Research Communication

High dose vitamin D supplementation is associated with an improvement in serum markers of liver function

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ABSTRACT

Limited studies have examined the effects of vitamin D on liver enzymes in patients with liver disease but none has explored its effects in the healthy subjects. The aim of present study was to evaluate the effects of a high dose vitamin D supplementation on measures of liver function. A total of 988 adolescent girls were recruited; all were assessed for liver function tests (LFTs) including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transferase (ϒGT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, albumin, and total protein before and after supplementation with 50,000 IU cholecalciferol perls. Significant reductions were observed for AST, ALT, direct bilirubin, total bilirubin, LDH, and ϒGT at the end of supplementation, only in the group with abnormal reference value. Serum levels of total protein and albumin were higher at the end of follow up in the group with abnormal value. No significant change was obtained for LFTs in the group with normal value. Our

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BMI, Body mass index; BUN, Blood urea nitrogen; Cr, Creatinine; DBP, Diastolic blood pressure; FBG, Fasting blood glucose; HDL-C, High density lipoprotein-cholesterol; hs-CRP, High sensitive-C reactive protein; LDH, Lactate dehydrogenase; LDL-C, Low density lipoprotein-cholesterol; LFTs, Liver function tests; SBP, Systolic blood pressure; TC, Total cholesterol; TG, Triglyceride; WC, Waist circumference; WHR, Waist hip ratio; ϒGT, Gamma-glutamyl transferase

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findings suggest that vitamin D supplementation may improve markers of liver function in adolescents with abnormal LFTs. More randomized controlled trial with longer follow-up time will be required. © 2019 BioFactors, 9999(9999):1–8, 2019

Keywords: *vitamin D; liver; adolescent; supplementation*

1. Introduction

Vitamin D is a fat-soluble vitamin derived in part from sun exposure of the skin, diet, and dietary supplements. It is converted to an active form (25 hydroxylated cholecalciferol) in the liver and kidney (1,25 dihydrocholicalciferol) [1]. Although mineral and skeletal homeostasis are key functions of vitamin D, several studies have indicated that vitamin D status is also related to risk of cancer, diabetes, autoimmune, infectious, and cardiovascular diseases [2]. Vitamin D deficiency is known to be a prevalent global health problem [3]. Using the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition criteria [4] for our population, we have found that 90% of subjects have vitamin D insufficiency.

Vitamin D deficiency is a common problem among patients with chronic liver diseases [5]. Measures of liver function are used for routine evaluation of patients included from the assessment to management of disease states [6]. These markers include alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transferase (ϒGT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, albumin, and total protein. Although abnormal liver function tests (LFTs) are associated with liver injury and dysfunction, these tests are not specific for the liver and may be affected by various conditions unrelated to liver disease such as bone or cardiovascular diseases [6].

Most studies have previously evaluated the association between vitamin D and LFTs in patients with serious liver dysfunction. Several studies have shown associations between low levels of serum vitamin D and liver dysfunction and elevated serum transaminases [7,8]. The key question is whether liver dysfunction contributes to vitamin D deficiency or is a consequence of low serum of vitamin D. In a recent multicenter study, which was conducted in Egyptian children, low serum vitamin D concentrations were associated with the presence of hepatosteatosis [9]. Although some studies have reported that vitamin D deficiency is related to liver dysfunction and abnormal LFTs [10–12], but the effects of vitamin D supplementation on LFTs is less clear. Some studies have indicated that vitamin D supplementation may improve liver enzymes [13,14] while no significant effects were observed in other reports [15,16]. It is possible that apparently healthy individuals without liver disease have LFTs results that are outside the reference range (6). Obesity or inflammation may be associated with abnormal LFTs [13]. We therefore aimed to examine the effects of vitamin D on LFTs in apparently healthy subjects. Improvement of LFTs can be as an indicator during treatment process of individuals [17].

