

Correlation between serum 25-OH vitamin D expression and non-alcoholic fatty liver disease

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Abstract. This study investigated changes in the level of serum 25-OH vitamin D [25-hydroxyvitamin D, 25(OH)D] in patients with non-alcoholic fatty liver disease (NAFLD) and the correlation between the severity of NAFLD and 25(OH)D. A retrospective analysis was performed on 385 NAFLD patients (NAFLD group) admitted to the Zhongshan Hospital Affiliated to Xiamen University from January 2015 to December 2017 and 347 healthy people with physical examination (control group). The height and weight of all subjects were measured, and BMI was calculated. Fasting venous blood was extracted for the determination of blood glucose, blood lipid and 25(OH)D. The indicator levels of patients in the two groups were compared and analyzed. Spearman's correlation analysis was used to investigate the correlation between the severity of NAFLD and the level of 25(OH)D. The levels of BMI, FPG, FPI, HbA1c, TG, TC and LDL-C of patients in the NAFLD group were significantly higher than those in control group ($P < 0.05$). The level of 25(OH)D in the NAFLD group was lower than that in control group ($P < 0.05$). There was a significant negative correlation between 25(OH)D and the severity of patients in the NAFLD group ($r = -0.868$, $P < 0.001$). BMI, FPG, FPI, HbA1c, TG, TC and LDL-C were independent risk factors for the low level of 25(OH)D ($P < 0.05$). Lowly expressed in the serum of NAFLD patients, 25(OH)D has a significant negative correlation with the severity of NAFLD, which is of guiding significance for the prevention and treatment. 25(OH)D is a novel biomarker for NAFLD diagnosis and a potential drug target.

Introduction

Non-alcoholic fatty liver disease (NAFLD), a kind of metabolic stress-induced liver function damage, is a syndrome of the excessive deposition of fat in hepatocytes, apart from that caused by alcohol (1). With changes in diet and living habit, it has become the most common liver disease around the world, the global prevalence rate of which is as high as 26%, seriously affecting people's health (2). Most NAFLD patients are not aware of clinical signs, and only a small number of patients have persistent or intermittent self-induced fatigue, dyspepsia and other symptoms. Due to the non-specific symptom its diagnosis is difficult. Therefore, it is often found through routine physical examination (3).

The main cause of NAFLD is closely related to insulin resistance and genetic susceptibility. Patients with metabolic syndrome, diabetes, obesity and dyslipidemia are the high-risk populations (4). 25-Hydroxyvitamin D [25(OH)D] is the main form of human vitamin D. Vitamin D, a sterol derivative that is synthesized by ultraviolet radiation from the body, can also be supplemented by food (5). Being a fat-soluble vitamin, it is closely related to the maintenance of the health, and growth and development of the body (6). 25(OH)D is the conversion of vitamin D from the hydroxylation of liver, closely associated with vitamin D deficiency. The level of serum 25(OH)D in the body can reflect whether the body lacks vitamins (7). The study of Della Corte *et al* (8) reported that the lack of 25(OH)D may be an important cause of insulin resistance in NAFLD patients. Its action mechanism on blood glucose can affect the function of islet cells by directly or indirectly acting on islet β cells (9). Therefore, in order to prevent and treat NAFLD, it is crucial to timely ingest active vitamin D. In this study, the expression levels of blood glucose, blood lipid and serum 25(OH)D in NAFLD and the correlation between the severity of NAFLD and 25(OH)D were investigated.

Patients and methods

Patient data. A retrospective analysis was performed on 385 NAFLD patients admitted to the Zhongshan Hospital Affiliated to Xiamen University (Xiamen, China) from January 2015 to December 2017 and 347 healthy people with physical examination. There were 385 NAFLD patients in the NAFLD group, including 237 males and 148 females, aged

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Table I. Basic data of patients in the NAFLD group and the control group [n(%)].

