per day for 3 months.
Results: 106 NAFLD patients were randomized to receive calcitriol, vitamin D3 and placebo pearls for 12 weeks and data for 91 patients were analyzed. After 12 weeks of treatment, serum alkaline phosphatase levels was significantly decreased from baseline levels in vitamin D and calcitriol treated groups ($P < 0.05$). Serum and gamma glutamyl transferase (GGT) level was also significantly decreased compared to the baseline levels after 12 weeks of treatment with vitamin D. There was no statistically significant difference between placebo, calcitriol, vitamin D groups in terms of serum aminotransferase, alkaline phosphatase, serum GGT and lipid profile ($P > 0.05$).
Conclusion: While significant reduction of serum alkaline phosphatase and GGT were seen with vitamin D and calcitriol supplementation from baseline levels, no beneficial effects was seen when comparing vitamin D, calcitriol and placebo groups at the end of trial.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum ranging from simple steatosis to steatohepatitis that may lead to liver cirrhosis [1]. It is a rapidly growing disease in many areas of the world with the highest prevalence in the Middle Eastern countries [2,3]. NAFLD is now the most common cause of liver enzyme abnormalities worldwide. It is usually considered as the hepatic manifestation of metabolic syndrome, however, there are patients with NAFLD without components of metabolic syndrome [4]. The precise pathophysiology of NAFLD and its progression to cirrhosis and hepatocellular carcinoma (HCC) is not still clear and several complex underlying mechanisms may involve.

On the other hand, NAFLD is not a liver limited disease and extrahepatic diseases have been associated with NAFLD. Cardiovascular diseases are more prevalent in NAFLD patients and are the main cause of mortality among these patients [5,6]. NAFLD is more prevalent in patients with diabetes mellitus and diabetic patients are more susceptible to cirrhosis secondary to NAFLD [7]. Thyroid hormone abnormalities have also been reported in patients with NAFLD in several previous studies [8,9]. Recently, association between NAFLD with bone and vitamin D metabolism have been reported [10].

Vitamin D is a fat-soluble vitamin which is primarily involved in bone and mineral metabolism [11]. In recent decades, other functions have been proposed and discovered for vitamin D in human body. Several previous studies have shown that low serum vitamin D have been associated with metabolic syndrome and diabetes [12–14]. Vitamin D supplementation has been shown to improve insulin resistance and glycemic control in patients with overt diabetes and those with impaired glucose tolerance [15,16]. Considering cumulative evidences about association of vitamin D and NAFLD, this study aimed to investigate the role of vitamin D therapy in amelioration of hepatic steatosis in patients with NAFLD.

* Corresponding author at: Endocrine and Metabolism Research Center, Namazi Hospital, PO Box: 71345-1744, Shiraz, Iran.
E-mail address: dabbaghm@sums.ac.ir (M.H. Dabbaghmanesh).

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2. Materials and methods

2.1. Study design and patients

A single center, double blind, randomized, placebo controlled trial was conducted in Endocrinology and Metabolism Research Center affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. Study participants were recruited among individuals participated in Kawar cohort study conducted between April 2011 and October 2013. Participants were eligible to be included in the study if fulfilling these criteria: men and women aged between 20 and 75 years with presence of hepatic steatosis diagnosed by ultrasound (US). Patients with liver cirrhosis, patients with positive results for hepatitis B virus surface antigen or hepatitis C virus antibody, patients with alcohol consumption (>10 gr/day), patients with autoimmune hepatitis or other causes of chronic liver diseases such as Wilson's disease and hemochromatosis were excluded from the study. Other exclusion criteria were: known cancer, nephrolithiasis, nephrocalcinosis, chronic renal failure, hypercalcemia, hypercalciurea, pregnancy, lactation, hypersensitivity to vitamin D3, patients receiving estrogen, tamoxifen, methotrexate, amiodarone, tetracycline and those receiving vitamin D and calcium supplementations in previous 6 months.

2.2. Randomization

Investigators, patients, radiologist and laboratory staff were all blinded to the treatment allocation. Block randomization with a block size of 5 was used through a computer based procedure and participants were randomized 1:1:1 to receive one weekly pearl of placebo, 50,000 U vitamin D3 (cholecalciferol) pearl per week and 0.25 mg calcitriol (1,25 dihydroxycholecalciferol) pearl per day. Treatments and placebo were provided in identical packages and were given to the participants by an educated person who was blinded to the drug and patients.

