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ORIGINAL ARTICLE

Low vitamin D status is associated with advanced liver fibrosis in patients with nonalcoholic fatty liver disease

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Abstract Several studies explored the association between vitamin D status and nonalcoholic fatty liver disease with contradictory results. We aimed to investigate the association between vitamin D status, inflammatory cytokines and liver fibrosis in nonalcoholic fatty liver disease patients. Two hundred nineteen nonalcoholic fatty liver disease patients and 166 age- and gender- matched healthy controls were recruited for this study. Serum 25(OH)D was measured by radioimmunoassay. Serum interleukin-8 and transforming growth factor-β1 were measured using ELISA. Serum 25(OH)D was only marginally decreased in nonalcoholic fatty liver disease patients. Interestingly, serum 25(OH)D was markedly reduced in nonalcoholic fatty liver disease patients with advanced liver fibrosis compared to nonalcoholic fatty liver disease patients with indeterminate liver fibrosis and no advanced fibrosis. Logistic regression analysis showed that there was an inverse association between serum 25(OH)D and severity of liver fibrosis in nonalcoholic fatty liver disease patients. Further analysis showed that serum interleukin-8 was elevated in nonalcoholic fatty liver disease patients, the highest interleukin-8 in patients with advanced fibrosis. An inverse correlation between serum 25(OH)D and interleukin-8 was observed in nonalcoholic fatty liver disease patients with and without liver fibrosis. Although serum transforming growth factor-β1 was slightly elevated in nonalcoholic fatty liver disease patients, serum transforming growth factor-β1 was reduced in nonalcoholic fatty liver disease patients with advanced fibrosis. Unexpectedly, a positive correlation between serum 25(OH)D and transforming growth factor-β1 was observed in nonalcoholic fatty liver disease patients with advanced fibrosis. In conclusion, low vitamin D status is associated with advanced liver fibrosis in nonalcoholic fatty liver disease patients. Interleukin-8 may be an important mediator for hepatic fibrosis in nonalcoholic fatty liver disease patients with low vitamin D status.

Keywords Vitamin D deficiency · Nonalcoholic fatty liver disease · Interleukin (IL)-8 · Advanced liver fibrosis

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Abbreviations

NAFLD	nonalcoholic fatty liver disease
25(OH)D	25-hydroxyvitamin D
BMI	body mass index
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ALP	alkaline phosphatase
GGT	glutamyltransferase
LDH	lactate dehydrogenase
HDL	high-density lipoprotein
LDL	low density lipoprotein
OR	odds ratio

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NASH	nonalcoholic steatohepatitis
IL-8	interleukin-8
TGF-β	transforming growth factor-β

Nonalcoholic fatty liver disease (NAFLD) is a very common disease. In Western countries, the prevalence of NAFLD is as high as 20–30 % in the general population [1], and even higher in patients with obesity or diabetes [2]. In China, NAFLD prevalence is rapidly increasing, especially in economically advanced areas. Recent studies indicated that the prevalence of NAFLD was 17 % in Shanghai, and 15 % in Guangdong [3]. The spectrum of NAFLD ranges from simple fatty liver to a potentially progressive form, nonalcoholic steatohepatitis (NASH), which may lead to liver fibrosis and cirrhosis [4]. Although its pathogenesis has not been fully elucidated, NAFLD is an acquired metabolic stress related liver disorder that is closely associated with insulin resistance and genetic susceptibility [5, 6].

Vitamin D is known as the sunshine vitamin and now is considered as a steroid hormone. Besides its classical functions in calcium uptake and bone metabolism [7], vitamin D has anti-inflammatory effects [8–10]. On the other hand, vitamin D deficiency is increasingly recognized as a global public health problem. According to US national data from 2001 to 2004, only 23 % of children and adults were considered vitamin D sufficient (higher than 30 ng/mL) as compared to 45 % in 1988–1994 [11]. Only 12.2 % of males and 6.4 % of females exhibited 25(OH)D sufficient from the Korean National Health and Nutrition Examination Survey 2008 [12]. A recent report from our laboratory showed that more than 70 % of pregnant women were insufficient (lower than 30 ng/mL) or vitamin D deficient [13].

