The relationship between 25-hydroxyvitamin D concentration and liver enzymes in healthy adults: A Cross-sectional Study

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Abstract

Background Studies have shown that serum 25-hydroxyvitamin D (25(OH)D), a product of exogenous vitamin D, plays an influential role in calcium and phosphorus balance, anti-inflammation, and cardiovascular protection. There were long-standing interests in the potential benefits of vitamin D for preventing liver diseases. Investigations showed that 25(OH)D was increased in adolescents with abnormal liver function. Yet data about the relationship of 25(OH)D concentration and liver enzymes from prospective cross-sectional study was limited. The present study is undertaken to examine whether serum 25-hydroxyvitamin D and glutamyl transferase (GGT), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were closely related. Methods The cross-sectional study of 368 healthy volunteers with no history of liver diseases was used to examine risk factors. Pearson correlation (or Spearman correlation for skewed variables) was performed to assess associations between GGT, ALT, and ALP and 25(OH)D. Linear regression analysis was conducted with GGT, ALT, and ALP as dependent variables in separate models. Results The results found no significant difference between ALT and ALP in different vitamin D groups (25(OH)D < 25 nmol/L, 25-50 nmol/L and > 50 nmol/L) (Kruskal-Wallis test, all $p > 0.05$). In multiple linear regression analysis, GGT, ALT and ALP levels remained unrelated to 25(OH)D concentration after adjusting covariates including age, BMI, sex, BuN, Cr, UA, AST, Gb, ALB, WBC, cholesterol, HDL, LDL, cholesterol, and total protein. (All $p > 0.1$). Conclusion This study suggested that there was no association between the 25(OH)D concentration and the levels of GGT, ALT or ALP in normal population.

Background

Vitamin D originates from skin synthesis as a result of exposure to sunlight in vivo, supplementary and dietary vitamin D intake in vivo[1]. It is a group of liposoluble steroid derivatives, responsible for maintaining the strength of bones. In addition, vitamin D is able to reduce the risk of heart diseases, diabetes, breast cancer[2]. As we all know, suboptimal 25-hydroxyvitamin D3 is produced by exogenous vitamin D through liver hydroxylation, which can be used as a biomarker of vitamin D levels owing to its long-term stable levels[3]. Farnik and colleagues have shown that vitamin D not only maintains the balance of calcium and phosphorus, but also regulates immune system, anti-inflammation, anti-fibrosis, and cardiovascular protection function[4]. Recently, scientists confirmed that serum 25(OH)D levels were beneficial to clinical prevention of chronic hepatitis B[4]. Low serum 25(OH)D levels may lead to chronic hepatitis B infection. With deep exploration about new activity of vitamin D, it draws more attention from scientists[5].

The correlation between vitamin D deficiency and non-alcoholic fatty liver disease (NAFLD) was reported by Jeon et al. The results demonstrated that liver enzymes can be used as biomarkers for NAFLD of metabolic syndrome[6]. Eliades et al. found decreased serum 25(OH)D concentration in NAFLD patients by meta-analysis of 17 studies, suggesting that vitamin D may play a role in the development of NAFLD[6]. One study showed that vitamin D could exert positive effects on NAFLD and NAFLD-related parameters, if well administered for medium–long periods[7]. However, current evidence on the

association of 25(OH)D concentration and liver enzymes was insufficient and inconsistent. Naderpoor et al. observed that vitamin D supplementation affected liver enzymes in 54 persons for 16 weeks. Consequently, 25(OH)D concentration was unlinked to liver enzymes in adults with no history of liver diseases, and vitamin D supplementation didn't impact the liver enzymes serum levels in vitamin D-deficient and overweight people[8]. However, Bahreynian et al. found that higher rates of vitamin D deficiency were observed among individuals with high levels of liver enzymes. Recent study has showed that NAFLD patients have decreased serum 25(OH)D concentration, and vitamin D may play a role in the development of NAFLD[9]. In order to verify this association between 25(OH)D and liver enzymes, exhaustive investigations were undertaken to examine their relationship in healthy people.

