

Vitamin D Levels in Egyptian Patients With Non-Alcoholic Fatty Liver Disease

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ABSTRACT

BACKGROUND/AIM: An association between non-alcoholic fatty liver disease (NAFLD) and serum vitamin D levels is thought to exist. However, the definite role and mechanisms are unknown. Aim: we aimed to investigate serum vitamin D levels in patients with NAFLD.

METHODS: Fifty patients with NAFLD and another 50 healthy volunteers without NAFLD or any clinically apparent liver diseases were enrolled in this study. Diagnosis of NAFLD is based on ultrasound (U.S) findings after exclusion of other possible causes of NAFLD and chronic liver diseases. Serum 25(OH) vitamin D levels were measured using ELISA based test.

RESULTS: There are statistically significant decrease of serum 25(OH) vitamin D levels in patients with NAFLD than those without NAFLD. Serum 25(OH) vitamin D level is inversely correlated with body mass index (BMI), total cholesterol, triglycerides, low density lipoprotein (LDL) and age.

CONCLUSION: Serum 25(OH) vitamin D level is inversely associated with NAFLD.

Key words: Vitamin D, NAFLD, Fatty liver

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INTRODUCTION

NAFLD is the most common liver disease in western countries^[1], the exact mechanism of NAFLD development is unknown. However, NAFLD has the potentiality of progression to non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (HCC). It is commonly associated with insulin resistance and metabolic syndrome^[2,3]. Vitamin D has many functions in many systems of the human body, including muscles, bone, heart, gut, liver and immune system^[4]. Vitamin D may have a role in development of autoimmune diseases and inflammatory conditions through production of inflammatory cytokines^[5-7]. Some studies found a significant association between vitamin D deficiency and obesity, metabolic syndrome, type 2 diabetes and insulin resistance^[6,7]. An increasing evidence is pointing to a close link between 25(OH) vitamin D and NAFLD^[8]. So, we aimed to investigate the possible association between NAFLD and serum 25(OH) vitamin D levels.

METHODS

Our study is a cross sectional study performed to investigate the

association between NAFLD and serum 25(OH) vitamin D. for this purpose we enrolled 50 patients with NAFLD (cases), and 50 healthy volunteers without NAFLD or any chronic liver disease as controls, cross matched with patients in age and sex.

Inclusion criteria

Any patient with NAFLD above 18 years.

Exclusion criteria

(1) Children; (2) Alcohol abuse: daily consumption of more than 20 grams for males and 10 grams for females; (3) Those with hepatitis B or C infection; (4) Patients with clinical, radiological or laboratory evidence of chronic liver disease; (5) Patients with Wilson's disease or hemochromatosis; (6) Patients with renal failure; (7) Patients who are taking drugs containing or affecting vitamin D level including: pure vitamin D drugs or multivitamins containing vitamin D formulas.

All subjects (cases and controls) are admitted to Al-Azhar-Assiut university hospital. Detailed history, clinical examination and BMI calculation were done for every patient.

Laboratory evaluation

All subjects are evaluated by the following laboratory tests after a mid-night fasting: Complete blood count, renal function, liver functions (AST, ALT, serum bilirubin, INR, total protein and albumin), fasting blood sugar and lipid profile.

Assessment of NAFLD

Diagnosis of NAFLD was done depending on ultrasound imaging of the liver^[9]. Ultrasound examination was done by the same operator -who was blind for the results of laboratory data and serum 25(OH) vitamin D level- for every subject in the same day of clinical examination and laboratory assessment. NAFLD was graded on a scale from 0-3 according to Saverymuttu *et al*^[10], where is 0 refers to absence of NAFLD, 1 mild, 2 moderate and 3 severe NAFLD. Ultrasound examination was done using a B-mode convex probe from 2.5-5 MHz on a Mindray DP-2200 ultrasound machine (Shenzhen, China).

Vitamin D level

Serum is separated and stored in -25°C for few days. 25(OH) vitamin D levels were measured using The Alegria® 25-OH Vitamin D3/D2 Test Strip (ORGENTEC Diagnostika GmbH Carl-Zeiss-Straße 49-51 Mainz – Germany). It is an ELISA based test.

Ethical consideration

The study was approved by AL-Azhar Assiut- Faculty of medicine ethical committee.

Verbal consent was taken from persons who participated in the study.

