The association between serum levels of 25-hydroxyvitamin D and nonalcoholic fatty liver disease in the Egyptian population

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Background and aim
Nonalcoholic fatty liver disease (NAFLD) and vitamin D deficiency are associated with insulin resistance, type 2 diabetes, and dyslipidemia. Many studies have examined the association between vitamin D and NAFLD, but the results have been contradictory. Our aim was to investigate the association of serum vitamin D assessed by 25-hydroxyvitamin D [25 (OH) vitamin D] with NAFLD and to analyze the role of insulin resistance and dyslipidemia.

Patients and methods
A total of 60 patients with NAFLD, chosen from the gastroenterology outpatient clinic of Internal Medicine, Kasr Al-Ainy Hospital, Cairo University, were included in the study. They were classified into three groups (group 1: 20 diabetic patients; group 2: 20 dyslipidemic patients; and group 3: 20 nondiabetic nondyslipidemic patients). In addition, 20 healthy control individuals were also included. For all participants, clinical and biochemical data were obtained, liver ultrasonography for the diagnosis of fatty liver disease was performed, and serum 25 (OH) vitamin D was measured.

Results
Our data showed that NAFLD patients had lower levels of 25 (OH) vitamin D (15.7 ± 10 nmol/l) compared with controls (43.4 ± 14 nmol/l), with a statistically significant difference between the two groups. There was a statistically significant inverse correlation between 25 (OH) vitamin D and low-density lipoprotein and triglyceride among the studied groups, whereas no significant correlation was detected with other variables. The receiver operating characteristic curve showed that 25 (OH) vitamin D was better positive than negative in discriminating between NAFLD patients and controls at a cutoff of 18. The sensitivity was 99% and specificity was 77%, with an excellent area under the curve of 0.94.

Conclusion
Low 25 (OH) vitamin D levels were associated with the presence of NAFLD independently of age, sex, type 2 diabetes mellitus, and insulin resistance.

Keywords:
dyslipidemia, insulin resistance, nonalcoholic fatty liver disease, vitamin D

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a wide disease spectrum ranging from simple steatosis and nonalcoholic steatohepatitis to cirrhosis and even hepatocellular carcinoma [1–3]. NAFLD has become a global epidemic with a high prevalence exceeding even that of viral hepatitis [4].

Vitamin D-deficient individuals are more likely to develop impaired glucose tolerance, metabolic syndrome, and type 2 diabetes mellitus [5,6]; however, the mechanisms underlying the association of vitamin D with NAFLD are not fully understood [7–9].

Therefore, we aimed to assess serum 25-hydroxyvitamin D [25 (OH) vitamin D] levels in Egyptian patients with NAFLD in relation to insulin resistance and dyslipidemia.

Patients and methods

Patients' characteristics

Sixty patients, selected from the gastroenterology outpatient clinic of Internal Medicine, Kasr Al-Ainy Hospital, Cairo University, were enrolled in the current study. In addition, 20 healthy individuals were recruited as controls. Written informed consent was obtained from all participants, and the study design was in accordance with the regulations of the Ethical Committee Research of Faculty of Medicine, Cairo University.

Selection criteria

The inclusion criteria for the study were age 40–60 years and absence of a history of current or past alcohol drinking. The following patients were excluded from the study: those with hepatitis B surface antigen (HBVsAg) or anti-hepatitis C virus antibody (HCV-Ab); patients with elevated serum liver enzymes or any laboratory investigation denoting autoimmunity (antinuclear antibody, antismooth muscle antibody, liver kidney microsomal antibody); patients with metabolic or other causes of hepatitis; those with malignancy; and patients who had ingested vitamin D supplements, or drugs known to produce hepatic steatosis, such as corticosteroids, high-dose estrogens, methotrexate, valproic acid, tetracycline hydrochloride, amiodarone, isoniazide, phenytoin, carbamazepine, or tamoxifen citrate in the previous 6 months.