Increasing evidence has demonstrated that vitamin D deficiency is associated with an increased risk of diabetes mellitus, cardiovascular diseases and metabolic syndrome [14–16]. Several studies explored the association between vitamin D status and NAFLD with contradictory results [17–21]. Recently, two animal experiments showed that vitamin D deficiency exacerbated the progression of high-fat diet-induced NAFLD in rats [22, 23]. In contrast, vitamin D3 supplementation ameliorated NASH progression [24]. In contrast, a recent report showed that vitamin D deficiency attenuates high-fat diet-induced hepatic lipid accumulation in male mice [25].

The aim of the present study is to investigate the association among vitamin D status, inflammatory cytokines and NAFLD prevalence in a population-based case–control study. Our results showed that serum 25(OH)D level in NAFLD patients was only marginally reduced as compared with control subjects. We found that serum vitamin D status was correlated negatively with IL-8 level in NAFLD patients. We demonstrate that low vitamin D status is associated with advanced liver fibrosis in NAFLD patients.

Materials and methods

Study participants

This was a population-based case–control study using physical examination data from the Fourth Affiliated Hospital of Anhui Medical University in China between March 1, 2013 and May 31, 2015. Exclusion criteria were as follows: age of less than 20 years or more than 70 years, alcohol consumption ≥140 gram/week in men and ≥70 gram/week in women, patients with hepatitis B or hepatitis C, obstructive bile duct diseases, biliary infection, pregnant and lactating women, kidney diseases, cardiocerebrovascular diseases, cancer, and taking drugs that could affect vitamin D metabolism. Total 219 eligible patients with NAFLD were recruited as cases. The controls were matched with these cases with regard to body mass index (BMI), age, as well as sex. In this study, 166 eligible healthy subjects were recruited as controls. This study obtained ethics approval from the ethics committee of Anhui Medical University. Oral and written consents were obtained from all subjects.

Clinical and biochemical measurements

Demographic and clinical characteristics were collected by a standard questionnaire, including gender, age, alcohol intake, and history of metabolic diseases. Height, weight, waist circumference, systolic blood pressure, and diastolic blood pressure were from record of physical examinations. Laboratory evaluation included complete blood count, routine liver biochemistry, and blood lipid profile. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glutamyltransferase (GGT), lactate dehydrogenase (LDH), creatinine, uric acid, total cholesterol, triglyceride, high-density lipoprotein (HDL), low density lipoprotein (LDL), and fasting blood glucose were measured with automatic biochemical analyzer.

Evaluation of hepatic steatosis

Abdominal ultrasound examination was used to evaluate the severity of hepatic steatosis by radiologists who were blinded to the aims of the study. Ultrasonographic steatosis scores were calculated as follows: normal liver echotexture (score 0); mild steatosis (score 1) as slight and diffuse increase in fine parenchymal echoes with normal visualization of diaphragm and portal vein borders; moderate steatosis (score 2) as moderate and diffuse increase in fine

echoes with slightly impaired visualization of portal vein borders and diaphragm; and severe steatosis (score 3) as fine echoes with poor or no visualization of portal vein borders, diaphragm, and posterior portion of the right lobe [26].

Identification of advanced liver fibrosis

NAFLD Fibrosis Score was used to identify the presence of advanced fibrosis. A scoring system is based on six available variables (age, BMI, hyperglycemia, platelet count, albumin, AST/ALT ratio) and calculated using published formula (http://nafldscore.com). NAFLD patients were divided into three groups based on NAFLD Fibrosis Score: no advanced liver fibrosis (score < -1.455), indeterminate liver fibrosis ($-1.455 \leq$ score ≤ 0.676) and advanced liver fibrosis (score $>$ 0.676) [27].

25(OH)D measurement

Serum 25(OH)D was measured by radioimmunoassay (DiaSorin Inc, Stillwater, MN, USA) according to previous protocol [13]. Vitamin D deficiency defined as lower than 15 ng/mL of 25(OH)D [28, 29].