Variables	NAFLD group (n=385)	Control group (n=347)	χ^2 value	P-value
Sex			0.188	0.665
Male	237 (61.56)	219 (63.11)		
Female	148 (38.44)	128 (36.89)		
Age (years)			0.001	0.972
<30	197 (51.17)	178 (51.30)		
≥30	188 (48.83)	169 (48.70)		
Smoking			0.038	0.846
Yes	218 (56.62)	194 (55.91)		
No	167 (43.38)	153 (44.09)		
Food habit			0.489	0.484
Low fiber	274 (71.17)	255 (73.49)		
High fiber	111 (28.83)	92 (26.51)		
Pathological diagnostic classification			-	-
Simple fatty liver disease	163 (42.34)	-		
NASH	127 (32.99)	-		
NASH-related cirrhosis	95 (24.68)	-		
Degree of CT diagnosis			-	-
Mild	191 (49.61)	-		
Moderate	128 (33.25)	-		
Severe	66 (17.14)	-		

NAFLD, non-alcoholic fatty liver disease.

from 22 to 76 years, with an average age of 43.57±5.75 years. In total 347 healthy people with physical examination were in the control group, including 219 males and 128 females, aged 19 to 71 years, with an average age of 41.64±6.16 years. Inclusion criteria: Pathological section diagnosis met the criteria for fatty liver disease, and the pathological change of NAFLD was evaluated according to the NASH Clinical Research Network Pathology Society (NASH-CRN) assessment protocol (10); having complete records; no history of drinking or alcohol content in male <140 g per week, in female <70 g; no relevant treatment in other hospitals. Exclusion criteria: Patients with viral hepatitis, alcoholic liver disease, drug-induced hepatitis and hepatocellular degeneration; patients during pregnancy and lactation; patients suffering from total parenteral nutrition and autoimmunity that can cause fatty liver disease; patients with other severe diseases or tumors; patients with communication impairment or cognitive dysfunction.

This study was approved by the Ethics Committee of Zhongshan Hospital Affiliated to Xiamen University. Patients who participated in this research had complete clinical data. All subjects and their family members signed an informed consent form and cooperated with medical staff to complete relevant medical treatments.

Methods. The height and weight of patients in the two groups were measured, and the body mass index (BMI) was calculated. Fasting venous blood was extracted to determine blood lipid, blood glucose and 25(OH)D. The indicator levels were compared and analyzed. Patients fasted for 12 h. The venous

blood was extracted, and was placed at room temperature for 30 min. Serum was centrifuged at 1,500 x g at 4°C for 10 min (Shanghai Pudong Tianben Centrifugal Machinery Co., Ltd.). The detection of fasting blood glucose (FPG), fasting proinsulin (FPI), triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) was performed using an automatic biochemical analyzer (American Beckman Coulter Co., Ltd.) detection of glycosylated hemoglobin (HbA1c) using glycosylated hemoglobin analyzer (Jiangsu Odikang Medical Technology). Enzyme linked immunosorbent assay (ELISA) was performed to detect 25(OH)D in strict accordance to the human 25 hydroxyvitamin D [25(OH)D] ELISA assay kit (Shanghai Guandao Biological Engineering Co., Ltd.; GD-E003266861).

Degree criteria for CT diagnosis. Mild, CT liver/spleen ratio of patients ≤1.0 but >0.7; moderate, CT liver/spleen ratio of patients ≤0.7 but >0.5; severe, CT liver/spleen ratio of patients ≤0.5 (11).

Statistical analysis. SPSS 17.4 (Beijing Boyizhixun Information Technology Co., Ltd.) software system was used for statistical analysis, and the basic enumeration data of patients were expressed as percentage (%), and tested using χ^2 . The expressions of BMI, FPG, FPI, HbA1c, TG, TC, LDL-C and 25(OH)D levels were expressed as standard deviation of mean ± SD. The t-test was used for difference between two groups, one-way ANOVA followed by LSD for comparison of multiple groups, Spearman's correlation analysis for correlation

Table II. Expression of BMI, FPG, FPI and HbA1c levels.