Study design

This cross-sectional, case–control study was carried on 60 consecutive patients with NAFLD. They were classified into three groups (group 1: 20 diabetic patients; group 2: 20 dyslipidemic patients; and group 3: 20 nondiabetic nondyslipidemic patients). In addition, 20 healthy control individuals without fatty liver were also included.

All participants underwent a complete workup, including a full history taking and thorough clinical examination, biochemical analysis, and abdominal ultrasound.

Analysis of laboratory and biochemical parameters

Venous blood samples were obtained from patients and controls after an overnight fast for the following biochemical tests: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), alkaline phosphatase, blood urea nitrogen and creatinine, serum iron, total iron binding capacity, and ferritin. Serum ceruloplasmin and copper (Cu) in urine were measured.

Fasting blood glucose at 2-h postprandial, serum triglycerides (TGs), total cholesterol, and high-density lipoprotein-cholesterol (HDL-C) were determined using a commercially available kit (New York, USA, supplied by the Eastern Co. for Eng. & Trade, Giza, Egypt). Low-density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald's equation [10].

Blood insulin (μU/ml) was also measured by means of radioimmunoassay. Diagnosis of diabetes mellitus was defined according to WHO [11]. Homeostatic model assessment-insulin resistance (HOMA-IR) was determined following the equation [12]:

HOMA-IR = Glucose × Insulin/405(Glucose in mass units)(mg/dl).

Diagnosis of nonalcoholic fatty liver disease

Participants underwent abdominal ultrasonography in accordance with a standardized protocol, taking into consideration that ultrasonography can detect increased liver echogenicity and confirm the diagnosis of NAFLD, particularly when hepatic fat infiltration surpasses 33% [13]. Real-time imaging of the liver was performed for each participant by an experienced radiologist, using a Hitachi (Voluson) ultrasound machine equipped with a convex 3.5 MHz. The radiologist performing the ultrasonography was unaware of the clinical and laboratory results. The ultrasonography diagnostic patterns of fatty liver disease were based on the presence of a 'bright' liver, with stronger echoes in the hepatic parenchyma than in the renal parenchyma, often associated with unusually fine liver echotexture and vessel blurring, in the absence of findings suggestive of other chronic liver diseases. In very rare instances, a liver biopsy was performed. Ultrasound is currently the most commonly used tool for screening asymptomatic patients with elevated liver enzymes and suspected NAFLD [13].

Serum 25-hydroxyvitamin D

Vitamin D status was evaluated by measuring serum 25 (OH) vitamin D, the most stable circulating form of this molecule [14], using a DRG assay for quantitative determination of 25 (OH) vitamin D in plasma and serum (DRG International Inc., USA).

Statistical analysis

Data were analyzed using an IBM computer with SPSS (Statistical Program for Social Science version 12). Quantitative variables were described as mean, SD, and range, and qualitative variables as number and percentage. The χ^2-test was used to compare qualitative variables between groups. The unpaired *t*-test was used to compare quantitative variables in parametric data (SD <50% mean). The Mann–Whitney test was used instead of the unpaired *t*-test for nonparametric data (SD >50% mean). One-way analysis of variance was used to compare more than two groups as regards quantitative variable (least significant difference). The Spearman correlation coefficient test was used to rank variables positively or inversely. The receiver operating characteristic (ROC) curve was used to determine the best cutoff value and the validity of variables: *P* values greater than 0.05 were considered insignificant; *P* values less than 0.05 were considered significant; and *P* values less than 0.01 were considered highly significant.

Results

Table 1 shows the demographic and laboratory data of all participants. There was no statistically significant difference between the four studied groups as regards sex, age, ALT, AST, blood urea, iron, ceruloplasmin, and copper. Ferritin levels (ng/ml) in G1, G2, and G3 were 97 ± 47, 84 ± 40, and 92.6 ± 37, respectively, with no significant difference when compared with the control group (82.4 ± 38); *P* value was greater than 0.05. Also, copper (μg/24 h) showed no significant difference in relation to the control group. Ceruloplasmin levels (mg/dl) in NAFLD patients were 29.5 ± 6.3, 32.8 ± 11, and 29.5 ± 6.3 in G1, G2, and G3, respectively, and 35.1 ± 8 in the control group, with no significant difference.