The Iranian Ministry of Health has recently recommended high-dose supplements of vitamin D for reducing vitamin D deficiency in adolescents. Approximately 100,000 adolescent girls took 9 weekly perls of high dose vitamin D supplements (50,000 IU). The main reason for the vitamin D supplementation in these subjects was based on recent reports of the high prevalence of vitamin D deficiency in Iranian children and adolescents [18,19]. Given the inconsistent reports of the effects of vitamin D on LFTs in previous studies, we conducted the current follow up study before and after supplementation on 988 apparently healthy adolescent girls. To the best of our knowledge, this is first study, which examines the effects of vitamin D supplementation on LFTs in apparently healthy individuals.

2. Experimental procedures

2.1. Study design and participants

This observational study was performed in the cities of Mashhad and Sabzevar, Iran between January and April 2015. The ethical committee of Mashhad University of Medical Sciences approved the study, and informed written consent was completed by all participants.

Vitamin D supplementation was provided to 1,00,000 girls aged between 12–18 years by Iran's Ministry of Health. Each girl took one perl of 50,000 IU vitamin D per week for consecutive 9 weeks. Of these participants, 1026 subjects were selected using a randomized clustering method and computer-generated random numbers. The participants were randomly recruited from different areas in the cities of Mashhad and Sabzevar. Written consent was obtained from students and their parents. We excluded those with any auto-immune diseases, cancer, metabolic bone disease, hepatic, or renal failure, cardiovascular disorders, malabsorption or thyroid, parathyroid, or adrenal diseases. Also, individuals with taking anti-inflammatory, antidepressant, anti-diabetic, or anti-obesity drugs, vitamin D or calcium supplement use and hormone therapy within the last 6 months were excluded. Of the 1026 subjects originally selected, 988 met the inclusion criteria. Overall, 940 girls completed the intervention; with a dropout rate of 4.8%, 48 subjects did not complete the course of supplements; 19 were ill or on holiday on the day of sampling, 29 students failed to deliver a blood sample.

Compliance with the vitamin D supplements was checked at weekly intervals by contacting individuals by phone or by face-to-face interviewers. A validated food frequency questionnaire was used for evaluating dietary intakes during the year [20,21]. To estimate energy and nutrient intakes, the reported

portion sizes in food frequency questionnaire were converted to grams using household measures and then were entered to the Nutritionist 4 software. Physical activity was assessed through validated questionnaire [22] and provided as metabolic equivalents (METs) in hours per day. Demographic data were collected by an expert interviewer and by use of the standard questionnaires.

2.2. Anthropometric measurements

Anthropometric parameters were obtained at baseline and at the end of the follow-up period in health centers, by trained paramedic. Body weight was measured by using of a digital scale to the nearest 0.1 kg, while subjects were in over-night fasting state and without shoes and with minimal clothing. A non-stretched tape measure to the nearest 0.1 cm was used for the assessment of height, body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (WC) was obtained at the minimum circumference between the iliac crest and the last rib. Systolic blood pressure (SBP) diastolic blood pressure (DBP) was measured twice with 5-min intervals between the measurements, by an experienced nurse.

2.3. Biochemical assessments

Fasting blood samples were obtained early in morning between 8 and 10 a.m. at baseline and after 9-weeks intervention by venipuncture of an antecubital vein while in a 14 h overnight fasting. The samples were collected into vacutainer tubes from subjects in a sitting position, according to a standard protocol. Blood samples were immediately centrifuged (Hettich model D-78532) for 10 min at room temperature to separate serum and plasma into two aliquots (0.5 mL). Then, after separation, aliquots of serum were stored at −80°C at the reference laboratory in Mashhad University of medical science until analyses. The electrochemi-luminescence method (ECL, Roche, Basel, Switzerland) was used for the measurement of serum 25-OH D. Limits of detection was considered 10 nmol/L for ECL (Roche) and intra-and inter-assay variations were <5.7% and <9.9%, respectively. Fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), high-sensitive C-reactive protein (hs-CRP) concentrations, creatinine, and blood urea nitrogen were measured by enzymatically method with the use of commercial kits (Pars Azmun, Karaj, Iran) and the BT-3000 auto-analyzer machine (Biotechnica, Rome, Italy). Low density lipoprotein-cholesterol (LDL-C) was calculated by using Friedewald formula if serum TGs concentrations were lower than 4.52 mmol/L [23].