Materials And Methods

Study design and participants

The study population consisted of 368 healthy volunteers (males and females) with no history of liver diseases who came from the province of Anhui, China. Inclusion criteria were as follows: aged 26–86 years, stable weight for at least 12 months (no weight change > 5 kg) and no intention to lose weight or change diet or physical activity for the trial duration, and no known comorbidities. Participants were required to no-vitamin D intake for at least three months prior to taking part in the study. This study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from all participants. Ethical approval for the present study was obtained from the ethical committee of Anhui Medical University (Protocol ID: 20170248).

Anthropometric measurements

All participants (n = 368) underwent physical examinations, including measurements of body weight, height and blood pressure. Body mass index (BMI) was calculated by weight (kg)/height square (m²). Physical health was assessed by a full-body dual-energy X-ray absorptiometer (DEXA) (Madison Lunar Radiation Company, Wisconsin, USA).

Metabolic Measurements

After resting for at least five minutes, average systolic and diastolic blood pressures were measured from three measurements using an automatic sphygmomanometer(M6 Automatic BP monitor, Omron, Japan).

Fasting venous blood samples were analyzed for 25 (OH) D concentration (direct competitive chemiluminescent immunoassays, DiaSorin Inc., MN, USA), inter- and intra-analysis coefficient of variation (< 10% and < 4%). The test included glucose (glucose oxidase assay, YSI 2300 Stat, YSI Inc., OH, USA), insulin, kidney and liver tests, lipid assay (all using commercial enzymatic immunoassays, Beckman Coulter, Australia) and whole blood cell count (Beckman Coulter LH750).

Insulin sensitivity was measured with hyperinsulinemia-normal glucose clamp as our protocol, including initial intravenous insulin pills (9 mU/kg), followed by continuous infusion of insulin at a rate of 40 mU/m²/min for at least 120 minutes. At the same time, adjust the glucose infusion to maintain blood sugar at about 5 millimole/liter. According to the last 30 minutes of clamp and the stable glucose infusion rate, the total insulin stimulated glucose treatment rate (M value) was calculated.

Statistical analysis

SPSS version 17 statistical package was used to perform the analyses. The baseline characteristics of the participants were presented as the mean $\pm$ standard deviation (SD) of the normal distribution variable and the median [interquartile range (IQR)] of the non-normal distribution variable. For all the above, a two-tailed p-value < 0.05 was considered statistically significant.

Results

The study population (n = 368) included males and females aged 26–86 years. There were no significant differences in age, fasting FBG, AST, Ghb, cholesterol, HDL, LDL, triglycerides, total protein, ALB, WBC, ALP and 25(OH)D (all p > 0.05). Male participants had a lower BMI (p = 0.01), higher diastolic blood pressure (p = 0.03), higher urea nitrogen (BuN) (p < 0.001), Cr (p = 0.02), uric acid (UA) (p = 0.01), GGT (p = 0.01) and ALT (p = 0.03). Baseline characteristics of participants are summarized in Table 1.

Table 1
Baseline characteristic of participants in the cross-sectional study.