Statistical analysis

Statistical analysis was done using SPSS version 22 (IBM SPSS Inc., Chicago, US) for windows 10. Baseline demographic, clinical, and laboratory characteristics were recorded as numbers and percentage for categorical data and means and standard deviation for continuous data. Student's T-test was used to compare results of continuous variables between groups and chi square test for categorical variables. Multivariable linear regression analysis was performed to identify independent risk factors of vitamin D. *P*-value considered significant if < 0.05.

RESULTS

Baseline characteristics of patients and control were given in (Table 1). Patients with NAFLD showed higher white blood cell count, BMI, fasting blood sugar (FBS), ALT, triglycerides and total cholesterol levels than controls. Grade 1 NAFLD was present in 13 patients (26%), grade 2 in 24 patients (48%), and grade 3 in 13 patients (26%). No significant differences between males and females in serum 25(OH) vitamin D levels (Table 2).

NAFLD and 25(OH) vitamin D

Serum 25(OH) vitamin D levels were significantly reduced in patients with NAFLD than those without NAFLD (18.76 vs 40.36 *p* value 0.000) (Table 1). Serum 25(OH) vitamin D levels decreased with the increase in the NAFLD grade (Table 3). However, no significant difference in serum 25(OH) vitamin D level between patients with grade 1 NAFLD and those without NAFLD, but, there are significant differences between patients with grade 2 and 3 on one hand and those with grade 1 and 0 on the other hand.

Multivariable linear regression analysis was used to assess predictors of serum 25(OH) vitamin D (Table 4). Total cholesterol levels were inversely associated with serum 25(OH) vitamin D levels and LDL levels were positively associated with serum 25(OH) vitamin D levels, otherwise no other factors affecting serum vitamin D levels.

Correlations between serum 25(OH) vitamin D levels and other parameters

Serum 25(OH) vitamin D levels were found to be correlated with age, BMI, ALT, AST, triglycerides, LDL and total cholesterol (Table 5 and 6).

Table 1 Baseline characteristics of cases and controls.

	Cases (<i>n</i> = 50) Mean ± SD	Control (<i>n</i> = 50) Mean ± SD	<i>P</i> -value
Age (years)	49.88 ± 9.126	47 ± 8.769	0.374
Males/Females	27/23	26/24	0.886
BMI (Kg/m ²)	26.18 ± 4.03	23.41 ± 2.64	0.045
HB (g/dl)	12.11 ± 1.44	11.99 ± 1.033	0.809
RBCs (10 ⁶ cells/mm ³)	4.64 ± 0.55	4.48 ± 0.64	0.453
WBCs (10 ³ /mm ³)	7.69 ± 1.92	6.13 ± 1.28	0.019
Platelets (10 ³ /mm ³)	266.08 ± 74.35	282 ± 59.48	0.533
FBS (mg/dl)	135.28 ± 93.51	93.50 ± 19.89	0.013
ALT (U/L)	54.42 ± 36.79	24.46 ± 10.43	0.008
AST (U/L)	43.97 ± 27.53	27.30 ± 9.32	0.067
Albumin (g/dl)	4.23 ± 0.34	4.02 ± 0.21	0.07
Total protein (g/dl)	6.99 ± 0.67	6.79 ± 0.69	0.409
Total bilirubin (mg/dl)	0.61 ± 0.24	0.51 ± 0.23	0.242
TG (mg/dl)	200.25 ± 140.13	74.70 ± 34.74	0.008
Cholesterol (mg/dl)	215.38 ± 50.81	150.30 ± 29.37	0
LDL (mg/dl)	131.94 ± 33.42	145.12 ± 42.37	0.296
HDL (mg/dl)	54.39 ± 17.41	54.70 ± 15.05	0.959
INR	1.03 ± 0.09	1.06 ± 0.15	0.496
Creatinine (mg/dl)	0.79 ± 0.28	0.77 ± 0.25	0.879
Vitamin D	18.76 ± 14.37	40.36 ± 22.24	0

BMI: body mass index, HB: Hemoglobin, RBCs: red blood cells, WBCs: white blood cells, FBS: fasting blood sugar, ALT: alanine transferase, AST: Aspartate transferase, TG: Triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein, INR: international normalized ratio.