2.4. Evaluation of liver function test

Serum levels of ALT, AST, LDH, ϒGT, ALP, total bilirubin, direct bilirubin, albumin, and total protein were determined using commercial kits (Pars Azmun, Karaj, Iran) and the BT-3000 auto-analyzer machine (Biotechnica, Rome, Italy). We categorized all individuals into two groups: those with normal or abnormal value at baseline. Individuals were categorized into two groups; those with test values within the reference range for age, and those with values above the reference range for ALT, AST, LDH, ϒGT, ALP, total bilirubin, and direct bilirubin and below the reference range for total protein and albumin. Abnormality of LFTs defined as following cut off: ALP >350 IU/L (n = 159), ALT >31 IU/L (n = 92), AST >31 IU/L (n = 106), LDH > 280 IU/L (n = 84), ϒGT >32 IU/L (n = 77), total protein <5.7 g/dL (n = 67), albumin <3.5 g/dL (n = 49), total bilirubin >1.2 mg/dL (n = 104), and direct bilirubin >0.3 mg/dL (n = 113).

2.5. Statistical analyses

Kolmogrov–Smirnow test was applied to ensure the normal distribution of variables. Independent sample t-test was used detect differences in general demographics and anthropometrics measurements between normal and abnormal value; also significant differences in clinical characteristics between normal and abnormal value were examined by use of the independent sample t-test. To examine the effects of vitamin D supplementation on LFTs, we used paired-sample t-test. P value <0.05 was considered statistically significant. All statistical analyses were performed using statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, IL).

3. Results

All 988 subjects received perls of 50,000 IU of vitamin D and 940 completed the 9 weeks follow up. On average, the rate of compliance was high, such that about 95% of perls were taken throughout the study. No serious side effect was effect during our study. Serum levels of 25-hydroxy vitamin D increased from 9.4 ± 8.8 ng/ml at baseline to 36.4 ± 15 ng/ml at the end of follow up (P < 0.05).

The demographic and anthropometric measurements of study participants between normal and abnormal value groups are shown in Table 1. At baseline, BMI, WC, and SBP were significantly higher in the individuals with abnormal value of ALT and ALP compared with the subjects with normal value. We observed the subjects with abnormal value of protein and albumin had higher levels of SBP and DBP compared with the subjects with normal value. No significant differences were seen for age, physical activity, and energy intakes between two groups. The clinical and biochemical differences between two groups are indicated in Table 2. Lower concentrations of serum 25-OH vitamin D were seen in individuals with abnormal value of ALT, total bilirubin, and LDH. The higher serum levels of TG were observed in subjects with abnormal value of AST and direct bilirubin. There was a significantly higher serum TC in the group with abnormal LFTs compared with normal value group.

The effects of vitamin D supplementation on LFTs are shown in Table 3. Serum levels of LFTs were compared at baseline and at the end of follow up by the abnormal and normal value groups. Significant reductions were observed for AST, ALT, direct bilirubin, total bilirubin, LDH, and ϒGT at the end of follow up compared with the baseline in the abnormal value group. Serum levels of total protein and albumin were increased at the end of follow up in the abnormal reference

TABLE 1 ***General characteristics of study population at baseline by abnormal and normal value groups***