Variables	Control group (n=347)	Mild NAFLD group (n=191)	Moderate NAFLD group (n=128)	Severe NAFLD group (n=66)	F-value	P-value
BMI (kg/m ²)	21.2±0.9	22.4±3.6 ^a	23.5±3.9 ^{a,b}	24.8±3.5 ^{a,c}	45.070	<0.001
FPG (mmol/l)	5.16±0.28	6.28±1.48 ^a	7.44±1.75 ^{a,b}	8.62±1.83 ^{a,c}	221.600	<0.001
FPI (pmol/l)	6.58±1.31	7.03±1.42 ^a	8.48±1.74 ^{a,b}	9.34±1.81 ^{a,c}	99.630	<0.001
HbA1c (%)	5.21±0.47	5.27±0.51	7.45±0.88 ^{a,b}	8.64±0.85 ^{a,c}	933.900	0.026

^aP<0.001, compared with control group; ^bP<0.05, compared with mild NAFLD group; ^cP<0.05, compared with moderate NAFLD group. BMI, body mass index; FPG, fasting blood glucose; FPI, fasting proinsulin; HbA1c, glycosylated hemoglobin; NAFLD, non-alcoholic fatty liver disease.

Table III. Expression of TG, TC, LDL-C and 25(OH)D levels.

Variables	Control group (n=347)	Mild NAFLD group (n=191)	Moderate NAFLD group (n=128)	Severe NAFLD group (n=66)	F-value	P-value
TG (mmol/l)	1.38±0.63	1.50±0.66 ^a	1.88±0.699 ^{a,b}	2.15±0.65 ^{a,c}	37.790	<0.001
LDL-C (mmol/l)	2.81±0.41	3.17±0.74 ^a	3.29±1.019 ^a	3.63±1.24 ^{a,c}	32.350	<0.001
TG (mmol/l)	1.28±0.63	1.49±0.68 ^a	1.54±0.73 ^a	1.67±0.75 ^{a,b}	10.080	<0.001
TC (mmol/l)	3.67±0.28	4.51±0.34 ^a	4.87±0.45 ^{a,b}	5.15±0.52 ^{a,c}	609.600	<0.001
LDL-C (mmol/l)	2.41±0.22	2.63±0.25 ^a	2.86±0.33 ^{a,b}	3.35±0.37 ^{a,c}	273.100	<0.001
25(OH)D (nmol/l)	19.62±3.07	17.23±2.61 ^a	15.47±2.38 ^{a,b}	13.83±2.26 ^{a,c}	127.400	<0.001

^aP<0.001, compared with control group; ^bP<0.05, compared with mild NAFLD group; ^cP<0.05, compared with moderate NAFLD group. TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; NAFLD, non-alcoholic fatty liver disease.

between the degree of patients in the NAFLD group and the level of 25(OH)D, and logistic regression analysis for risk factors. P<0.05, was considered statistically significant.

Results

Comparison of clinical data of patients. In order to make the experimental results accurate and credible, the sex, age, smoking and food habit of patients in the two groups were compared. There was no significant difference in them (P>0.05), indicating that the two groups are comparable (Table I).

Changes in expression of BMI and blood glucose levels between the NAFLD group and the control group. The levels of BMI, FPG, FPI and HbA1c of patients in the NAFLD group were significantly higher than those in the control group (P<0.05). The levels of BMI, FPG, FPI and HbA1c in the mild NAFLD group were significantly lower than those in the moderate and severe NAFLD groups (P<0.05), while the levels of BMI, FPG, FPI and HbA1c in the moderate NAFLD group were significantly lower than those in the severe NAFLD group (P<0.05) (Table II).

Changes in expression of blood lipid and 25(OH)D levels between the NAFLD group and the control group. The levels of TG, TC and LDL-C of patients in the NAFLD group of patients were significantly higher than those in the control

group, with a statistically significant difference (P<0.05). The level of 25(OH)D in the NAFLD group was lower than that in the control group (P<0.05). TG, TC and LDL-C levels were significantly lower in the mild NAFLD group than in the moderate and severe NAFLD groups (P<0.05), while 25(OH)D level in the mild NAFLD group was significantly higher than that in the moderate and severe NAFLD groups (P<0.05). The levels of TG, TC and LDL-C in the moderate NAFLD group were significantly lower than those in the severe NAFLD group (P<0.05), while the level of 25(OH)D in the moderate NAFLD group was significantly higher than that in the severe NAFLD group (P<0.05) (Table III).