DISCUSSION

In our study, patients with NAFLD showed significantly decreased serum 25(OH) vitamin D levels than those without NAFLD. Serum 25(OH) vitamin D levels were found to be progressively decreased with the increase in the ultrasound grades of NAFLD. The mechanisms by which 25(OH) vitamin D may induce NAFLD is not clear. The liver converts vitamin D to its active form, 25 (OH) vitamin D, so in liver diseases the 25 (OH) vitamin D level

Table 2 Vitamin D levels in males and females.

Vitamin D (ng/dL)		Mean ± SD	P-value
Sex	Female	20.45 ± 16.42	0.234
	Male	24.71 ± 20.27	

Table 3 Vitamin D levels according to U.S grades of NAFLD.

	Grade 0 (50)	Grade 1 (13)	Grade 2 (24)	Grade 3 (13)
Vitamin D level	40.36 ± 22.24 ^a	35.56 ± 20.83 ^a	13.39 ± 2.06 ^b	12.74 ± 5.56 ^b

a, b: there are significant differences between groups with different letters but not among groups with the same letter.

Table 4 Multivariable linear regression analysis of determinants of vitamin D level, Dependent variable: vitamin D.

Determinant	B	S. E	Beta	P-value
Age	-0.245	0.284	-0.121	0.395
BMI	-1.274	0.697	-0.281	0.075
FBS	-0.034	0.044	-0.16	0.447
TC	-0.144	0.055	-0.435	0.013
HDL	-71	0.156	-0.067	0.65
LDL	0.203	0.07	0.401	0.006
TG	0.021	0.03	0.157	0.493

BMI: body mass index, FBS: fasting blood sugar, TC: total cholesterol, HDL: high density lipoproteins, LDL: low density lipoprotein, TG: triglycerides

Table 5 correlation between Age & sex and mean vitamin D levels.

	Vitamin D (ng/dl)	
	R	P-value
Age (years)	-0.3	0.034
BMI (kg/m ²)	-0.186	0.01

BMI: body mass index.

Table 6 Correlation between laboratory data and mean Vitamin D levels.

	Vit.D(ng/dl)	
	r	P-value
HB (g/dl)	0.223	0.12
Glucose (mg/dl)	-0.232	0.109
ALT (U/L)	-0.383	0.006
AST (U/L)	-0.409	0.003
Serum albumin (g/dl)	-0.037	0.8
Total protein (g/dl)	-0.025	0.862
Total bilirubin (mg/dl)	0.056	0.7
TG (mg/dl)	-0.324	0.022
Cholesterol (mg/dl)	-0.309	0.029
LDL (mg/dl)	0.329	0.02
HDL (mg/dl)	0.176	0.22
Serum creatinine (g/dl)	-0.031	0.83

HB: hemoglobin, ALT: alanine transferase, AST: aspartate transferase, TG: triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein

is expected to decrease due to synthetic dysfunction of the liver^[11]. However, patients in our study had normal synthetic function, with no significant differences between NAFLD patients and control group in albumin, INR or bilirubin levels. So, low levels of 25 (OH) vitamin D in NAFLD group in our study is not due to synthetic deficiency of vitamin D by the liver. Also, vitamin D deficiency may induce NAFLD by impairing hepatic lipid metabolism^[12]. Patients with vitamin D deficiency found to have high rates of insulin resistance, metabolic syndrome and inflammatory mediators including IL-4, IL-6 and TNF- α ^[13,14]. It was reported that vitamin D receptors widely exist in liver tissue with negative association between vitamin D receptors expression and necro-inflammatory grades of NASH^[15]. Vitamin D may be sequestered in the adipose tissue in obese patients^[16]. Also, NAFLD patients may have a sedentary life with low sunlight exposure and nutritional imbalance.

Table 7 summary of studies results on vitamin D in NAFLD patients.