Group		*Age (y)*	*BMI (kg/m^2)*	*WC (cm)*	*WHR*	*SBP (mm Hg)*	*DBP (mm Hg)*	*Physical activity (MET.h/day)*	*Energy intake (Kcal)*
AST (IU/l)	Normal value group	14.5 ± 1.5	21.1 ± 4.2	70.1 ± 9.1	0.76 ± 0.06	96.3 ± 14.3	61.8 ± 14.03	45.4 ± 3.7	2707 ± 860
	Abnormal value group	14.4 ± 1.3	21.2 ± 4.1	71.5 ± 9.2	0.77 ± 0.08	96.9 ± 13.4	63.1 ± 10.8	45.3 ± 3.8	2794 ± 736
ALT (IU/l)	Normal value group	14.5 ± 1.5	21.09 ± 4.2	70.2 ± 9.04	0.76 ± 0.06	96.1 ± 14.2	61.8 ± 13.7	45.4 ± 3.7	2707 ± 853
	Abnormal value group	14.5 ± 1.5	23.4 ± 4.3**	76.5 ± 11**	0.81 ± 0.14	103 ± 12.07*	67.1 ± 10.05	45.5 ± 4.3	2729 ± 581
ALP (IU/l)	Normal value group	14.5 ± 1.5	20.3 ± 4.2	68.7 ± 9.08	0.77 ± 0.07	92.4 ± 14.09	58.02 ± 14.3	45.3 ± 3.7	2708 ± 876
	Abnormal value group	14.5 ± 1.5	21.7 ± 4.2****	71.6 ± 9.02***	0.76 ± 0.06	98.8 ± 13.6***	64.7 ± 12.4***	45.4 ± 3.6	2724 ± 834
Direct bilirubin (mg/dl)	Normal value group	14.7 ± 1.5	21.1 ± 4.1	70.4 ± 9.2	0.76 ± 0.06	97.6 ± 13.7	63.8 ± 12.1	45.5 ± 3.45	2713 ± 821
	Abnormal value group	14.4 ± 1.6	21.4 ± 4.7	70.7 ± 9.2	0.76 ± 0.05	96.7 ± 13.2	63.1 ± 11.2	46 ± 4.6	2778 ± 781
Total bilirubin (mg/dl)	Normal value group	14.6 ± 1.5	21.4 ± 4.1	70.7 ± 9.1	0.77 ± 0.06	97.3 ± 13.4	63.6 ± 12.01	45.4 ± 3.3	2709 ± 824
	Abnormal value group	14.7 ± 1.6	20.8 ± 4.8	69.7 ± 9.6	0.75 ± 0.05	97.6 ± 14.6	63.8 ± 11.2	46.1 ± 4.9	2721 ± 756
LDH (IU/l)	Normal value group	14.7 ± 1.5	21.2 ± 4.06	70.5 ± 8.9	0.76 ± 0.06	94.4.3 ± 13.4	62.03 ± 12.1	45.3 ± 3.3	2691 ± 818
	Abnormal value group	14.2 ± 1.6	20.6 ± 4.4	69.2 ± 9	0.77 ± 0.07	96.5 ± 12.9	63.6 ± 11.9	46.02 ± 4.5	2837 ± 802
GGT (IU/l)	Normal value group	14.5 ± 1.5	21.1 ± 4.3	70.2 ± 9.3	0.76 ± 0.06	97.06 ± 13.7	63.2 ± 12.1	45.5 ± 3.7	2731 ± 812
	Abnormal value group	14.1 ± 1.3	22.9 ± 3.6***	73.5 ± 8.8	0.78 ± 0.07	97.9 ± 16.8	62.5 ± 9.2	46.2 ± 3.3	2700 ± 809
Total protein (g/dl)	Normal value group	14.5 ± 1.5	21.08 ± 4.1	70.3 ± 9.1	0.76 ± 0.06	96.7 ± 13.9	63.1 ± 12.3	45.5 ± 3.7	2745 ± 805
	Abnormal value group	14.8 ± 1.4	21.8 ± 4.9	70.5 ± 9.6	0.76 ± 0.05	99.8 ± 11.4**	66.6 ± 9.7**	45.9 ± 3.7	2635 ± 873
Albumin (g/dl)	Normal value group	14.5 ± 1.5	21.1 ± 4.1	70.5 ± 9.2	0.76 ± 0.06	96.9 ± 14.2	63.06 ± 12.1	45.5 ± 3.7	2755 ± 795
	Abnormal value group	14.7 ± 1.4	21.5 ± 4.6	70.3 ± 8.8	0.76 ± 0.06	99.4 ± 11.3*	66.04 ± 10.7*	46.07 ± 3.7	2614 ± 869

Obtained from independent-sample t*-test. Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; LDH, Lactate dehydrogenase; ϒGT, Gamma-glutamyl transferase; BMI, Body mass index; WC, Waist circumference; WHR, Waist hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; Kcal, Kilocalorie.*

* P *< 0.05.*

** P *< 0.01.*

*** P *< 0.001.*

TABLE 2 ***Clinical characteristics of study population at baseline by abnormal and normal value groups***