Correlation analysis between degree of patients in the NAFLD group and level of 25(OH)D. The 25(OH)D levels of patients with mild, moderate, and severe NAFLD were 17.23±2.61 nmol/l, 15.47±2.38 nmol/l and 13.83±2.26 nmol/l, respectively, and the differences were statistically significant (P<0.05). The 25(OH)D levels of patients with moderate and severe NAFLD were significantly lower than that of patients with mild NAFLD (P<0.05). The 25(OH)D level of patients with severe NAFLD was significantly lower than that of patients with moderate NAFLD (P<0.05). According to Spearman's correlation analysis, there was a significant negative correlation between 25(OH)D and the degree of patients in the NAFLD group (r=-0.868, P<0.001) (Figs. 1 and 2).

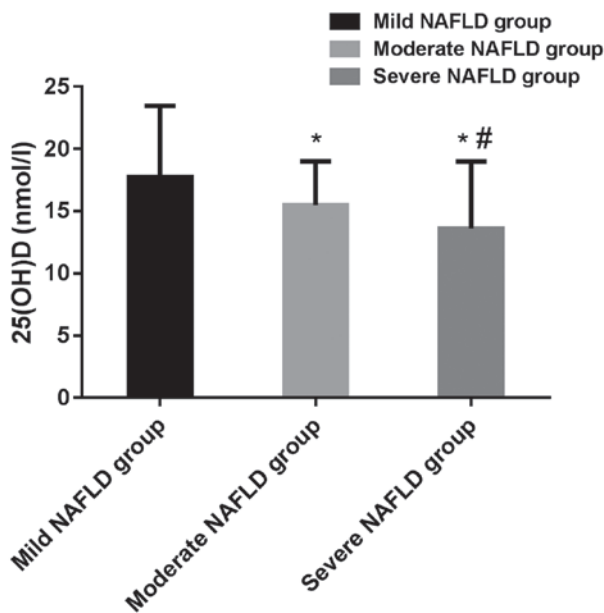


Figure 1. Expression level of 25(OH)D in patients with mild, moderate and severe NAFLD. The level of serum 25(OH)D in control group of patients was 19.62±6.07 nmol/l, and those of patients with mild, moderate and severe NAFLD were 17.75±5.71 nmol/l, 15.48±3.52 nmol/l and 13.61±5.38 nmol/l, respectively. The level of 25(OH)D in patients with mild, moderate and severe NAFLD was significantly lower than that in the control group (P<0.05). Patients with different severity in NAFLD group were compared. The level of 25(OH)D in patients with moderate and severe NAFLD was significantly lower than that of patients with mild NAFLD (P<0.05). That of patients with severe NAFLD was significantly lower than of patients with moderate NAFLD (P<0.05). *P<0.05, compared with the mild NAFLD group; #P<0.05, compared with the moderate NAFLD group.

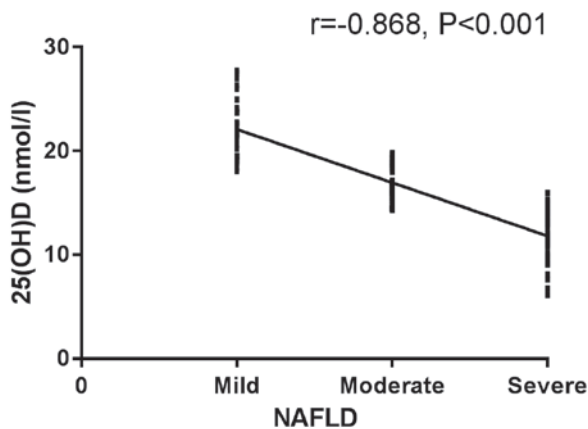


Figure 2. Correlation analysis between degree of patients in NAFLD group and level of 25(OH)D. Spearman's correlation analysis showed a significant negative correlation between 25(OH)D and the degree of patients in NAFLD group (r=-0.868, P<0.001).