Serum IL-8 and TGF-β1 measurement

Serum IL-8 and TGF-β1 were measured using an ELISA kit (R&D Systems, Abingdon, Oxon, UK) according to manufacturer's instructions.

Statistical analysis

The difference between two independent groups was compared using two independent sampling *t* test or using the Mann-Whitney U-test. Comparative analyses of categorical variables were carried out by the chi-square test. The data in multiple groups were tested using ANOVA analysis. Multivariable logistic regression was carried out to identify association between 25(OH)D and NAFLD after adjustment for confounding factors. The relationship between 25(OH)D and IL-8 and TGF-β1 were analyzed using scatter plots and linear correlation. A *P*-value of less than 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

Demographic and clinical characteristics were shown in Table 1. As expected, BMI, waist circumference, systolic blood pressure and diastolic blood pressure, ALT, AST, ALP, uric acid and fasting blood glucose were significantly increased in NAFLD patients.

Serum 25(OH)D concentration among NAFLD patients

Serum 25(OH)D was analyzed among all NAFLD patients. As shown in Table 1, serum 25(OH)D in NAFLD patients was slightly reduced. Serum 25(OH)D was analyzed among NAFLD patients with mild, moderate, and severe steatosis. As shown in Fig. 1a, no significant difference on serum 25 (OH)D was observed among different groups.

Association between serum 25(OH)D and severity of liver fibrosis in NAFLD patients

Serum 25(OH)D was analyzed among NAFLD patients with no advanced fibrosis, indeterminate liver fibrosis and advanced liver fibrosis. As shown in Fig. 1b, serum 25(OH) D was significantly reduced in NAFLD patients with indeterminate liver fibrosis and advanced liver fibrosis. In addition, serum 25(OH)D was lower in NAFLD patients with advanced liver fibrosis than in NAFLD patients with indeterminate liver fibrosis and no advanced fibrosis (Fig. 1b). Interestingly, there was no significant difference on serum 25(OH)D between patients with no advanced fibrosis and controls (Fig. 1b). Logistic regression analysis was conducted to further evaluate association between serum 25(OH)D and the severity of hepatic fibrosis in NAFLD patients. As shown in Table 2, there was an inverse association between serum 25(OH)D and severity of liver fibrosis.

Association between vitamin D deficiency and severity of liver fibrosis in NAFLD patients

The association between vitamin D deficiency and severity of liver fibrosis was analyzed. As shown in Table 3, no significant association between vitamin D deficiency and no advanced fibrosis, indeterminate fibrosis was observed. Moreover, there was a positive association between vitamin D deficiency and advanced liver fibrosis (OR: 3.95; 95 %CI: 1.81, 8.63) (Table 3).

Serum IL-8 and TGF-β1 levels among NAFLD patients

Firstly, serum IL-8 was analyzed among NAFLD patients. Results showed that serum IL-8 was significantly elevated in NAFLD patients as compared with controls (1126.98 ± 92.95 vs. 361.80 ± 42.38 pg/ml, $P < 0.01$). Next, serum IL-8 was analyzed among NAFLD patients with and without liver fibrosis. As shown in Fig. 2a, serum IL-8 was significantly elevated in all NAFLD patients with liver fibrosis. Moreover, the highest serum IL-8 level was observed in

NAFLD patients with advanced fibrosis (Fig. 2a). Serum TGF-β1 was then analyzed among NAFLD patients. Results showed that serum TGF-β1 was slightly elevated in NAFLD patients as compared with controls (31.96 ± 1.24 ng/ml vs. 25.68 ± 1.03, $P < 0.01$). Finally, serum TGF-β1 was analyzed among NAFLD patients with and without liver fibrosis. As shown in Fig. 2b, serum TGF-β1 was significantly elevated in NAFLD patients with no advanced fibrosis and indeterminate liver fibrosis. Unexpectedly, serum TGF-β1 was significantly reduced in patients with advanced fibrosis (Fig. 2b).