		Vitamin D (ng/ml)	*TG (mg/dl)*	*TC (mg/dl)*	*LDL (mg/dl)*	*HDL (mg/dl)*	*FBG (mg/dl)*	*hs-CRP (mg/L)*	*Cr (mg/dl)*	*BUN (mg/dl)*
AST (IU/l)	Normal value group	9.2 ± 8.4	83.4 ± 36.7	161.3 ± 28.7	99.1 ± 25.3	46.5 ± 8.6	85.8 ± 11.9	1.5 ± 1.7	0.68 ± 0.11	12.01 ± 3.9
	Abnormal value group	8.6 ± 8.4	99.01 ± 55.5**	157.6 ± 30.1	99.3 ± 28.2	46.2 ± 8.6	87.3 ± 12.6	1.5 ± 1.8	0.68 ± 1	12.3 ± 3.4
ALT (IU/l)	Normal value group	9.2 ± 8.4	85.1 ± 39.8	160.9 ± 29	99.1 ± 25.9	46.5 ± 8.5	86.01 ± 12	1.6 ± 1.7	0.68 ± 0.11	12.9 ± 3.9
	Abnormal value group	7.5 ± 7.1*	90.1 ± 38.1	159.5 ± 24.6	99.9 ± 16	45.8 ± 8.9	87.5 ± 11.5	1.3 ± 2.1	0.64 ± 0.11	11.6 ± 2.9
ALP (IU/l)	Normal value group	9.03 ± 8.06	85.7 ± 40.1	158.07 ± 30	94.7 ± 26.3	46.05 ± 9	86.04 ± 13.5	1.5 ± 1.8	0.69 ± 0.12	13.06 ± 3.6
	Abnormal value group	9.6 ± 8.8	85.1 ± 39.9	163.7 ± 27.2**	102.9 ± 24.6**	46.9 ± 8.5	86.3 ± 10.7	1.6 ± 1.8	0.68 ± 0.1	12.7 ± 3.1
Direct bilirubin (mg/dl)	Normal value group	9.8 ± 9.6	83.7 ± 38.07	161.4 ± 29	100.4 ± 25.9	47.2 ± 9.5	85.9 ± 12	1.4 ± 1.7	0.68 ± 0.1	12.9 ± 3.4
	Abnormal value group	9.4 ± 7.9	110.8 ± 43.6***	160.9 ± 27.7	97.03 ± 26.8	44.9 ± 7.4**	89.6 ± 12.9*	1.7 ± 1.9	0.66 ± 0.12	12.4 ± 3.3
Total bilirubin (mg/dl)	Normal value group	9.9 ± 8.3	85.7 ± 40.2	161.6 ± 28.4	99.6 ± 25.5	47.05 ± 9.4	86.1 ± 12.1	1.5 ± 1.8	0.67 ± 0.11	12.9 ± 3.4
	Abnormal value group	7.05 ± 6.03*	87.1 ± 45.7	161.5 ± 28.9	101.3 ± 28.2	45.9 ± 8.2	87.08 ± 11.9	1.4 ± 1.7	0.66 ± 0.11	12.5 ± 3.06
LDH (IU/l)	Normal value group	11.4 ± 10.8	84.3 ± 36.6	161.3 ± 27.2	99.7 ± 24.2	47.2 ± 8.4	87.7 ± 10.7	1.4 ± 1.7	0.66 ± 0.09	12.5 ± 3.4
	Abnormal value group	8.8 ± 8.1**	88.07 ± 44.2	162 ± 30.6	100.4 ± 28.7	46.6 ± 10	86.8 ± 13.3	1.6 ± 1.8	0.67 ± 0.11	13.1 ± 3.3*
GGT (IU/l)	Normal value group	9.9 ± 7.6	84.7 ± 40.8	159.8 ± 28.6	99.1 ± 25.6	49.5 ± 9.08	85.8 ± 11.8	1.5 ± 1.7	0.67 ± 0.11	12.9 ± 3.4
	Abnormal value group	9.1 ± 6.9	100.8 ± 51.1	173.2 ± 34.9*	103.7 ± 27.4	50.1 ± 10.9	89.3 ± 18.1	1.7 ± 1.8	0.66 ± 0.07	12.6 ± 3.7
Total protein (g/dl)	Normal value group	9.8 ± 7.6	86.4 ± 42.3	160.7 ± 28.8	99.05 ± 26.2	46.7 ± 9.2	86.3 ± 12.01	1.5 ± 1.8	0.67 ± 0.11	12.8 ± 3.4
	Abnormal value group	9.1 ± 6.9	85.6 ± 33.5	165.1 ± 21.2	104.2 ± 20.2	45.07 ± 6.6	87.03 ± 13.6	1.7 ± 1.9	0.66 ± 0.09	12.8 ± 3.1
Albumin (g/dl)	Normal value group	9.9 ± 8.5	86.2 ± 41.9	161.1 ± 28.8	99.4 ± 26.3	46.7 ± 9.2	86.3 ± 12.09	1.5 ± 1.3	0.67 ± 0.11	12.8 ± 3.4
	Abnormal value group	8.9 ± 7.9	85.6 ± 39.2	166.8 ± 22.7	107.4 ± 24.05	44.4 ± 7.7	85.1 ± 12.5	1.7 ± 1.4	0.66	12.4 ± 3.1