Total cholesterol and HDL were significantly higher in groups 1 and 2 compared with controls. TGs were significantly higher in the patient groups compared with controls.

Moreover, there was a statistically significant difference between the studied groups and the control group as regards creatinine and GGT.

Insulin and blood sugar (fasting blood glucose and 2-h postprandial) levels were significantly higher in group 1 than in group 2, and each of them showed a significantly elevated insulin level ($P < 0.01$) versus the control group ($P < 0.01$).

Insulin resistance (HOMA-IR) was significantly higher in group 1 than in other groups and controls ($P < 0.01$) (post-hoc Tukey's test). In addition, controls had the highest level of 25 (OH) vitamin D compared with the other groups, and groups 1 and 2 also showed significant difference compared with group 3. Figure 1 shows that fatty liver patients had lower levels of 25 (OH) vitamin D compared with the control group, with statistically significant difference between the two. The level of 25 (OH) vitamin D (nmol/l) in all NAFLD patients was 15.7 ± 10, whereas among controls it was 43.4 ± 14, with a highly significant statistical difference between the two groups, on using the Mann–Whitney test ($P < 0.01$).

No correlation was found between HOMA-IR and all studied parameters, as shown in Table 2. There was a statistically significant inverse correlation between

Table 1 Comparison between the studied groups as regards demographic and laboratory data

Variables	Group 1 (*n* = 20)	Group 2 (*n* = 20)	Group 3 (*n* = 20)	Control (*n* = 20)	*F*	*P*	LSD
Sex [*n* (%)]							
Male	14 (70)	13 (65)	11 (55)	15 (75)	1.9	>0.05*	
Female	6 (30)	7 (35)	9 (45)	5 (25)			
Age	47.8 ± 3.32	46.8 ± 5.23	46.6 ± 4.21	44.25 ± 4.25	2	>0.05*	
FBG (mg/dl)	208 ± 56	101 ± 14	89 ± 9.9	85 ± 9	14	<0.01‡	1, 2 versus c1 versus 2, 3
2HPP (mg/dl)	294 ± 37	140 ± 16.7	137 ± 13	136 ± 12	10.5	<0.001‡	1, 2 versus c1 versus 2, 3
TG (mg/dl)	111.6 ± 50	313 ± 62	84 ± 22	78 ± 25	22	<0.001‡	1, 2, 3 versus c1, 2 versus 3
Cholesterol (mg/dl)	173 ± 44	244 ± 76	152 ± 21	157 ± 17	12	<0.001‡	1, 2 versus c1, 2 versus 3
HDL (mg/dl)	33 ± 15	34 ± 14	17 ± 8	20 ± 7.8	16	<0.01‡	1, 2 versus c1, 2 versus 3
LDL (mg/dl)	124 ± 38	124.7 ± 45	122 ± 30.3	132.9 ± 32	2	>0.05	
AST (U/l)	15.4 ± 6	17.9 ± 8	19.5 ± 7	18.3 ± 8	1.1	>0.05*	
ALT (U/l)	18 ± 5	16.8 ± 6	19 ± 4.5	19.6 ± 8	1.8	>0.05*NS	
GGT (U/l)	23.4 ± 9	26.4 ± 7.7	23.2 ± 11	17.1 ± 6	5	<0.05†	1, 2, 3 versus c2 versus 1
ALP (U/l)	76 ± 14.8	86.5 ± 14	95.9 ± 19	103 ± 19	0.8	>0.05*	
Urea (mg/dl)	17 ± 7	16.9 ± 7	14.9 ± 6	17.7 ± 7	1.2	>0.05*	
Creatinine (mg/dl)	1.2 ± 0.4	1.1 ± 0.4	1.02 ± 0.3	0.79 ± 0.2	6	<0.05†	1, 2 versus c
Insulin (mIU/ml)	38.4 ± 15	25.7 ± 10	12.1 ± 5	11.4 ± 4	11	<0.001‡	1, 2 versus c1 versus 2
HOMA-IR	18.7 ± 10.8	6.2 ± 2.4	2.7 ± 1.2	2.4 ± 1.0	37.3	<0.001‡	
25 (OH) vitamin D (nmol/l)	16.5 ± 7.3	16.7 ± 7.5	14 ± 7	43.4 ± 14	27	<0.001‡	Controls versus 1, 2, 3Group 1 and 2 versus 3

