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ORIGINAL ARTICLE

Relationship between Vitamin D Concentration and Lipid Concentration in Patients with NAFLD in the Hulunbuir Region of China

Shihua Zhang [1, *], Xiaoyu Jia [2, *], Zhanzhu Dong [1], Xia Li [3], Xin Li [4], Hongyan Ge [5], Chunyu Li [1]

[*] Shihua Zhang and Xiaoyu Jia contributed equally to this work
[1] Department of Clinical Laboratory, Inner Mongolia Forestry General Hospital, The Second Clinical Medical College of Inner Mongolia Minzu University, Yakeshi, China
[2] Department of Biochemistry and Molecular Biology, Shenyang Medical College, Shenyang, China
[3] Department of Internal Medicine, Inner Mongolia Forestry General Hospital, The Second Clinical Medical College of Inner Mongolia Minzu University, Yakeshi, China
[4] Department of Ultrasound, Inner Mongolia Forestry General Hospital, The Second Clinical Medical College of Inner Mongolia Minzu University, Yakeshi, China
[5] Medical College, Inner Mongolia Minzu University, Tongliao, China

SUMMARY

Background: **We aimed to characterize the relationship between the serum 25-hydroxyvitamin D concentration and the circulating lipid concentrations of patients with NAFLD in the Hulunbuir region of China.**
Methods: **One hundred fifty-six patients, who were diagnosed with NAFLD in the Physical Examination Department of the Second Clinical College of Inner Mongolia University for the Nationalities between January 2021 and March 2023, were recruited as NAFLD group, and 160 healthy people were recruited as a control group during the same period. The serum 25(OH)VitD, TBIL, TG, TC, LDL-C, HDL-C, AST, ALT, GGT, and FPG activities of the participants were measured, and hepatic ultrasonography was performed.**
Results: **The BMI of the NAFLD group was higher than of the control group ($p < 0.05$). The serum 25(OH)VitD3 ($p < 0.05$) and the HDL-C concentrations of the NAFLD group were lower than those of the normal control group. However, the AST ($p < 0.05$), ALT ($p < 0.05$), and GGT ($p < 0.05$) activities, and the serum TG ($p < 0.05$), TC ($p < 0.05$), LDL-C ($p < 0.05$), and the fasting glucose ($p < 0.05$) concentrations of the NAFLD group were higher than those of the normal control group. The serum 25(OH)VitD3 concentrations of the NAFLD group significantly correlated negatively with BMI ($r = -0.302$, $p < 0.01$), TG ($r = -0.221$, $p < 0.05$), and fasting glucose ($r = -0.236$, $p < 0.05$). The BMI, TG, and fasting glucose of vitamin D-deficient participants were higher than of the participants with adequate or insufficient levels of vitamin D ($p < 0.05$). Finally, the BMI of vitamin D-deficient participants was higher than of those with an adequate vitamin D status ($p < 0.05$).**
Conclusions: **A deficiency of 25(OH)VitD is more common in people from the Hulunbuir region of China than elsewhere. In addition, the vitamin D status is significantly associated with NAFLD; as the serum vitamin D concentration decreases, patients with NAFLD show greater dyslipidemia and hyperglycemia and a higher BMI.**
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Correspondence:
Hongyan Ge
Medical College
Inner Mongolia Minzu University Tongliao
China
Email: zhangshihua1128@126.com

Chunyu Li
Department of Clinical Laboratory
Inner Mongolia Forestry General Hospital
81 Lincheng Road, Yakeshi, Hulunbuir
Inner Mongolia, China
Email: 568258908@qq.com

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KEYWORDS

25-hydroxyvitamin D, nonalcoholic fatty liver disease, lipid metabolism

LIST OF ABBREVIATIONS

NAFLD - nonalcoholic fatty liver disease
AST - aspartate aminotransferase
ALT - alanine aminotransferase
GGT - gamma-glutamyltransferase
TBIL - total bilirubin
TG - triglyceride
TC - total cholesterol
LDL-C - low-density lipoprotein cholesterol
HDL-C - high-density lipoprotein cholesterol
FPG - fasting glucose
25(OH)VitD - 25-hydroxyvitamin D
BMI - body mass index

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is associated with an insulin resistance (IR) and hepatic metabolic stress, and is subject to genetic susceptibility [1,2]. The pathological features mainly manifest as hepatic steatosis, accompanied by cellular inflammatory response, which can further develop and lead to serious consequences such as cirrhosis and liver cancer. In recent years, with the increases in the prevalences of obesity and metabolic syndrome (MetS), NAFLD has become an increasingly recognized cause of chronic liver disease and high circulating liver enzyme activities. NAFLD is associated with cellular oxidative stress, insulin resistance, and pancreatic cell dysfunction, manifesting in dyslipidemia and hyperglycemia, which seriously endanger health [3,4].