Table 1 Demographic and clinical characteristics of study participants

Variable	NAFLD patients ($n = 219$)	Control subjects ($n = 166$)	*P*-value
Age (year)	47.27 ± 11.88	47.83 ± 11.74	0.644
Sex (M/F)*	139/80	96/70	0.261
BMI (kg/m^2)	26.16 ± 3.52	22.18 ± 2.11	<0.001
Waist circumference (cm)	92.10 ± 7.87	82.48 ± 5.72	<0.001
Systolic blood pressure (mmHg)	131.22 ± 15.87	119.28 ± 14.36	<0.001
Diastolic blood pressure (mmHg)	84.86 ± 11.50	75.83 ± 8.65	<0.001
ALT (U/L)	47.30 ± 45.80	16.33 ± 7.63	<0.001
AST (U/L)	37.89 ± 33.10	21.60 ± 5.12	<0.001
ALP (U/L)	90.47 ± 74.55	77.87 ± 18.33	0.017
GGT (U/L)	67.25 ± 96.47	21.61 ± 13.46	<0.001
LDH (U/L)	197.38 ± 75.77	180.96 ± 27.90	0.003
Creatinine (μmol/L)	70.92 ± 20.14	68.08 ± 16.06	0.137
Uric acid (mmol/L)	369.06 ± 117.33	322.16 ± 77.97	<0.001
Triglyceride (mmol/L)	2.41 ± 1.94	1.19 ± 0.57	<0.001
Total cholesterol (mmol/L)	5.06 ± 1.09	4.91 ± 0.88	0.174
LDL cholesterol (mmol/L)	2.96 ± 0.96	2.95 ± 0.72	0.905
HDL cholesterol (mmol/L)	1.23 ± 0.35	1.37 ± 0.37	<0.001
Fasting glucose (mmol/L)	6.96 ± 2.58	5.64 ± 0.44	<0.001
25(OH)D (ng/ml)	16.63 ± 6.57	18.44 ± 7.30	0.011

Note: Values are given as the mean ± standard deviation

NAFLD nonalcoholic fatty liver disease, *M/F* male/female, *BMI* body mass index, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *GGT* glutamyltransferase, *LDH* lactate dehydrogenase, *LDL* low density lipoprotein, *HDL* high-density lipoprotein, *25(OH)D* 25-hydroxyvitamin D

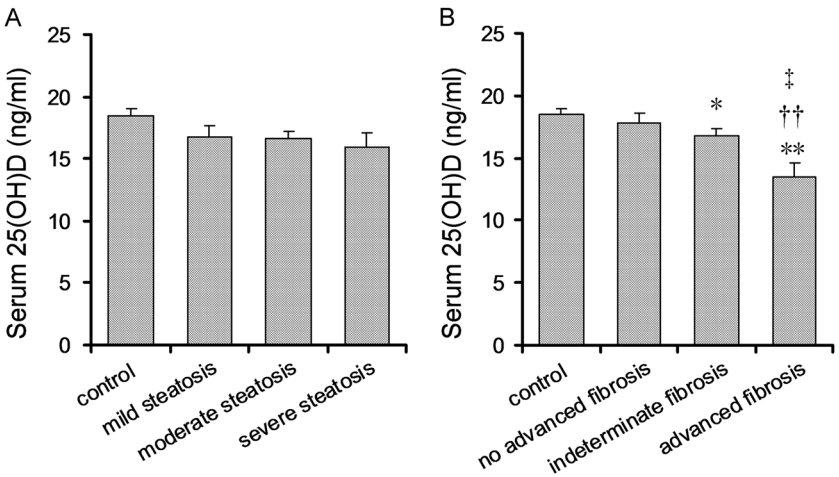

Fig. 1 Serum 25(OH)D among NAFLD patients. Serum 25(OH)D in NAFLD patients and controls ($n = 166$) were measured by radioimmunoassay. **a** NAFLD patients were divided into three groups: mild steatosis ($n = 91$), moderate steatosis ($n = 102$) and severe steatosis ($n = 26$). **b** NAFLD patients were divided into three groups: no advanced fibrosis group ($n = 76$), indeterminate liver fibrosis group ($n = 110$) and advanced liver fibrosis group ($n = 33$). All data were expressed as means ± S.E.M. $^{**}P < 0.01$ vs. controls; †† $P < 0.01$ vs. no advanced liver fibrosis; ‡ $P < 0.05$ vs. indeterminate liver fibrosis