Variable	All participants	Males	Female	p-value
^a Age (year)	49.00 [12.00]	48.00 [11.75]	49.00 [12.00]	0.76
BMI (kg/m ²)	24.61±3.16	24.61±3.16	25.27±3.38	0.01
^a SBP (mmHg)	119.0 [14.0]	123.0 [16.0]	118.0 [13.0]	0.12
^a DBP (mmHg)	75.00 [11.00]	79.00 [12.00]	73.00 [10.00]	0.03
^a GLU (mmol/L)	5.28 [0.84]	5.36 [0.88]	5.15 [0.66]	0.59
^a BUN (mmol/L)	5.37 [1.75]	5.59 [1.50]	4.85 [1.60]	< 0.001
^a Creatinine (umol/L)	70.00 [21.00]	76.00 [13.00]	53.00 [16.00]	0.02
UA (umol/L)	332.4±86.9	366.5±77.8	265.2±61.0	0.01
^a AST (U/L)	21.00 [9.00]	21.00 [9.00]	19.50 [7.75]	0.06
^a HbA1C (%)	5.50 [0.55]	5.50 [0.55]	5.50 [0.50]	0.29
HDL (mmol/L)	1.27 [0.45]	1.21 [0.35]	1.32 [0.52]	0.06
^a LDL (mmol/L)	2.93 [1.05]	3.09 [1.28]	2.84 [1.06]	0.15
^a Triglyceride (mmol/L)	1.38 [1.10]	1.93 [1.36]	1.11 [1.03]	0.06
TP (g/L)	74.70±3.80	74.49±3.69	75.12±3.99	0.71
Alb (g/L)	47.86±2.67	44.52±3.94	49.18±2.15	0.55
^a WBC (10 ⁹ /L)	5.92 [1.90]	5.99 [1.26]	5.63 [1.47]	0.39
^a GGT (U/L)	24.00 [27.75]	25.50 [16.75]	15.5 [12.75]	0.01
^a ALT (U/L)	21.00 [18.75]	25.00 [20.75]	15.00 [12.75]	0.03
^a ALP (U/L)	74.00 [25.00]	75.00 [24.00]	67.00 [24.00]	0.06
^a 25(OH)D (nmol/L)	22.85 [18.25]	22.65 [19.35]	23.95 [15.65]	0.83
ALT: alanine aminotransferase; ALP: alkaline phosphatase; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; GGT: γ-glutamyl transferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; 25(OH)D: 25-hydroxyvitamin D.				
^a Data are presented as median [IQR] due to non-normal distribution				

Table 2 presented the bivariate correlations with GGT, ALT, and ALP. There was no correlation between 25(OH)D and any liver enzyme levels (all $p > 0.05$). GGT was positively related to age and BMI ($p = 0.039$ and 0.041 , respectively). ALP was positively related to diastolic blood pressure ($p < 0.05$) and there was also a positive relationship between GGT and diastolic blood pressure ($p = 0.045$). The three liver enzymes were positively related to each other and to FBG (all $p < 0.05$). However, there were no correlations between any liver enzyme levels and BuN, the same with UA (all $p > 0.05$). There was no relationship between the three enzymes and the lipid profile (all $p > 0.05$).

Table 2

Correlations of 25(OH)D, anthropometric, metabolic and inflammatory markers with GGT, ALT and ALP.

	GGT r (p-value)	ALT r (p-value)	ALP r (p-value)
25(OH)D (nmol/L)	0.036 (0.489)	-0.038 (0.466)	0.026 (0.623)
Age (year)	0.042 (0.039)	-0.156 (0.218)	0.047 (0.122)
BMI (kg/m ²)	0.128 (0.041)	-0.057 (0.233)	0.031 (0.059)
SBP (mmHg)	0.063 (0.051)	0.103 (0.154)	0.164 (0.201)
DBP (mmHg)	0.131 (0.043)	-0.097 (0.058)	0.212 (0.049)
GLU (mmol/L)	0.122 (0.031)	0.631 (0.007)	0.245 (0.010)
BUN (mmol/L)	0.283 (0.242)	0.156 (0.214)	0.334 (0.257)
Creatinine (umol/L)	0.327 (0.839)	0.324 (0.127)	0.442 (0.041)
UA (umol/L)	0.083 (0.443)	0.051 (0.147)	0.304 (0.759)
AST (U/L)	0.029 (0.334)	0.024 (0.165)	0.543 (0.148)
HbA1C (%)	0.423 (0.047)	0.068 (0.205)	0.121 (0.032)
HDL (mmol/L)	0.214 (0.326)	0.540 (0.005)	0.129 (0.504)
LDL (mmol/L)	0.229 (0.235)	0.115 (0.608)	-0.045 (0.123)
Triglyceride (mmol/L)	0.255 (0.011)	0.129 (0.094)	0.216 (0.026)
TP (g/L)	0.034 (0.059)	0.128 (0.031)	0.112 (0.066)
Alb (g/L)	0.133 (0.061)	0.072 (0.147)	0.056 (0.043)
WBC (10 ⁹ /L)	0.058 (0.129)	0.320 (0.071)	0.132 (0.095)
GGT (U/L)		0.322 (< 0.001)	0.320 (< 0.001)
ALT (U/L)	0.322 (< 0.001)		0.166 (0.001)
ALP (U/L)	0.320 (< 0.001)	0.166 (0.001)	
ALT: alanine aminotransferase; ALP: alkaline phosphatase; BMI: body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; GGT: γ -glutamyl transferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; 25(OH)D: 25-hydroxyvitamin D.			