Recent studies have shown that the vitamin D status is closely associated with NAFLD, and the degree of vitamin D deficiency positively correlates with circulating TGF-β concentration and the severities of fatty liver and liver cirrhosis [5]. A high BMI is associated with low circulating vitamin D concentration, and vitamin D deficiency may increase the risks of an impaired glucose regulation and dyslipidemia. Vitamin D is an essential steroid hormone, that has numerous physiological roles, including the regulation of calcium and phosphorus metabolism and the immune response [6]. In addition, vitamin D plays a role in chronic diseases, such as diabetes [7] and coronary heart disease [8]. Vitamin D3 can be synthesized in the skin from 7-dehydrocholesterol through a photochemical reaction in the sunlight.

Hulunbuir, Inner Mongolia, is located in the northernmost part of China, which has a cold climate and limited sunshine, meaning that the inhabitants typically have a poor vitamin D status. In addition, the prevalence of NAFLD is relatively high in this region, owing to the high-fat content of the inhabitants' diet, which includes a lot of beef and mutton. Therefore, we aimed to study the relationship of 25-hydroxyvitamin D status with NAFLD and lipid metabolism in this region, in order to provide guidance for people with NAFLD and a vitamin D deficiency, who live in cold climates and consume a high-fat diet.

MATERIALS AND METHODS

Subject selection and sample collection

We recruited patients who attended the Physical Examination Center of the Second Clinical Medical College of Inner Mongolia University for the Nationalities between January 2021 and March 2023, all of whom underwent blood testing, hepatic ultrasonography, and the measurement of BMI. One hundred fifty-six of the participants (81 men and 75 women) were diagnosed with NAFLD, and a control group was created, that consisted of 160 healthy people without fatty liver (78 men and 82 women), who were recruited during the same period. The study was approved by the Ethics Committee of the Second Clinical Medical School of Inner Mongolia University for the Nationalities.

Inclusion criteria

All patients met the diagnostic criteria of the Guidelines for the Prevention and Treatment of Nonalcoholic Fatty Liver Disease, formulated by the Chinese Medical Association in 2018 [9-12]: 1) Imaging or pathological findings showed a steatosis of hepatocytes, patients with no history of alcohol consumption or an alcohol consumption equivalent to the alcohol consumption standards (male alcohol consumption < 30 g/d, female < 20 g/d), patients with alcohol, autoimmune, or other clear causes for their fatty liver disease were excluded, as well as patients with special drugs being applied to their liver cell steatosis; 2) the diagnostic criteria of abdominal B-ultrasonography for fatty liver are enhanced near field echo of liver, higher than that of spleen and kidney, showing "bright liver", far field echo attenuation, and an unclear intrahepatic duct structure; 3) residents, aged 20 - 69 years, with complete clinical data and as long-term residents in the Hulunbuir area; and 4) none of the participants took vitamin D supplements during the study period, and the sampling times were parallel between the two groups.

Exclusion criteria

Patients were excluded if they had a history of excessive alcohol consumption (> 30 g/day or > 20 g/day for men and women, respectively), if they had a history of acute or chronic hepatitis, cirrhosis, or liver cancer, if they had experienced metabolic infectious gastrointestinal diseases affecting the vitamin D absorption, if they were orally administered drugs affecting the vitamin D absorption, or if they were pregnant or lactating.

Measurement of serum parameters
Five milliliter fasting venous blood samples were drawn from patients into tubes, containing 5 mL of inert separation gel and coagulation enhancers. After standing for 30 minutes, they were centrifuged at 1,509 x g for 15 minutes, and the 25(OH)VitD3 (nmol/L) concentrations of the serum samples were measured by the German Roche Cobas e602 electrochemical luminescence analyzer. The serum AST (U/L), ALT (U/L), and GGT (U/L) activities and the serum TBIL (μmol/L), TG (mmol/L), TC (mmol/L), LDL-C (mmol/L), HDL-C (mmol/L), and glucose (mmol/L) concentrations were measured by Beckman AU5800 of Beckman Kurt Limited, USA.

Categorization of the participants according to serum VitD concentration
There is no standard definition for vitamin D deficiency, so we used previously published criteria [13]. The participants with NAFLD were categorized according to their serum vitamin D concentration as follows: 1) Vitamin D deficiency subgroup: serum 25(OH)VitD < 50 nmol/L; 2) Vitamin D insufficiency subgroup: serum 25(OH)VitD 50 - 74.9 nmol/L; and 3) Vitamin D sufficiency subgroup: serum 25(OH)VitD ≥ 75 nmol/L.