Association between serum 25(OH)D and IL-8 levels

The correlation between serum 25(OH)D and IL-8 were analyzed among controls. As shown in Fig. 3a, no significant correlation between serum 25(OH)D and IL-8 was observed among controls. The correlation between serum 25(OH)D and TGF-β1 was then analyzed in NAFLD patients. As shown in Fig. 3c, serum 25(OH)D was negatively correlated with serum IL-8 in NAFLD patients ($r = -0.214$). Finally, the correlation between serum 25(OH)D and IL-8 was analyzed in NAFLD patients with liver fibrosis. As shown in Table 4, there was an inverse correlation between serum 25(OH)D and IL-8 in NAFLD patients with no advanced fibrosis group, indeterminate liver fibrosis and advanced liver fibrosis.

Association between serum 25(OH)D and TGF-β1 levels

The association between serum 25(OH)D and TGF-β1 was analyzed among controls. As shown in Fig. 3b, there were no significant correlation between serum 25(OH)D and TGF-β1 among controls. Next, the association between serum 25(OH)D and TGF-β1 was analyzed in NAFLD

Table 2 Multivariable logistic regression analysis correlation between 25(OH)D and NAFLD

Variable	β	Wald	*P*-value	OR	95 % CI	
					Lower	Upper
NAFLD vs. control						
Unadjusted	−0.039	6.075	0.014	0.962	0.932	0.992
Adjusted[a]	−0.067	7.779	0.005	0.935	0.892	0.980
No advanced fibrosis vs. control						
Unadjusted	0.142	0.247	0.619	1.152	0.659	2.014
Adjusted[a]	−0.028	1.039	0.308	0.972	0.921	1.026
Indeterminate fibrosis vs. control						
Unadjusted	−0.322	1.476	0.224	0.725	0.431	1.218
Adjusted[a]	−0.061	4.625	0.032	0.941	0.890	0.995
Advanced fibrosis vs. control						
Unadjusted	−1.154	5.084	0.024	0.315	0.116	0.860
Adjusted[a]	−0.160	14.129	<0.001	0.852	0.784	0.926

Note *β* regression coefficient, *Wald* Wald chi-square value, OR odds ratio

*Adjusted for gender, age, BMI and fasting blood glucose

Table 3 Association between vitamin D deficiency and severity of liver fibrosis in NAFLD patients

Groups	*N*	25(OH)D (<15/≥15) ng/ml	*χ2*-value	OR (95 % CI)	*P*-value
Control	166	51/115			
No advanced fibrosis	76	21/55	0.238	0.86 (0.47–1.57)	0.625
Indeterminate fibrosis	110	45/65	3.026	1.56 (0.94–2.58)	0.082
Advanced fibrosis	33	21/12	12.915	3.95 (1.81–8.63)	<0.001