Obtained from independent-sample t*-test. Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; LDH, Lactate dehydrogenase; ϒGT, Gamma-glutamyl transferase; TG, Triglyceride; TC, Total cholesterol; LDL-C, Low density lipoprotein-cholesterol; HDL-C, High density lipoprotein-cholesterol; FBG, Fasting blood glucose; hs-CRP, High sensitive-C reactive protein; Cr, Creatinine; BUN, Blood urea nitrogen.*

* P *< 0.05.*

** P *< 0.01.*

*** P *< 0.001.*

TABLE 3 ***Serum liver markers at baseline and after intervention by abnormal and normal value groups***

	Abnormal value group			*Normal value group*		
Liver markers	*Baseline*	*At the end of follow up*	P *value*	*Baseline*	*At the end of follow up*	P *value*
AST (IU/l)	36.6 ± 9.8	28.7 ± 9.6	<0.001	18.1 ± 4.1	20.6 ± 5.8	0.59
ALT (IU/l)	41.6 ± 9.1	35.7 ± 8.8	<0.001	12.4 ± 4.5	12.9 ± 5.9	0.16
ALP (IU/l)	359.5 ± 161.7	356.9 ± 170.1	0.73	345.9 ± 125.2	352.2 ± 124.9	0.11
Direct bilirubin (mg/dl)	0.44 ± 0.09	0.35 ± 0.11	<0.001	0.23 ± 0.07	0.24 ± 0.08	0.36
Total bilirubin (mg/dl)	1.35 ± 0.34	1.14 ± 0.33	<0.001	0.79 ± 0.15	0.79 ± 0.19	0.32
LDH (IU/l)	342.4 ± 72.4	317.4 ± 75.4	<0.001	239.2 ± 28.4	232.2 ± 26.8	0.002
GGT (IU/l)	36.9 ± 15.06	28.5 ± 13.2	0.001	11.3 ± 5.4	11.6 ± 5.9	0.52
Total protein (g/dl)	5.5 ± 0.82	6.5 ± 0.89	0.02	7.04 ± 0.74	6.9 ± 0.82	0.27
Albumin (g/dl)	3.4 ± 0.33	4.4 ± 0.48	0.03	4.5 ± 0.39	4.4 ± 0.44	0.13

Obtained from pair-sample t*-test. Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; LDH, Lactate dehydrogenase; ϒGT, Gamma-glutamyl transferase*

value. We did not observe any significant differences in the LFTs in the normal value group between before and after supplementation. In addition, there was no significant effect of vitamin D on ALP in either group.

4. Discussion

To the best of our knowledge, this is the first study showing that a high-dose supplementation of vitamin D improves serum markers of liver function in a large population of adolescent girls, which is in line with previous findings [24,25]. Furthermore, vitamin D metabolism is often disrupted in patients with liver diseases [24]. On the other hand, it has been shown that there is a significant association between low level of vitamin D and liver dysfunction and mortality. Moreover, the role of vitamin D deficiency in development and evolution of non-alcoholic fatty liver (NAFLD) and chronic hepatitis C (CHC) virus infection has also been shown. Moreover, vitamin D supplementation is suggested as a possible treatment for liver fibrosis in several epidemiological and longitudinal studies [26–28]. Therefore, it is important to consider the crucial role of vitamin D deficiency on development and evolution of liver diseases and important role of liver on vitamin D metabolism.