Statistical analyses
SPSS 26.0 was used for data analysis. Normally distributed continuous data are expressed as mean ± standard deviation, and one-way analysis of variance was used for comparisons among the groups, followed by the LSD test. Abnormally distributed continuous data are expressed as median and interquartile range. Categorical datasets were compared by using the rank-sum test. The chi-squared test was used for count data. The Spearman analysis method was used for correlation analysis. $p < 0.05$ was considered to represent a statistical significance.

RESULTS

Comparison of the general characteristics of the NAFLD and control groups
The 156 participants in the NAFLD group had a mean age of 47.1 ± 10.5 years and the 160 participants in the control group had a mean age of 46.3 ± 11.7 years. There were no differences in the age or gender distributions of the two groups. The BMI of the NAFLD group was higher than of the control group, and the difference was statistically significant (Table 1).

Serum concentrations of 25(OH)VitD3 and biochemical indices of the two groups
The serum concentrations of 25(OH)VitD3 and HDL-C were lower in the NAFLD group than in the control group ($p < 0.05$). However, the AST, ALT, and GGT activities and the TG, TC, LDL-C, and glucose concentrations of the NAFLD group were higher than those of the control group ($p < 0.05$). There was no difference in the TBIL concentrations of the two groups, as is shown in Table 2.

Correlations between 25(OH)VitD3 concentration and biochemical indices in the NAFLD group
The serum 25(OH)VitD3 concentrations of the participants in the NAFLD group significantly, negatively correlated with BMI ($r = -0.302$, $p < 0.01$), TG ($r = -0.221$, $p < 0.05$), and fasting glucose ($r = -0.236$, $p < 0.05$). However, they did not correlate with AST, ALT, GGT, TBIL, TC, LDL-C, or HDL-C, as is shown in Table 3.

Comparison of BMI, TG, and fasting glucose concentrations among the 25(OH)VitD3 status subgroups of the NAFLD group
There were significant differences in the BMI, TG, and fasting glucose concentrations among the three subgroups ($p < 0.05$). There were statistically significant differences in the BMI between the NAFLD group and the control group ($p < 0.05$). There was no significant difference in TG or glucose between the vitamin D sufficiency and insufficiency groups ($p < 0.05$). There were significant differences in TG and glucose between the vitamin D sufficiency and vitamin D deficiency groups ($p < 0.05$) (Table 4).

DISCUSSION

In the present study, we found that the serum AST, ALT, and GGT activities of patients with NAFLD are higher than those of healthy individuals, which is consistent with the findings of many previous studies [14]. Hepatocyte disorders caused by the excessive deposition of fat in the liver of patients with NAFLD, result in high circulating levels of transaminases. Therefore, patients with NAFLD should have their transaminase activities measured regularly. NAFLD is associated with a high level of oxidation stress, IR, beta-cell dysfunction, and dyslipidemia. In the present study, the serum HDL-C concentrations of participants with NAFLD were lower than of the control group, whereas their BMI, TG, TC, LDL-C, and glucose were higher. This is consistent with the close relationships of NAFLD with diabetes, dyslipidemia, obesity, and IR. NAFLD usually coexists with lipid metabolism disorders. Due to the impact of lipid metabolism disorders on liver cell function, liver cells undergo steatosis to form fatty liver, which transforms into hyperlipidemia and further exacerbates liver cell steatosis [15].
NAFLD is closely associated with a poor lifestyle. In recent years, owing to factors such as a sedentary lifestyle, the consumption of high-fat foods, and the consequent obesity, the prevalence of NAFLD has been increasing, and the disease is increasingly affecting younger people. A large number of studies have shown that vitamin D is closely associated with fatty liver and metabolic syndrome [16], but the mechanisms involved

Table 1. Comparison of the general characteristics of the NAFLD and the control group.

Parameter	NAFLD group (n = 156)	Control group (n = 160)	t/χ²	p
Grade [n (male/female)]	81/75	78/82	0.32	0.573
Age (years)	47.06 ± 10.53	46.34 ± 11.65	0.57	0.369
BMI (kg/m^2)	25.69 ± 1.89	23.08 ± 2.02	11.87	0.000

Table 2. Comparison of the serum 25(OH)D3 concentrations and of the biochemical indices of the NAFLD and the control group.