Logistic regression analysis of low level of 25(OH)D and levels of BMI, blood glucose and blood lipid. Indicators with differences in clinical pathology analysis were assigned values (Table IV). Multi-factor logistic regression analysis showed that BMI (OR, 0.712; 95% CI, 0.513-0.988), FPG (OR, 1.357; 95% CI, 1.041-1.770), FPI (OR, 0.678; 95% CI, 0.526-0.873), HbA1c (OR, 2.001; 95% CI, 0.172-0.957), TG (OR, 0.668; 95% CI, 0.483-0.926), TC (OR, 0.564; 95% CI, 0.323-0.985), and LDL-C (OR, 1.723; 95% CI, 1.051-2.825)

Table IV. Assigned values.

Variables	Value
BMI	<22=1; ≥22=0
FPG	<7.00=1; ≥7.00=0
FPI	<8.15=1; ≥8.15=0
HbA1c	<6.53=1; ≥6.53=0
TG	<1.52=1; ≥1.52=0
TC	<4.69=1; ≥4.69=0
LDL-C	<2.85=1; ≥2.85=0

BMI, body mass index; FPG, fasting blood glucose; FPI, fasting proinsulin; HbA1c, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table V. Logistic regression analysis of low level of 25(OH)D and levels of BMI, blood glucose and blood lipid.

Variables	Regression coefficient	OR	95% CI	P-value
BMI	2.064	0.712	0.513-0.988	0.042
FPG	1.576	1.357	1.041-1.770	0.024
FPI	1.015	0.678	0.526-0.873	0.003
HbA1c	2.001	0.406	0.172-0.957	0.039
TG	1.247	0.668	0.483-0.926	0.015
TC	2.154	0.564	0.323-0.985	0.044
LDL-C	1.875	1.723	1.051-2.825	0.031

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; FPG, fasting blood glucose; FPI, fasting proinsulin; HbA1c, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol.

were independent risk factors for the low level of 25(OH)D (P<0.05) (Table V).

Discussion

Due to the prevalence of obesity and metabolic syndrome, the incidence of NAFLD has increased year by year, having become an important cause of liver failure and hepatocellular carcinoma in developed countries (12). Most NAFLD patients have metabolic syndrome that refers to the metabolic disorder of protein, fat and carbohydrate in the body, causing the occurrence of hyperlipidemia, hyperglycemia and obesity. It is even closely associated with atherosclerotic cardio-cerebrovascular disease and extrahepatic malignant carcinoma (13,14). NAFLD is regarded as a disease that interacts with various diseases, not an isolated disease. Therefore, it has become a new challenge in current medical field.

In this study, a retrospective analysis was performed on 385 patients in the NAFLD group and 347 healthy people with physical examination in the control group. First, the levels of BMI, blood glucose, blood lipid and 25(OH)D between the two

groups were compared. The BMI level in the NAFLD group was significantly higher than that in the control group, with a statistically significant difference. BMI, a current standard for obesity, is closely related to total body fat, so its level indicator is closely correlated with NAFLD (15,16). The levels of TG, TC and LDL-C in the NAFLD group were significantly higher than those in the control group, with a statistically significant difference. The lipids contained in human plasma are collectively referred to as blood lipids. After the digestion and absorption of food through the gastrointestinal tract, the lipids enter the blood to form blood lipid, the content of which can reflect the metabolism of lipids in the body (17). If the body is affected by a high-fat and high-calorie diet for a long time, it will cause an increase in blood lipid, so as to induce NAFLD (18). According to a study (19) the increased levels of TG, TC and LDL-C are associated with the occurrence of NAFLD. The levels of FPG, FPI and HbA1c in the NAFLD group were significantly higher than those in the control group, with a statistically significant difference. Glucose in the blood is called blood sugar. The operation of various tissues and organs of the body requires glucose to provide energy (20). The normal body maintains blood glucose at a relatively stable level through hormonal regulation and nerve regulation. This is due to the fact that the production and utilization of blood glucose are in a dynamic equilibrium. If regulatory dysfunction is caused by the combination of genetic factors and environmental factors, the level of blood glucose will pathologically increase (21). According to the study of Alwahsh and Gebhardt (22), hyperglycemia is a trigger factor for NAFLD. It may reduce the non-esterified fatty acid in the blood circulation through inhibiting the lipolysis of adipose tissues by insulin, thereby leading to fat deposition in the liver. Therefore, it is a key factor in promoting fatty liver to fatty hepatitis and even cirrhosis. The study (22) is consistent with the views expressed in our study, even corroborating it.