Lipid profile and AST were not associated with any liver enzymes (all $p > 0.05$). GGT and ALP were positively associated with Ghb ($p = 0.047$ and 0.032 respectively). ALT was associated with HDL ($p = 0.047$). GGT and ALP were positively related to triglycerides ($p < 0.05$). However, three liver enzymes had no correlations with the levels of ALB and WBC (all $p > 0.05$).

In multiple linear regression analysis, GGT, ALT or ALP levels remained unrelated to 25(OH)D concentration after adjustments for covariates including age, BMI, sex, BuN, Cr, UA, AST, Ghb, cholesterol, HDL, LDL, and total protein, ALB and WBC (Table 3a–3c) (all $p > 0.1$). In these models, GGT was associated with sex ($p < 0.01$) (Table 3a). ALT was related to sex and BuN (all $p < 0.05$) (Table 3b). ALP was associated with AST and triglycerides (all $p < 0.05$). When categorized based on 25(OH)D concentration, 203 participants had 25(OH)D < 25 nmol/L, 159 participants had 25(OH)D of 25–50 nmol/L, and 6 participants had 25(OH)D > 50 nmol/L. There were no significant differences in GGT, ALT or ALP according to vitamin D categories (Kruskal-Wallis test, all $p > 0.05$).

Table 3
a. Multiple linear regression analyses for GGT.

Dependent variable	Independent variable	Model 1		Model 2	
		β	p-value	β	p-value
GGT	25(OH)D	0.23	0.12	0.07	0.45
	Age	-0.02	0.57	0.31	0.15
	BMI	0.03	0.45	0.18	0.06
	Sex	0.39	< 0.01	0.27	0.44
	SBP				
	DBP				
	GLU			0.07	0.06
	BUN	0.16	0.25	0.29	0.37
	Creatinine	-0.07	0.44	0.31	0.45
	UA	0.31	0.15	0.18	0.19
	AST	0.19	0.07	0.27	0.56
	HbA1C	0.04	0.05	0.25	0.20
	HDL	0.28	0.27	0.13	0.55
	LDL	0.37	0.42	0.24	0.05
	Triglyceride	0.15	0.29		
	TP	0.07	0.53	0.22	0.38
	Alb	0.24	0.10	0.21	0.41
	WBC	0.11	0.61	0.39	0.57
	R ² (p-value)	0.46	< 0.01	0.45	< 0.01
Model 1: adjusted for age, sex, BMI, SBP, DBP, BUN, Creatinine, UA, AST, HbA1C, HDL, LDL, Triglyceride, TP, Alb, WBC.					
Model 2 adjusted for age, sex, BMI, SBP, DBP, GLU, BUN, Creatinine, UA, AST, HbA1C, HDL, LDL, TP, Alb, WBC.					

Table 3
b. Multiple linear regression analyses for ALT.

Dependent variable	Independent variable	Model 1		Model 2	
		β	p-value	β	p-value
ALT	25(OH)D	0.14	0.26	0.08	0.34
	Age	0.05	0.39	0.22	0.17
	BMI	0.16	0.41	0.35	0.19
	Sex	0.34	0.02	-0.07	0.31
	SBP	0.07	0.23	0.14	0.07
	DBP	-0.14	0.12	-0.12	0.26
	GLU			0.18	0.05
	BUN	0.17	0.02	0.13	0.30
	Creatinine	0.29	0.38	0.26	0.14
	UA	0.28	0.43	-0.02	0.29
	AST	0.17	0.06	0.03	0.57
	HbA1C	0.09	0.45	0.38	0.11
	HDL	0.31	0.15		
	LDL	0.18	0.06	0.27	0.18
	Triglyceride	0.27	0.34	0.24	0.03
	TP	-0.02	0.13	0.39	0.21
	Alb	0.42	0.15	0.46	0.35
	WBC	0.33	0.27	0.48	0.23
	R ² (p-value)	0.31	< 0.01	0.16	< 0.01
Model 1: adjusted for age, sex, BMI, SBP, DBP, BUN, Creatinine, UA, AST, HbA1C, HDL, LDL, Triglyceride, TP, Alb, WBC.					
Model 2 Model 1: adjusted for age, sex, BMI, SBP, DBP, GLU, BUN, Creatinine, UA, AST, HbA1C, LDL, Triglyceride, TP, Alb, WBC.					