2.3. Procedures

All patients with NAFLD were recruited. Serum vitamin D was checked and those with vitamin D level below 30 ng/ml were included. After randomization, patients had a baseline clinical visit. A comprehensive history taking and physical examination were made and 10 cc blood was taken and freezed at −70 °C after plasma separation. Body weight was measured while patients had light clothing and having no shoes. Height was measured in standing position with patients wearing no shoes. The body mass index (BMI) was calculated using this formula: weight (kilograms)/height $(m)^2$. Waist circumference was measured using a tape meter at the site of iliac crest. Hip circumference was measured by a tape meter. Blood pressure was measured with the patient sitting on a chair and after 5 min of rest with a standardized blood pressure recorder.

Fasting plasma glucose, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), calcium and phosphorus by standard methods.

NAFLD was defined based on ultrasonographic criteria. Metabolic syndrome was defined as presence of three or more of the following metabolic components according to the National Cholesterol Education Program and Adult Treatment Panel III (NCEP: ATPIII) criteria: 1) central obesity: waist circumference >102 cm for men and >88 cm for women, 2) hypertriglyceridemia: triglyceride ≥ 150 mg/dL or taking specific medications, 3) low HDL cholesterol: <40 mg/dL in men and <50 mg/dL in women or taking specific medication, 4) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or taking specific medication, 5) fasting plasma glucose ≥ 100 mg/dL or taking specific medication or previously diagnosed with type II diabetes. Ultrasonographic examinations were performed by an expert radiologist who was unaware of laboratory tests and clinical evaluations of the participants.

Medications and placebo were administered for 12 weeks. At the end of 6th week, a follow up visit was made and patients were asked about compliance and possible adverse events. After completion of 12 weeks of the study, patients were recruited and re visited, clinical data were recorded and blood sampling was also performed to measure lab data.

2.4. Primary and secondary outcomes

The primary outcome was reduction of serum ALT, AST, GGT from baseline to 12 weeks. Secondary outcomes were improvement of metabolic component of patients including fasting plasma glucose, LDL, HDL, triglyceride and total cholesterol.

2.5. Statistical analysis

Data was analyzed as both continuous and categorical variables. Data were presented using means ± standard deviation for numeric variables, and percents and counts for categorical variables. Comparisons of continuous variables was performed with student's *t*-test. Non-parametric Mann- Whitney and Kruskal-Wallis tests were used when appropriate. A one-way analysis of variance (ANOVA) and post-hoc were used to evaluate the differences between three groups. Statistical analysis was performed with SPSS 18.0 (SPSS Inc.; Chicago, IL, USA). A P- values of <0.05 were considered statistically significant.

3. Results

3.1. Basic information

180 individuals were diagnosed with NAFLD, among them 131 individuals had vitamin D levels below 30 ng/ml. Three individuals had severe vitamin D deficiency (<10 ng/ml), 58 individuals had vitamin D deficiency (<20 ng/ml) and 70 individuals had vitamin D insufficiency (vitamin D level between 20 and 29.9 ng/ml). 106 NAFLD patients were randomized to receive calcitriol, vitamin D3 and placebo pearls for 12 weeks. Fifteen individuals were excluded from the study (4 patients in vitamin D group, 7 patients in calcitriol group and 4 patients in placebo group). From these 15 excluded patients, 8 patients did not return to us at the end of study and 7 patients had not followed the study protocol or had discontinued drugs due to side effects. After completion of the drug allocation, data of 91 NAFLD patients were analyzed. Flow diagram of the study was outlined in Fig. 1. There were 37 men and 54 women. Baseline demographic characteristics of study participants were outlined in Table 1.

3.2. Effect of vitamin D and calcitriol supplementation

There was no statistically significant difference in baseline laboratory data of patients in 3 groups (Table 2). After 12 weeks of treatment, serum alkaline phosphatase level was significantly decreased from baseline levels in vitamin D and calcitriol treated groups ($P < 0.05$) (Table 3). Serum gamma glutamyl transferase (GGT) level was also significantly decreased compared to the baseline levels after 12 weeks of treatment with vitamin D (Table 3).