All values are expressed as mean ± SD; 2HPP, 2-h postprandial; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBG, fasting blood glucose; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment-insulin resistance; LDL, low-density lipoprotein; LSD, least significant difference; TG, triglyceride; *Nonsignificant ($P > 0.05$); †Significant ($P < 0.05$); ‡Highly significant ($P < 0.01$).

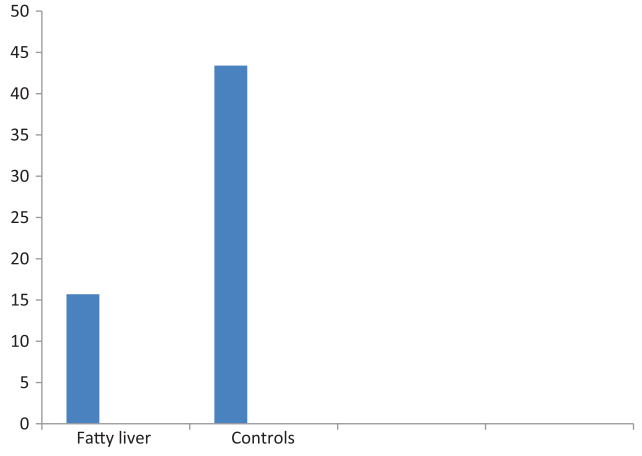
25 (OH) vitamin D and LDL and TG among the studied groups. No significant correlation versus other variables was found (Table 3).

Table 2 Correlations between insulin resistance (homeostatic model assessment-insulin resistance) and age, fasting blood glucose, 2-h postprandial, lipid profile, and vitamin D in each group

Variables	Insulin resistance (HOMA-IR)			
	Group 1	Group 2	Group 3	Control
Age				
P	0.169	0.075	0.025	−0.382
r	0.476	0.754	0.917	0.097
FBG				
r	0.007	0.309	0.342	0.133
P	0.977	0.185	0.140	0.575
2-h PPG				
r	−0.020	−0.283	0.284	−0.122
P	0.935	0.227	0.225	0.610
Triglycerides				
r	−0.041	0.358	0.052	0.377
P	0.865	0.121	0.828	0.102
Total cholesterol				
r	−0.290	−0.041	0.235	−0.159
P	0.214	0.865	0.319	0.502
HDL				
r	−0.190	0.241	−0.230	0.049
P	0.423	0.307	0.329	0.838
LDL				
r	0.008	−0.157	0.005	0.434
P	0.975	0.508	0.985	0.056
25 (OH) vitamin D				
r	0.177	0.214	−0.174	0.031
P	0.454	0.364	0.464	0.897

It shows no significant correlation between insulin resistance (HOMA-IR) and different variables among the studied groups; 2HPP, 2-h postprandial; FBG, fasting blood glucose; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment-insulin resistance; LDL, low-density lipoprotein; *P*, probability value; *r*, Spearman's correlation coefficient.

Figure 1



Comparison between nonalcoholic fatty liver disease (NAFLD) patients and the control group regarding 25-hydroxyvitamin D [25 (OH) vitamin D] level.

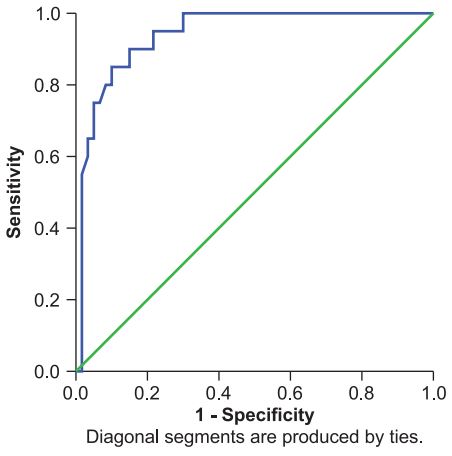
As shown in Table 4, 25 (OH) vitamin D was considered a better positive than a negative predictor of fatty liver. At a cutoff value of 18, its sensitivity was 99% and specificity was 77%. The ROC curve shows that the marker is better positive than negative, with higher sensitivity and excellent area under the curve of 0.94 (Fig. 2).