Note 25(OH)D 25-hydroxyvitamin D, *OR* odds ratio

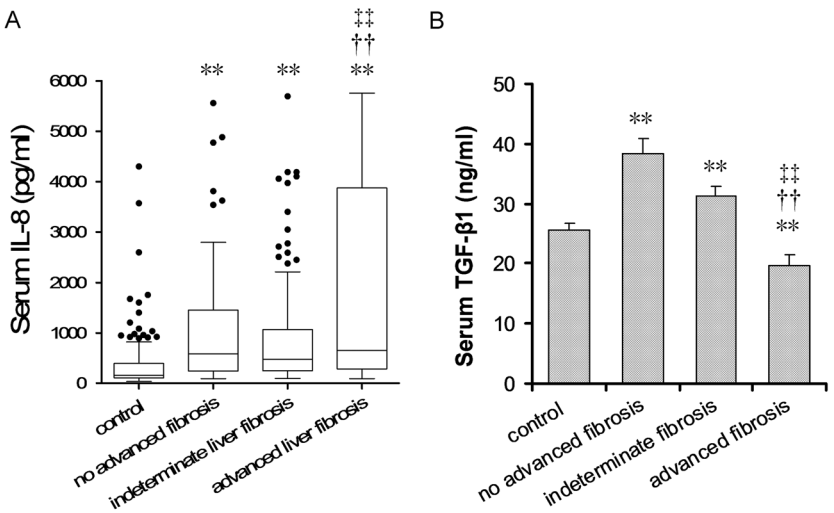

Fig. 2 Serum IL-8 and TGF-β1 in NAFLD patients with and without liver fibrosis. Serum IL-8 and TGF-β1 in NAFLD patients and controls ($n = 166$) were measured using ELISA. NAFLD patients were divided into three groups: no advanced fibrosis group ($n = 76$), indeterminate liver fibrosis group ($n = 110$) and advanced liver fibrosis group ($n = 33$). **a** IL-8. The median and 25th and 75th percentiles were used for variables with skewed distribution. **b** TGF-β1. Data were expressed as means ± S.E.M. $^{**}P < 0.01$ vs. controls; †† $P < 0.01$ vs. no advanced liver fibrosis; ‡‡ $P < 0.01$ vs. indeterminate liver fibrosis

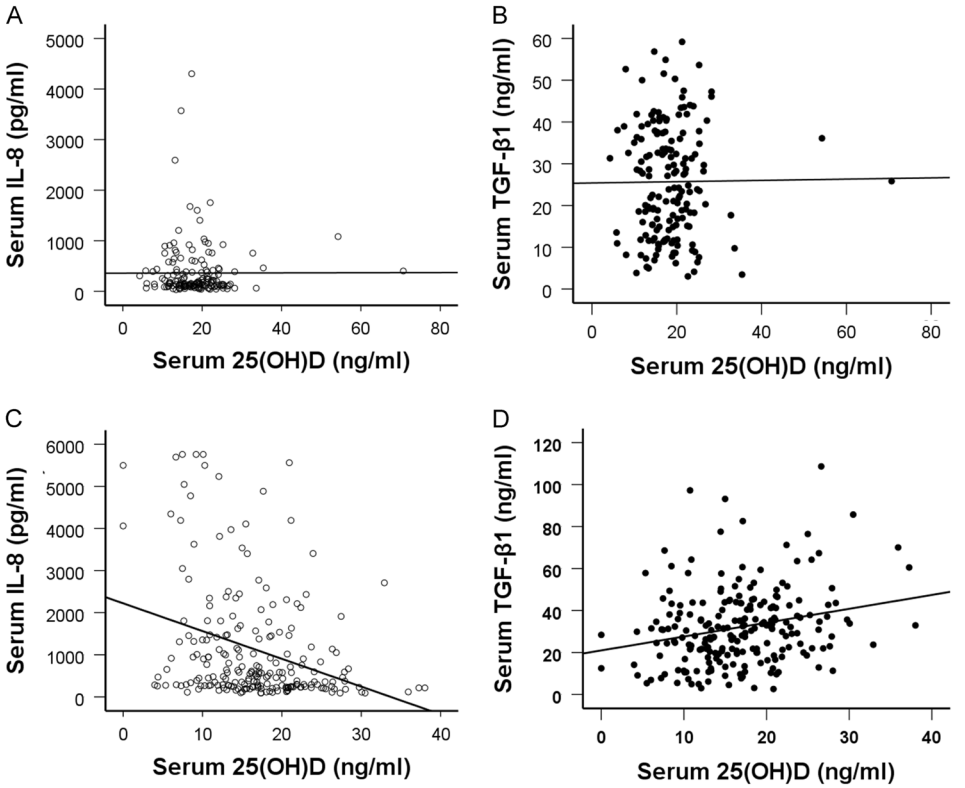

Fig. 3 Association between serum 25(OH)D and IL-8 and TGF-β1. **a** Correlation between serum 25(OH)D and IL-8 among controls. **b** Correlation between serum 25(OH)D and TGF-β1 among controls. **c** Correlation between serum 25(OH)D and IL-8 among NAFLD patients. **d** Correlation between serum 25 (OH)D and TGF-β1 among NAFLD patients