Table 3
b. Multiple linear regression analyses for ALT.

Dependent variable	Independent variable	Model 1		Model 2	
		β	p-value	β	p-value
ALT	25(OH)D	0.14	0.26	0.08	0.34
	Age	0.05	0.39	0.22	0.17
	BMI	0.16	0.41	0.35	0.19
	Sex	0.34	0.02	-0.07	0.31
	SBP	0.07	0.23	0.14	0.07
	DBP	-0.14	0.12	-0.12	0.26
	GLU			0.18	0.05
	BUN	0.17	0.02	0.13	0.30
	Creatinine	0.29	0.38	0.26	0.14
	UA	0.28	0.43	-0.02	0.29
	AST	0.17	0.06	0.03	0.57
	HbA1C	0.09	0.45	0.38	0.11
	HDL	0.31	0.15		
	LDL	0.18	0.06	0.27	0.18
	Triglyceride	0.27	0.34	0.24	0.03
	TP	-0.02	0.13	0.39	0.21
	Alb	0.42	0.15	0.46	0.35
	WBC	0.33	0.27	0.48	0.23
	R ² (p-value)	0.31	< 0.01	0.16	< 0.01
Model 1: adjusted for age, sex, BMI, SBP, DBP, BUN, Creatinine, UA, AST, HbA1C, HDL, LDL, Triglyceride, TP, Alb, WBC.					
Model 2 Model 1: adjusted for age, sex, BMI, SBP, DBP, GLU, BUN, Creatinine, UA, AST, HbA1C, LDL, Triglyceride, TP, Alb, WBC.					

Discussion

The cross-sectional study was conducted to examine the association between 25(OH)D concentration and liver enzymes, showing no association with healthy adults, before and after adjustment for potential

confounding factors, including age, sex, markers of adiposity, insulin sensitivity, lipids and inflammation. Liver enzymes were related to the metabolic syndrome (MS) factors including FBG and triglycerides. Additionally, GGT, ALT and ALP were positively interacted to each other. Similarly, Ballestri et al. demonstrated that liver histology changes could be predicted significantly using ALT, AST, homeostasis model of assessment-insulin resistance (HOMA-IR), serum uric acid (SUA), MS, total cholesterol (TCH) and serum iron[10].

Vitamin D can be acquired from the diet, but it is mainly produced by endogenous sun exposure[11]. Transported to the liver, vitamin D is hydroxylated to 25(OH)D by monooxygenase system in microsome. It is generally accepted that serum 25(OH)D concentration could be used to assess status of vitamin D and musculoskeletal growth. What's more, nutritional guidelines of vitamin D should be based on effects of vitamin D[12]. It is well known that vitamin D is beneficial to calcium and bone metabolism. Studies have confirmed that vitamin D has a series of physiological functions and its deficiency can lead to many diseases. Recent studies have shown that it regulates adaptive immune responses to various inflammatory and autoimmune diseases. Both vitamin D hydroxylase and vitamin D receptor (VDR) were expressed in immune cells, which lay a foundation for vitamin D to play its role in inflammatory diseases. 25(OH)D acts as a ligand for the VDR and participates in diverse physiological processes[13]. Furthermore, increased ALT, GGT and ALP, which were closely associated with liver fat deposition, were considered as indices of liver injury[14–17]. As mentioned earlier, ALT can serve as an index of hepatocyte death and an indirect maker of liver injury[15, 18]. GGT involving in the extracellular catabolism of the antioxidant glutathione was regarded as a marker of oxidative stress and sub-clinical inflammation[16]. ALP was responsible for hydrolysis of phosphate esters. An up to triple improvement of ALP was common to see in hepatocellular diseases, accompanied with elevated aminotransferases[19]. At the same time, ALP was significantly increased in cholestatic hepatobiliary diseases as well, for instance, primary biliary intrahepatic cholestasis[13]. However, the real fact was that AST was a less specific index of liver injury on account of wide secretion from other organs and cells, such as the heart, skeletal muscle, kidney, pancreas, lung, leukocyte, and erythrocyte[18–20].