Parameter	NAFLD group	Control group	Z/t	p
25(OH)D3 (nmol/L)	38.00 [30.00, 44.00]	45.00 [35.00, 50.00]	-4.88	< 0.001
AST (U/L)	24.11 ± 3.93	22.73 ± 2.93	3.53	< 0.001
ALT (U/L)	27.53 ± 7.77	23.20 ± 6.85	5.25	< 0.001
GGT (U/L)	40.00 [34.00, 43.00]	25.00 [23.00, 30.00]	-10.82	< 0.001
TBIL (μmol/L)	14.03 [12.12, 16.38]	13.98 [11.99, 16.84]	-0.47	> 0.05
TG (mmol/L)	2.41 [1.77, 2.62]	1.16 [0.95, 1.46]	-13.23	< 0.001
TC (mmol/L)	5.29 ± 0.88	4.05 ± 0.95	11.88	< 0.001
LDL-C (mmol/L)	3.75 ± 0.85	2.86 ± 0.42	11.84	< 0.001
HDL-C (mmol/L)	1.19 ± 0.14	1.36 ± 0.22	-7.82	< 0.001
FPG (mmol/L)	5.82 [5.21, 6.11]	5.17 [4.99, 5.43]	-6.97	< 0.001

Table 3. Pearson correlations for the relationships between serum 25(OH)D3 concentration and other biochemical indices in the NAFLD group.

Parameter	25(OH)D3	
	r	p
BMI	-0.302	< 0.01
AST (U/L)	-0.048	> 0.05
ALT (U/L)	0.017	> 0.05
GGT (U/L)	-0.046	> 0.05
TBIL (μmol/L)	0.148	> 0.05
TG (mmol/L)	-0.221	> 0.05
TC (mmol/L)	-0.021	> 0.05
LDL-C (mmol/L)	0.105	> 0.05
HDL-C (mmol/L)	-0.041	> 0.05
FPG (mmol/L)	-0.236	< 0.05

have not been firmly established. In particular, people with obesity and adolescents with fatty liver have been shown to lack vitamin D. The biological role of vitamin D is not limited to calcium and phosphorus homeostasis and the regulation of bone turnover, but is also involved in cell differentiation, immune regulation, and carbohydrate metabolism [17]. The level of vitamin D deficiency positively correlates with TGF-β concentration and the severities of fatty liver and cirrhosis. The level of 25 hydroxyvitamin D was significantly lower in patients

Table 4. Comparison of the BMI, TG, and FPG of the vitamin D deficiency, insufficiency, and sufficiency subgroups of the NAFLD patients.

Parameter	VD sufficiency group	VD insufficiency group	VD deficiency group	F	p
BMI (kg/m^2)	22.85 ± 0.69	24.19 ± 1.47 [a]	26.26 ± 1.65 [a, b]	32.34	< 0.05
TG (mmol/L)	1.32 ± 0.30	1.99 ± 0.32	2.59 ± 1.67 [a, b]	4.16	< 0.05
FPG (mmol/L)	4.36 ± 0.32	4.97 ± 0.49	6.13 ± 1.26 [a, b]	19.67	< 0.05
Total cases (n)	7	31	118	-	-

[a] - p < 0.05 vs. the 25(OH)VitD sufficiency subgroup, [b] - p < 0.05 vs. the 25(OH)VitD insufficiency subgroup.

with NAFLD compared to the normal control group. These low levels may be the result of a lacking exposure to ultraviolet light, because there are only a few hours of sunshine in the Hulunbuir area. In addition, the inhabitants of the Hulunbuir region have a relatively high prevalence of NAFLD, owing to their substantial consumption of beef and mutton, and NAFLD is considered to be a manifestation of IR and MS in the liver. Therefore, it is important to study the relationship of the 25(OH)VitD status with NAFLD and lipid metabolism in the inhabitants of this area.

In the present study, we found that the BMI of the NAFLD group was higher than of the control group, and that the serum 25(OH)VitD3 concentrations of the patients with NAFLD negatively correlated with BMI and TG, while there was no correlation between 25(OH)D3 and BMI and TG in the normal control group. It is suggested that the absorption of vitamin D was affected by the NAFLD, which lead to the decrease in the vitamin D level. Vitamin D deficiency is closely related to liver metabolic syndrome. The vitamin D receptor (VDR) in the nucleus can mediate intracellular signal transduction, regulate more than 200 genes, and participate in the glucose and lipid metabolism. Therefore, the vitamin D level may be an important factor in affecting body mass index and TG metabolism. Previous studies have shown that the serum 25(OH)VitD3 concentration is closely associated with NAFLD and that it is negatively associated with the severity of the disease [18]. In the present study, we categorized the participants with NAFLD according to their vitamin D status as deficient, insufficient, and sufficient, then compared their BMI, TG, and glucose concentrations. The patients with vitamin D deficiency had higher BMI and serum TG and glucose concentrations. High BMI is closely associated with poor vitamin D status, and vitamin D deficiency may increase the risks of metabolic abnormalities, such as hyperglycemia and dyslipidemia. Furthermore, obesity has a deleterious effect on the vitamin D status [19]. Therefore, clarifying the relationship between the vitamin D status and the circulating lipid concentrations provides a theoretical and experimental basis for researchers to determine the role of vitamin D in the lipid metabolism of patients with NAFLD.

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Declaration of Interest:

The authors have no conflicts of interest to declare.

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