The level of 25(OH)D in the NAFLD group was lower than that in the control group, with a statistically significant difference. Promoting the absorption of calcium in the small intestine, vitamin D can increase or maintain and regulate the concentrations of calcium and phosphorus in plasma. It is converted to 25(OH)D by hydroxylation in the liver (23). The storage level of vitamin D in human body can be reflected by the level of serum 25(OH)D that is closely related to body fat metabolism. 25(OH)D can stimulate the secretion of adiponectin by promoting its gene expression. Adiponectin can promote fatty acid oxidation, thereby reducing TG and TC in the liver (24). Research has shown that (25) 25(OH)D deficiency increases the risk of metabolic syndrome. Therefore, it is important for the diagnosis and treatment of NAFLD to investigate the level of serum 25(OH)D in NAFLD patients and its correlation with the severity of NAFLD.

Results of our study showed that the level of 25(OH)D in patients with mild, moderate and severe NAFLD was significantly lower than that in the control group, with a statistically significant difference. Patients in the NAFLD group were compared. That of patients with moderate and severe NAFLD was significantly lower than that of patients with mild NAFLD, with a statistically significant difference. That of patients with severe NAFLD was significantly lower than of patients with moderate NAFLD, with a statistically significant difference. Spearman's correlation analysis showed that there

was a significant negative correlation between 25(OH)D and the degree of patients in the NAFLD group, suggesting that 25(OH)D can be used as a monitoring indicator for the severity of NAFLD. Pacifico *et al* (26) found that the incidence of vitamin D deficiency was significantly higher in patients with cirrhosis than in patients without cirrhosis (86.3 vs. 49.0%, $P=0.0001$). In the liver function score, the level of vitamin D in Child C group was significantly lower than that in Child A group. Logistic regression analysis showed that BMI, FPG, FPI, HbA1c, TG, TC and LDL-C were independent risk factors for the low level of 25(OH)D, with a statistical significance. It has been reported (27) that the increased blood lipid and blood sugar are risk factors for fatty liver disease. This is similar to the results of the present study.

The results of this experiment show that 25(OH)D is closely related to the occurrence of NAFLD, which is consistent with the results of studies of vitamin D and NAFLD by Chung *et al* (28). However, Patel *et al* (29) considered that 25(OH)D was not related to the severity of NAFLD, and we speculated that the inconsistency may be caused by the difference in the detection method and the test sample. Patel *et al* selected patient liver tissue for gene expression profiling, while in this study, patient blood were used for ELISA analysis. It is possible that 25(OH)D is more sensitive in peripheral blood, leading to differences between studies. Further experimental analysis will be performed to explore the difference. In this investigation the small number of subjects may cause some contingency in the results. A longer-term tracing investigation of subjects will be conducted.

In conclusion, the 25(OH)D low expression in the serum of NAFLD patients, has a significant negative correlation with the severity of NAFLD, which is of guiding significance for the prevention and treatment.

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Availability of data and materials

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

Authors' contributions

JC and ZZ conceived the study and drafted the manuscript. JL, XX and CW acquired the data. MD and LC analyzed the data and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zhongshan Hospital Affiliated to Xiamen University (Xiamen, China). Patients who participated in this research had complete

clinical data. All subjects and their family members signed an informed consent form and cooperated with the medical staff to complete the relevant medical treatments.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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