Discussion

NAFLD has a prevalence as high as 30% of the general adult population, which exceeds that of viral hepatitis and alcoholic fatty liver disease [15]. The exact cause of NAFLD has not been fully elucidated, and it is almost certainly not the same in every patient [16]. Although most closely related to insulin resistance, obesity, and the metabolic syndrome, it was not proved by some studies [9,17].

The present study showed a statistically significant decrease in serum 25 (OH) vitamin D level in all of the studied NAFLD patients compared with the control group, with lowest values detected in group 3 (nondiabetic nondyslipidemia) with NAFLD. Several studies have investigated the association between NAFLD and 25 (OH) vitamin D, and found significantly low vitamin D levels in NAFLD patients when compared with healthy controls, suggesting that vitamin D may play a role in the development of NAFLD [18–21]. Moreover, we found a statistically significant inverse correlation between the low serum 25 (OH) vitamin D and the levels of TG and LDL in diabetic NAFLD patients (group 1). This agrees with previous data [18].

Figure 2



The receiver operating characteristic (ROC) curve shows that the marker is a better positive than a negative predictor with higher sensitivity and an area under the curve of 0.94.

Table 3 Correlation between 25 (OH) vitamin D and different variables in group 1

Variables	25 (OH) vitamin D	
	r	*P*
Age	0.22	>0.05*
Insulin	0.16	>0.05*
FBS	0.09	>0.05*
2HPP	0.02	>0.05*
TG	−0.46	<0.05†
Cholesterol	0.19	>0.05*
HDL	0.02	>0.05*
LDL	−0.43	<0.05†
AST	0.01	>0.05*
ALT	0.13	>0.05*
GGT	−0.23	>0.05*
ALP	0.03	>0.05*
Urea	0.15	>0.05*
Creatinine	0.16	>0.05*
Ferritin	0.25	>0.05*

2HPP, 2-h postprandial; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; *P*, probability; *r*, rank or equation or *r*-test results; TG, triglyceride; *Nonsignificant (*P* > 0.05); †Significant (*P* < 0.05).

Table 4 Validity of 25 (OH) vitamin D deficiency in the prediction of fatty change

Variables	%
Best cutoff value of 18	
Area under the curve	0.94
Sensitivity	99
Specificity	77
Positive predictive value	82
Negative predictive value	99
Accuracy	82

There is epidemiological as well as biochemical and therapeutic evidence that supports the premise that the primary pathophysiological derangement in most patients with NAFLD is insulin resistance. Insulin resistance leads to increased lipolysis, TG synthesis, increased hepatic uptake of free fatty acids, and accumulation of hepatic TG [22]. Even in nondiabetic Whites, low vitamin D levels were independently associated with insulin resistance and metabolic syndrome [23], and were a predictor of increased 10-year risk of developing hyperglycemia and insulin resistance [24]. A vitamin D response element is present in the insulin gene promoter region, and 1a, 25 $(OH)_2$ D activates the transcription of the insulin gene. Both 1a-hydroxylase and the vitamin D receptor are expressed on pancreatic b cells, with an association between low vitamin D levels and impaired b-cell function having been suggested [25]. It was concluded that vitamin D is capable of reducing free fatty acids (FFA)-induced insulin resistance both in peripheral tissues and in hepatocytes. Therefore, low serum vitamin D may predispose to intrahepatic lipid accumulation, leading to NAFLD [8]. Furthermore, two randomized placebo-controlled trials have shown that a high dose of vitamin D supplementation improves insulin sensitivity in nondiabetic South Asians [26,27].