After completion of study period, there was no statistically significant difference between placebo, calcitriol, vitamin D groups

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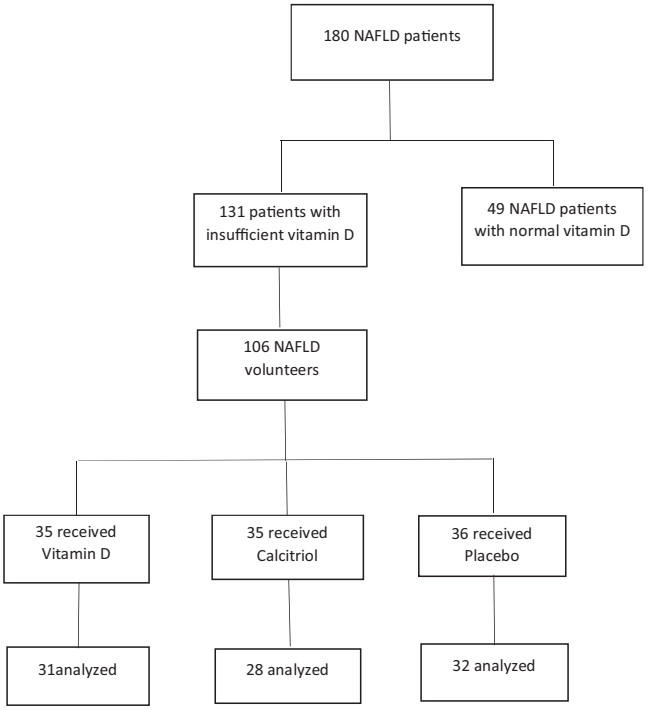

Fig. 1. Flow diagram of the study.

in terms of serum aminotransferase, alkaline phosphatase, serum GGT and lipid profile ($P > 0.05$) (Table 4).

4. Discussion

This study is the first randomized, double blind, placebo controlled trial investigating the effect of high dose oral vitamin D3 supplementation and calcitriol in non-diabetic, vitamin D deficient patients with NAFLD. Neither vitamin D3 nor calcitriol were capable of reducing serum aminotransferase, alkaline phosphatase and GGT in patients with NAFLD. Although mean serum fasting plasma glucose was decreased in calcitriol and vitamin D3 treated groups it was not statistically significant as compared to the placebo group. The alterations in serum level of TG, cholesterol and LDL were not statistically significant among these 3 groups. When compared to the baselines measures, 12 weeks of calcitriol therapy significantly increased serum HDL level and decreased alkaline phosphatase from baseline levels. Serum alkaline phosphatase and GGT levels were also significantly deceased in vitamin D treated group when compared with baseline levels. No significant alterations in lipid profile, liver enzymes or blood sugar was observed in placebo treated group.

Hepatic osteodystrophy is the term usually used to define abnormalities of bone in patients with chronic liver disease. Epidemiological evidences are now available that have proposed hypovitaminosis D as an independent risk factor for NAFLD [17,18]. It has been reported that low 25(OH) D is strongly and independently associated with NAFLD in adult normal population with normal liver enzymes [19]. Furthermore, the severity of vitamin D deficiency has been associated with NAFLD severity in histopathology and patients with lower vitamin D had more advanced liver steatosis and fibrosis [20,21]. NAFLD patients had 1.26 times higher likelihood to be vitamin D deficient in a recent meta-analysis [22]. Vitamin D receptor (VDR) is expressed on all hepatic cells and vitamin D have a direct anti-inflammatory action on hepatic cells in patients with chronic liver disease via this

Table 1
Baseline demographic characteristics of study participants.

	Placebo	Calcitriol	Vitamin D3	P-value
Age	45.7 ± 14.8	44.4 ± 11.1	44.7 ± 7.6	0.897
Weight	73.8 ± 13.1	77.1 ± 12.8	75.9 ± 12.4	0.561
Height	160.5 ± 9.2	161.4 ± 11.7	160.9 ± 11.0	0.953
BMI	28.6 ± 4.9	29.8 ± 5.2	29.4 ± 4.9	0.646
Hip Circumference	103.5 ± 12.8	107.7 ± 14.6	106 ± 15.01	0.483
Waist Circumference	97.2 ± 12.9	100.6 ± 11.2	97.4 ± 9.6	0.414
Diastolic BP	84.4 ± 12.1	81.8 ± 12.6	84.3 ± 12.2	0.636
Systolic BP	131.5 ± 20.7	122.5 ± 17.9	124.5 ± 24.6	0.198

BMI: Body mass index, BP: Blood pressure.