Table 4 Correlation between serum 25(OH)D content and serum IL-8 and TGF-β1 levels

Groups	*N*	*r*-value	*P*-value
IL-8 (LN)	219	−0.214	<0.01
No advanced fibrosis	76	−0.270	<0.05
Indeterminate fibrosis	110	−0.269	<0.01
Advanced fibrosis	33	−0.179	<0.05
TGF-β1	219	0.229	<0.01
No advanced fibrosis	76	0.144	0.214
Indeterminate fibrosis	110	0.114	0.236
Advanced fibrosis	33	0.565	0.001

Note LN natural logarithm, *IL-8* interleukin-8, *TGF-β* transforming growth factor-β

patients. As shown in Fig. 3d, serum 25(OH)D was positively correlated with serum TGF-β1 in NAFLD patients ($r = 0.229$). The correlation between serum 25(OH)D and TGF-β1 was then analyzed in NAFLD patients with liver fibrosis. Although there was no significant association between serum 25(OH)D and TGF-β1 in NAFLD patients with no advanced fibrosis and indeterminate liver fibrosis, a positive correlation between serum 25(OH)D and TGF-β1 was observed in patients with advanced liver fibrosis (Table 4).

Discussion

Several studies explored the association between vitamin D status and NAFLD with contradictory results. Recently, several studies demonstrated that low vitamin D status was positively associated with NAFLD [16, 30]. Other report showed that serum 25(OH)D were not significantly different between subjects with and without NAFLD [19]. A report from rodent animals found that vitamin D deficiency did not directly induce hepatic lipid accumulation but attenuate high-fat diet-induced hepatic lipid accumulation in mice [25]. The present study compared vitamin D status among NAFLD patients and controls in a population-based case–control study. We showed that serum 25(OH)D in NAFLD patients was only marginally reduced as compared with controls. Further analysis observed no significant difference on serum 25(OH)D among patients with mild, moderate, and severe steatosis. These results suggest that low vitamin D status is not significantly associated with hepatic steatosis.

An early study demonstrated that low serum 25(OH)D was associated with the severity of liver fibrosis in HIV/HCV co-infected patients [31]. Additional report showed that low serum 25(OH)D status was associated with hepatic dysfunction [32, 33]. According to a recent report from Non-alcoholic Steatohepatitis Clinical Research Network

(NASH CRN) cohort, low vitamin D status was associated with an increased risk of non-alcoholic steatohepatitis in NAFLD patients [34]. To explore the association between low vitamin D status and NAFLD-induced liver fibrosis, the present study compared serum 25(OH)D among controls and NAFLD patients with no advanced, indeterminate and advanced liver fibrosis. As expected, serum 25(OH)D was lower in NAFLD patients with indeterminate and advanced liver fibrosis than in controls. Interestingly, serum 25(OH)D was lower in NAFLD patients with advanced liver fibrosis than in NAFLD patients with indeterminate and no advanced liver fibrosis. Moreover, an inverse association was observed between serum 25(OH)D and the severity of liver fibrosis in NAFLD patients. These results are in agreement with a report from 148 consecutive outpatients with NAFLD, in which low vitamin D status was associated with the severity of hepatic fibrosis in NAFLD patients [35]. These results suggest that vitamin D deficiency may be an additional cofactor for hepatic fibrosis in NAFLD patients.

The mechanism through which vitamin D deficiency promotes liver fibrosis in NAFLD patients remains obscure. According to two clinical reports, serum inflammatory cytokines, such as IL-8, were significantly elevated in NASH patients, which was likely to contribute to inflammation and liver fibrosis [36, 37]. On the other hand, a recent report from rodent animals showed that vitamin D deficiency exacerbated progression of NAFLD and hepatic inflammation through activation of Toll-like receptor signaling [22]. Indeed, vitamin D receptor (VDR) is widely expressed in parenchymal and inflammatory cells and is negatively associated with severe lobular inflammation in NASH patients [38]. Increasing evidence demonstrates that active vitamin D3 has an anti-inflammatory activity and inhibits inflammatory cytokines including IL-8 in VDR-dependent manner [8, 10, 39]. To explore the association among serum vitamin D status, IL-8 and liver fibrosis, the present study analyzed serum IL-8 in NAFLD patients with liver fibrosis. As expected, serum IL-8 was elevated in all NAFLD patients with liver fibrosis. Interestingly, the highest serum IL-8 level was observed in NAFLD patients with advanced fibrosis. Further analysis showed that serum 25(OH)D status was negatively correlated with serum IL-8 in all NAFLD patients. These results suggest that IL-8 may be an important mediator for hepatic fibrosis in NAFLD patients with low vitamin D status.