However, data are contradictory on the cause of the association between NAFLD and vitamin D, as some studies report that it is independent of insulin resistance, type 2 diabetes mellitus, and metabolic syndrome [4,18]. In our study, there was no correlation between serum 25 (OH) vitamin D and insulin resistance (HOMA-IR) in any of the patients with NAFLD. In addition, 25 (OH) vitamin D was significantly lower in NAFLD patients in the absence of diabetes and dyslipidemia (group 3). These findings may point to possible underlying mechanisms for NAFLD other than insulin resistance. The association between low serum vitamin D level and the presence of NAFLD in the absence of insulin resistance was also demonstrated in previous studies [4,18]. They found that low vitamin D levels were closely associated with histologic severity of steatosis, necroinflammation, and fibrosis in NAFLD, independent of age, sex, BMI, HOMA-IR score, and the presence of metabolic syndrome. These findings have also been confirmed in children with NAFLD [24].

As regards liver enzymes, there were concordant values of AST and ALT in the NAFLD group in relation to controls, whereas serum GGT was elevated in NAFLD patients: group 1, 23.4 ± 9; group 2, 26.4 ± 7.7; and group 3, 23.2 ± 11; versus control, 17.1 ± 6, with significant difference (*P*<, w5).

These results were in agreement with those of Barchetta *et al.* [18], who demonstrated a strong independent association between low 25 (OH) vitamin D levels and NAFLD in a population of adults with normal serum aminotransferases, but in another study higher ALT levels were found to be the significant determinants of NAFLD [28]. The difference could be attributed to the different grades of NAFLD in these studies.

In agreement with Barchetta *et al.* [18], our findings show that there were no correlations between vitamin D, age, sex, AST, ALT, GGT, blood glucose, or HDL in the different NAFLD groups.

A plausible explanation for our findings is that the low levels of 25 (OH) vitamin D found in NAFLD may simply reflect an inadequate diet or poor sunlight exposure, which, it could be hypothesized, may promote the development and progression of NAFLD. Previous data have shown that vitamin D plays an important role in the regulation of oxidative stress, inflammatory cytokines, hepatocyte apoptosis, and liver fibrosis [9,28].

Furthermore, across various adult populations, lower serum 25 (OH) vitamin D levels are associated with higher circulating markers of inflammation [29], and vitamin D_3 supplementation can effectively reduce these inflammatory markers, including C-reactive protein, tumor necrosis factor-α, and interleukin-6 [29,30], and increase some anti-inflammatory cytokines, such as interleukin-10 [30]. Hence, low serum 25 (OH) vitamin D levels may promote these inflammatory processes that are also involved in the development and progression of NAFLD.

The role of vitamin D in hepatic stellate cell proliferation appears to be an inhibitory one. Abramovitch *et al.* [31] demonstrated that inhibition of hepatic stellate cell proliferation by vitamin D was associated with antifibrotic effects in an in-vivo murine model. Further study *in vitro* has suggested a benefit of vitamin D supplementation in suppressing the activity of hepatic stellate cells even in the presence of free fatty acids [32].

In the current study, the ROC curve showed that at a cutoff of 18 vitamin D was better positive than negative, with higher sensitivity (99%) and an excellent area under the curve of 0.94, in discriminating NAFLD patients from controls. This in concordance with the results of many previous studies [5,9,18,20].

Conclusion

This study found a strong association between vitamin D deficiency and NAFLD diagnosed by ultrasound examination. This association was independent of age, sex, type 2 diabetes mellitus, insulin resistance, and liver functions. An inverse correlation between vitamin D and serum lipids (LDL and TG) was evident only in the NAFLD diabetic group. Furthermore, the contribution of vitamin D deficiency to the pathogenesis of NAFLD and the role of vitamin D supplementation for treatment or even prevention of NAFLD need further studies. Our study has some limitations. First, it was conducted using a cross-sectional design and thus did not identify a causal relationship between clinical markers and hepatic steatosis; second, the total number of enrolled participants in both sexes was relatively small.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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