Table 2
Baseline laboratory data of three groups.

	Placebo	Calcitriol	Vitamin D3	P-value
Vit D	21.1 ± 5.2	18.6 ± 5.5	18.9 ± 6.2	0.14
FBS	122 ± 51.1	116.9 ± 16.1	119 ± 28.9	0.82
Tch	206.5 ± 40.4	212.1 ± 47	218 ± 28	0.52
LDL	87 ± 24.2	94.5 ± 22.9	92.1 ± 23.6	0.45
HDL	36.5 ± 9	33.6 ± 5.3	37.8 ± 8.8	0.13
TG	162.9 ± 92	152.2 ± 72	190.7 ± 98.5	0.24
ALK	192 ± 55.9	197.2 ± 65.4	202.9 ± 53.5	0.87
ALT	16.4 ± 7.6	16.1 ± 4.8	15.1 ± 4.3	0.45
AST	22.6 ± 10	20 ± 7.2	20.5 ± 5.1	0.96
GGT	36.5 ± 29.3	28.8 ± 13.4	31 ± 19.3	0.73
Calcium	9.1 ± 1.1	9.1 ± 0.8	9.4 ± 0.7	0.42
Phosphorus	4.2 ± 0.5	4 ± 0.6	5.7 ± 6.2	0.15

FBS: Fasting blood suger, Tch: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglyceride, ALK: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase.

Table 3
Comparison between baseline laboratory parameters and after 12 weeks of therapy.

Variable	Placebo			Calcitriol			Vit D3		
	Baseline	After 12W	P-value	Baseline	After 12W	P-value	Baseline	After 12W	P-value
Vit D	21.1 ± 5.2	18.8 ± 7	0.04	18.6 ± 5.5	22.9 ± 19.8	0.2	18.9 ± 6.2	32.2 ± 14.1	0.00
FBS	122 ± 51.1	129 ± 71.7	0.23	116.9 ± 16.1	110.5 ± 55.9	0.22	119 ± 28.9	113 ± 33.5	0.28
Tch	206.5 ± 40.4	195.6 ± 43.8	0.13	212.1 ± 47	202.1 ± 33.9	0.08	218 ± 28	213.4 ± 46.7	0.51
LDL	87 ± 24.2	92.5 ± 29.8	0.29	94.5 ± 22.9	100.1 ± 21.2	0.05	92.1 ± 23.6	100.7 ± 25.9	0.06
HDL	36.5 ± 9	38.4 ± 8.6	0.26	33.6 ± 5.3	38 ± 7	0.01	37.8 ± 8.8	38.7 ± 7.6	0.55
TG	162.9 ± 92	166.1 ± 99	0.8	152.2 ± 72	166.8 ± 69.3	0.3	190.7 ± 98.5	191.4 ± 96.6	0.94
ALK	192 ± 55.9	185 ± 51.4	0.08	197.2 ± 65.4	181.3 ± 59.4	0.03	202.9 ± 53.5	181.7 ± 49.2	0.001
ALT	16.4 ± 7.6	16.5 ± 5.4	0.88	16.1 ± 4.8	17.9 ± 4.6	0.07	15.1 ± 4.3	16.5 ± 7.3	0.3
AST	22.6 ± 10	24.5 ± 7.7	0.23	20 ± 7.2	25.6 ± 4.9	0.01	20.5 ± 5.1	24.2 ± 7.9	0.05
GGT	30.6 ± 29.3	29.1 ± 2.6	0.41	28.8 ± 13.4	25.7 ± 8.9	0.2	31 ± 19.3	26.1 ± 12.8	0.01
Ca	9.1 ± 1.1	9.4 ± 0.4	0.2	9.1 ± 0.8	9.3 ± 0.2	0.2	9.4 ± 0.7	9.2 ± 0.9	0.2
P	4.2 ± 0.5	4.1 ± 0.7	0.62	4 ± 0.6	4.2 ± 0.5	0.03	5.7 ± 6.2	4.5 ± 1.2	0.2

FBS: Fasting blood suger, Tch: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglyceride, ALK: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, Ca: Calcium, P: Phosphorus.

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Table 4
Comparison between 3 groups after completion of the study.