TGF-β is a key cytokine that is involved in cell survival, proliferation, differentiation, and wound healing. In the liver, TGF-β regulates all stages of chronic liver disease, from liver injury and regeneration through hepatic inflammation and fibrosis to cirrhosis and hepatocellular carcinoma [40]. Numerous results from rodent animals demonstrate that TGF-β mediates fibrogensis through TGF-β/Smad2/3 activation in hepatic stellate cells (HSCs) [41, 42]. Nevertheless, whether TGF-β mediates liver fibrogenesis in NAFLD patients remains obscure. According to several early reports, hepatic TGF-β1 mRNA and serum TGF-β1 were elevated in NASH patients with liver fibrosis [43, 44]. Other report showed no significant difference on serum TGF-β1 between patients with simple fatty liver and NASH patients [45]. The present study showed that serum TGF-β1 was slightly elevated in NAFLD patients. Further analysis showed that serum TGF-β1 was elevated in NAFLD patients with no advanced fibrosis and indeterminate liver fibrosis. In contrast, serum TGF-β1 was reduced in patients with advanced fibrosis. Moreover, we found that serum 25(OH)D was positively associated with serum TGF-β1 in all NAFLD patients. In addition, a positive correlation between serum 25(OH)D and TGF-β1 was observed among NAFLD patients with advanced liver fibrosis. Indeed, an early study demonstrated that serum TGF-β1 was not associated with the stages of liver fibrosis in NASH patients [46]. These results suggest that serum TGF-β1 is not a key biomarker for liver fibrosis in NAFLD patients with low vitamin D status. Recently, two studies showed that active vitamin D3 ameliorated type I collagen formation in TGFβ1-stimulated human stellate cells [47, 48]. Thus, additional research is necessary to determine whether vitamin D3 supplementation counteracts TGFβ1-mediated HSC activation in NAFLD patients.

The present study had several limitations. Firstly, the present study had not considered the influence of hepatic inflammation and fibrosis on vitamin D metabolism. Indeed, vitamin D itself is devoid of biological activity. Vitamin D is converted to 25(OH)D by hepatic cytochrome P450 (CYP)2R1 and is then converted into 1,25(OH)2D3, the active form of vitamin D, by CYP27B1 in the kidney [49, 50]. Several studies indicated that serum 25(OH)D level was reduced among patients with liver and kidney diseases [33, 51, 52]. Thus, a prospective study is needed to establish causal relationship between vitamin D deficiency and advanced liver fibrosis in patients with NAFLD. Secondly, the present study had not considered the influence of the metabolic syndrome on vitamin D status. Several reports suggest that vitamin D status is associated with the metabolic syndrome [53, 54]. Additional work is required to further analyze the association among vitamin D deficiency, NAFLD and metabolic syndrome.

In summary, the present study analyzed the association of vitamin D status with liver fibrosis in NAFLD patients in a population-based case-control study. We found that low vitamin D status was associated with liver fibrosis in NAFLD patients. Our results showed that serum IL-8 was elevated in all NAFLD patients, the highest IL-8 in NAFLD patients with advanced fibrosis. Moreover, an inverse correlation between serum 25(OH)D and IL-8 was observed in NAFLD patients with and without liver fibrosis. We

demonstrate that IL-8 may be an important mediator for liver fibrosis in NAFLD patients with low vitamin D status.

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Author Contributions XC and DXX conceived study. BBY collected data and carried out experiments. YHC and CZ carried out experiments and analyzed data. CES, KFH and JZ collected data. DXX and YHC wrote the paper. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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