Study	NAFLD	Controls	P-value
Targher <i>et al</i> 2007	51.0 ± 22	74.5 ± 15	< 0.001
Barchetta <i>et al</i> 2011	14.8 ± 9.2	20.5 ± 9.7	< 0.001
Chung <i>et al</i> 2016	21.75 ± 7.54	22.02 ± 8.43	0.253
Zhai <i>et al</i> 2016	40.05 ± 9.83	40.98 ± 10.80	< 0.01
Yang <i>et al</i> 2017	16.63 ± 6.57	18.44 ± 7.30	0.011
Eliades <i>et al</i> 2013 meta-analysis	Reported that vitamin D deficient patients have 1.26-fold increased risk for NAFLD than vitamin D sufficient patients		
Li <i>et al</i> 2013	22.8 ± 8.4	22.1 ± 8.1	0.21
Patel <i>et al</i> 2016	27.9 ± 12.8	27.7 ± 11.9	0.878
Ha <i>et al</i> 2017	16.2 ± 6.2	16.2 ± 6.5	0.95
De Paula <i>et al</i> 2017	Steatosis	28.66 ± 8.40	0.55
	27.6 ± 12.49		
Park <i>et al</i> 2017	NASH	21.5 ± 8.1	0.151
	26.5 ± 7.38		
	21.8 ± 8.7		

Table 8 Characteristics of the study participants according to serum 25 (OH) vitamin D state.

	NVD	VDD	P value
Age	45.37 ± 9.13	51.71 ± 8.22	0.014
Sex (male/female)	25/15	28/32	0
BMI	24.73 ± 2.68	26.17 ± 4.49	0.213
ALT	28.42 ± 17.81	61.98 ± 34.96	0
AST	25.70 ± 11.24	49.79 ± 27.96	0.001
Bilirubin	0.58 ± 0.23	0.59 ± 0.24	0.813
Albumin	4.17 ± 0.26	4.19 ± 0.37	0.856
Total protein	6.90 ± 0.66	6.98 ± 0.69	0.687
FBS	103.00 ± 48.59	140.55 ± 98.92	0.139
TG	112.00 ± 82.00	213.84 ± 148.28	0.009
Total cholesterol	168.84 ± 35.30	222.90 ± 53.37	0
LDL	145.43 ± 32.29	127.92 ± 35.93	0.089
HDL	57.30 ± 16.36	52.71 ± 17.12	0.354
INR	01.04 ± 0.11	1.03 ± 0.10	0.946
Creatinine	0.82 ± 0.25	0.77 ± 0.28	0.53
HB	12.38 ± 1.21	11.90 ± 1.43	0.228
Platelets	284.74 ± 68.66	259.77 ± 72.41	0.234
WBCs	6.76 ± 1.33	7.75 ± 2.12	0.077
RBCs	4.56 ± 0.56	4.64 ± 0.58	0.64

NVD: normal vitamin D, VDD: vitamin D deficiency, BMI: body mass index, ALT: alanine transferase, AST: aspartate transferase, FBS: fasting blood sugar, TG: triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein, INR: international normalized ratio, HB: hemoglobin, WBCs: white blood cells, RBCs: red blood cells.

Our results are in agreement with Targher *et al*^[17] 2007 who found significant decrease of vitamin D levels in patients with NAFLD than healthy controls, which was associated with NAFLD histopathological features. Barchetta *et al*^[18] 2011, found strong association between hypovitaminosis D and NAFLD which was independent on age, sex, BMI, lipid profile or glucose level. Chung *et al*^[19] 2016, found that serum vitamin D levels were correlated inversely with NAFLD in a dose dependent manner independently on known NAFLD risk factors. Also, Nelson *et al*^[20] 2016, found that low levels of vitamin D are associated with high risk of NASH in patients with NAFLD. Our results are also in agreement with Zhai *et al*^[21] 2016, Wang *et al*^[22] 2016, Yang *et al*^[23] 2017 and Eliades *et al*^[8] meta-analysis 2013 who reported that vitamin D deficient patients have 1.26-fold increased risk for NAFLD than those with sufficient vitamin D.

However, our results are in contrary with Li *et al*^[24] 2013, who found no significant differences between patients with NAFLD and those without NAFLD in serum vitamin D levels. Also, Patel *et al*^[25] 2016, Ha *et al*^[26] 2017, De Paula *et al*^[27] 2017 and Park *et al*^[28] 2017 found no association between vitamin D levels and NAFLD (Table 7 summarizes these studies).

These contradictory results among studies may be related to differences in the studied population, nutritional and environmental factors.

In our study, vitamin deficient patients (serum vitamin D less than 20 ng/mL^[20]) were significantly older in age, more were females, with significantly higher aminotransferases levels than normal vitamin D subjects (serum vitamin D more than 30 ng/mL^[20]). They also have significantly higher levels of triglycerides and total cholesterol than subjects with normal levels of vitamin D (Table 8).

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