	Placebo	Calcitriol	Vitamin D3	P-value
Vit D	18.8 ± 7	22.9 ± 19.8	32.2 ± 14.1	0.000
FBS	129 ± 71.7	110.5 ± 55.9	113 ± 33.5	0.297
Tch	195.6 ± 43.8	202.1 ± 33.9	213.4 ± 46.7	0.257
LDL	92.5 ± 29.8	100.1 ± 21.2	100.7 ± 25.9	0.397
HDL	38.4 ± 8.6	38 ± 7	38.7 ± 7.6	0.954
TG	166.1 ± 99	166.8 ± 69.3	191.4 ± 96.6	0.423
ALK	185 ± 51.4	181.3 ± 59.4	181.7 ± 49.2	0.908
ALT	16.5 ± 5.4	17.9 ± 4.6	16.5 ± 7.3	0.589
AST	24.5 ± 7.7	25.6 ± 4.9	24.2 ± 7.9	0.738
GGT	29.1 ± 2.6	25.7 ± 8.9	26.1 ± 12.8	0.822
Calcium	9.4 ± 0.4	9.3 ± 0.2	9.2 ± 0.9	0.287
Phosphorus	4.1 ± 0.7	4.2 ± 0.5	4.5 ± 1.2	0.189

FBS: Fasting blood sugar, Tch: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglyceride, ALK: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase.

receptor [23].These evidences support the role of vitamin D deficiency in pathogenesis of NAFLD.

Few human studies have been recently investigated efficacy of vitamin D supplementation as a treatment modality in patients with NAFLD. In a randomized placebo controlled clinical trial, Sharifi et al. administered bi-monthly oral 50000 IU vitamin D capsule for 4 months to NAFLD patients. While vitamin D therapy could decrease serum malondialdehyde, it was not effective in reduction of serum aminotransferase levels [24]. In another randomized trial, weekly 50000 IU vitamin D capsule failed to reduce serum aminotransferase and C reactive protein (CRP) in patients with NAFLD [25]. In an interventional non-randomized study, weekly vitamin D therapy with dose of 20,000 IU for six months could decrease hepatic fat content from baseline levels as measured by controlled attenuation parameter (CAP) during transient elastography (TE) [26]. In a recent published randomized trial, daily 25 μg calcitriol therapy for 12 weeks was compared to placebo with promising results. Calcitriol therapy reduced AST, ALT levels and improved HOMA-IR and lipid profile in patients with NAFLD [27]. In a very recent randomized trial; daily oral 2000 IU vitamin D therapy failed to show any beneficial effect on hepatic steatosis or cardiovascular/metabolic parameters in patients with type II diabetes [28]. These studies were non- homogenous in terms of inclusion criteria, severity of NAFLD, and outcome measurements resulting in conflicting results.

Vitamin D is a fat soluble vitamin that is derived from 7-dehydrocholestrol. Activation of vitamin D is started in the liver and is completed in kidney [29]. It has been suggested that patients with chronic liver disease may have impaired hepatic hydroxylation of vitamin to 25(OH) D [30]. Calcitriol is the active form of vitamin D and we hypothesized that calcitriol may be more effective than vitamin D for amelioration of steatosis since there is no need for hepatic hydroxylation step. Calcitriol therapy had beneficial effects on glycemic indices compared to placebo tablets [31]. and reduced lipid profile and markers of oxidative stress in patients with type II DM [32].

Our findings failed to demonstrate beneficial effects of vitamin D and calcitriol therapy in patients with NAFLD. We did not also measure steatosis and fibrosis scores using liver biopsy or transient elastography- controlled attenuation parameter. It should be noted that mean ALT and AST levels were normal in our study population before allocation and the duration of therapy was 12 weeks. More positive results may be achieved with longer duration of treatment and allocation of vitamin D to a group of NAFLD patients with elevated serum aminotransferase.

Other randomized trials with longer duration of therapy among patients with NASH may be required for further investigation of the issue.

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Authors contribution

Dabbaghmanesh MH: Concept, design, performing the study and analyzing data and drafting the manuscript and final approval.

Danafar F: Design, performing the study and gathering data and drafting the manuscript and final approval.

Eshraghian A: Design, gathering and analyzing data, drafting the manuscript and final approval.

Omrani GR: Design, gathering data, drafting the manuscript and final approval.

Conflict of interest

All authors declare that there is no conflict of interest.

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