Our data show that high dose vitamin D supplementation was associated with a reduction in serum ALT, AST, LDH, and ϒGT in girls who had abnormal LFTs at baseline. In a previous study conducted among Italian adults, it has been shown that BMI, WC, ALT, and AST in NAFLD patients were significantly higher than among non-NAFLD subjects. In this study, a significant lower amount of vitamin D was observed among NAFLD patients in compare to non-NAFLD subjects, after adjusting for potential confounders [29]. Similarly, the associations between low levels of serum vitamin D and liver dysfunction and elevated serum transaminases was reported in several studies previously [7,8,30]. Moreover, a study has previously showed that vitamin D deficient rats had a higher amount of LDH activity, which might be an indicator of cardiac damages [31]. It is suggested that chronic inflammation is an important cause of NAFLD progression, and it is shown that vitamin D has anti-inflammatory properties. Indeed, it is proposed that vitamin D may act as an “immune-modulator” to reduce inflammation and suppress fibroblast proliferation and collagen production in the liver [29,32–34]. Hence, a raised level of serum hepatic enzymes is not surprising in individuals with insufficient values of vitamin D. There are very limited data that have evaluated the effects of vitamin D supplementation on liver function enzymes; it seems that vitamin D supplementation might be useful in the treatment of patients with elevated LFTs.

In our study, both direct and total bilirubin decreased in the abnormal value group after vitamin D supplementation. The results of a previous double-center, double-blind, placebo-controlled study conducted among 36 cirrhosis patients showed that daily vitamin D supplementation for 8 weeks decreased bilirubin in the intervention group though this was not significant in comparison to the control group. Both albumin and total protein increased notably in abnormal group after intake of mega doses of vitamin D suggesting vitamin D supplementary might be beneficial for patients with reduced amounts of albumin and

total protein, which may be helpful in improvement of health status especially among critically ill patients. In a previous trial study, it has been shown that albumin and total protein were in the normal value after 6 months vitamin D supplementation (400 IU per day) in children with burn injuries [35]. More studies, particularly longitudinal randomized controlled trial studies will be needed to explore the relationship between vitamin D supplementation and blood protein status.

In this study, serum levels of ALP were not changed after supplementation in normal and abnormal value groups. Sadiya et al. indicated which serum concentrations of ALP were not changed after 3 months vitamin D supplementation in obese type 2 diabetes subjects [36]. No significant relationship was seen between serum vitamin D and ALP in a cross-sectional study in healthy adults [37]. In another study, single high dose of oral vitamin D3 had not any significant effect in serum bone-specific alkaline phosphatase [38]. Current randomized controlled trial study confirmed which vitamin D supplementation did not relate to change serum ALP in cirrhotic patients [16].

Despite the large and representative sample of adolescents, this study has a major limitation. This is an observational study, in which we followed adolescent girls who received mega doses of vitamin D for a short time. It is strongly suggested to design and implement double blind randomized controlled trial with longer follow-up time in future. Some limitations need to be considered in the interpretation of our findings. Owing to advice of our ethics committee, we were unable to include a control group in current study. We have not any non-supplemented group in this study. The main strength of the present study is large sample size for intervention. Second strength of the present study design was that it was performed in apparently healthy adolescent girl's aged 12–18 year.

5. Conclusion

Totally, we found that after 9-weeks supplementation with high doses of vitamin D LFTs include ALT, AST, LDH, YGT, direct bilirubin and total bilirubin decrease and albumin, and total protein increased in the subjects with abnormal LFTS. Our findings indicated that vitamin D supplementation may improve LFTs in the adolescents with abnormal value, which could prevent liver diseases during adulthood. More randomized controlled trial with longer follow-up time will be required in the future.

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Conflicts of Interest

The authors have no conflict of interest to disclose.

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