Similar to our findings, three articles were presented to support our conclusion. The first one from the third National Health and Nutrition Examination Survey (NHANES III) showed no correlation between 25(OH)D concentration and serum ALT levels. The result of this study was consistent with previous irrelevance outcome between ALT and 25(OH)D concentration[15]. However, the study showed that the lower quartile of 25(OH)D had a higher prevalence of unexplained ALT, individuals with elevated ALT levels (n = 308) had a lower concentration of 25(OH)D, compared to BMI-matched controls (n = 976). Notably, individuals with different comorbidities such as diabetes and hypertension were included, and drug use was not adjusted, which may have affected their results. The second study consisted of 654 individuals with risk factors for diabetes(aged ≥ 30 years) reported an inverse association of 25(OH)D concentration and ALT, after adjusting for BMI, waist circumference, and lipids[16]. In contrast, the third study using NHANES III data for 12,155 participants with normal ALT levels, demonstrated a positive association between 25(OH)D concentration and ALT levels, after adjusting for confounding factors, such as gender, race, BMI, diabetes, high blood pressure, smoking and drinking history. One thing to be noted

was that all individuals with serum ALT levels > 39 U/L were excluded in their study. However, our study did not require ALT levels and everyone included. Therefore, their findings were not comparable to other conclusions, as higher ALT levels may be related to liver damage and severe comorbidities.

There were some limitations in our study. Firstly, due to the cross-sectional design of our study, we can't conclude cause and effect relationships and evaluate the impact of vitamin D status over time. Secondly, vitamin D concentration is measured for one time. However, a previous study declared that a single measurement of serum 25(OH)D had reasonable validity over a 5-year period[21]. Multiple measurements of 25(OH)D would estimate the vitamin D status better and reduce the extent of non-differential measurement errors. Thirdly, the status of vitamin D may be confusing because vitamin D concentrations may generally be influenced by a different lifestyles[22, 23]. In our model, we have adjusted other determinants. However, possible residual confusion may not be ruled out. Finally, adverse effects of vitamin D use were not discussed in this article. More deep research for liver injury should be carried out to re-evaluate and determine relationships between 25-hydroxyvitamin D concentration and liver enzymes, using larger sample sizes, longer durations and more sensitive screening tools.

Conclusion

In conclusion, evidence from some but not all cross-sectional studies suggested a relationship between 25(OH)D concentration and liver enzymes, which were within the normal ranges. The reason why our results were not related to the 25(OH)D concentration and ALT levels were that our sample size was large and the participants were older (our sample median age = 49). In our study performed in Chinese population within 368 healthy drug-naïve individuals, the results showed there was no association between 25(OH)D concentration and GGT, ALT or ALP levels.

Abbreviations

ALP

alkaline phosphatase; ALT:alanine aminotransferase; BMI:Body mass index;

FBG

fasting blood-glucose; Ghb:glycated hemoglobin; GGT:glutamyl transferase;

NAFLD

non-alcoholic fatty liver disease; NHANES III:the third National Health and Nutrition Examination Survey;

WBC:white blood cell

Declarations

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Authors' contributions

TX and FP designed the study. JW, LL and HH participated in the collecting and analyzing of the data. QY and PL finished the manuscript. YL revised and edited the manuscript. SC reviewed the manuscript. All authors approved the final version of the manuscript for publication.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethical approval was obtained from the Ethical Committee of Anhui Medical University (Protocol ID: 20170248). All patients and healthy persons included in this study provided written informed consent.

The clinical trial information sheath was given to all patients that include the objectives, methodology and purpose of the study using a simple layman terms.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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