Original Article

Vitamin D Is Associated with Severity and Mortality of Non-alcoholic Fatty Liver Disease: A US Population-based Study

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Abstract

Background and Aims: There has been increasing evidence that vitamin D deficiency may increase the risk of metabolic syndrome. Since metabolic syndrome is a major risk factor for non-alcoholic fatty liver disease (NAFLD), we aimed to investigate the association between vitamin D and the severity and mortality of NAFLD. *Methods:* Data was obtained from the United States Third National Health and Nutrition Examination Survey conducted in 1988–1994, with follow-up mortality data through 2011. NAFLD was defined by ultra-sonographic detection of hepatic steatosis in the absence of other liver diseases and categorized as normal, mild, moderate or severe. The severity of hepatic fibrosis was determined by NAFLD fibrosis score (NFS). ANOVA (F-test) was used to evaluate the association between vitamin D level and degree of NAFLD, and Cox proportional hazards regression analysis was used for survival analyses. *Results:* Vitamin D levels for normal, mild, moderate and severe steatosis were 25.1 ± 0.29 ng/mL, 24.7 ± 0.42 ng/mL, 23.7 ± 0.37 ng/mL and 23.6 ± 0.60 ng/mL, respectively (trend $p < 0.001$). Likewise, vitamin D levels for low, intermediate and high NFS categories were 24.7 ± 0.38 ng/mL, 23.4 ± 0.42 ng/mL and 21.5 ± 0.57 ng/mL, respectively (trend $p < 0.001$). After median-follow up over 19 years, vitamin D deficiency was significantly associated with diabetes- and Alzheimer's disease-related mortality (hazard ratio (HR): 3.64, 95%CI: 1.51–8.82 and HR: 4.80, 95%CI: 1.53–15.1, respectively), with a borderline significance in overall mortality (HR: 1.16, 95%CI: 0.99–1.36, $p = 0.06$). *Conclusion:* Vitamin D level was inversely related to the degree of liver steatosis and fibrosis.

Moreover, vitamin D deficiency was associated with diabetes- and Alzheimer's disease-related mortality in NAFLD patients.

Introduction

Vitamin D and its possible role in the development of chronic diseases has been the focus of recent studies. Long recognized as important for maintaining bone health, it has also been found to be associated with cancer, cardiovascular disease, type 2 diabetes mellitus, insulin resistance, and metabolic syndrome.[1–4] Along with this, there is growing attention to non-alcoholic fatty liver disease (NAFLD) as a hepatic manifestation of metabolic syndrome.[5] NAFLD represents a wide spectrum of clinicopathologic conditions, from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis; currently, it is the most common cause of chronic liver disease.[6]

Although a few studies have reported the possible role of vitamin D deficiency in the degree of NAFLD, these studies evaluated a small patient population, and the findings are inconsistent regarding whether or not the level of vitamin D affects the severity of NAFLD.[7–9] Moreover, to our knowledge, there is no published United States population-based study that represents the entire national household. It has been reported that NAFLD is a major cause of death associated with obesity, diabetes and cardiovascular disease.[10–12] Since vitamin D level has been associated with these metabolic disorders, it would be important to investigate whether vitamin D level can be a prognostic factor in NAFLD. Currently, the role of vitamin D in the mortality of NAFLD remains to be elucidated.

Thus, the purpose of this study was to (i) investigate the association between vitamin D level and severity of NAFLD and (ii) explore whether vitamin D level can predict mortality in NAFLD patients, using nationally representative cross-sectional data with subsequent follow-up mortality data.

Methods

Study population

The Third National Health and Nutrition Examination Survey (NHANES III) is a cross-sectional, population-based sample survey of the civilian non-institutionalized population of the United States conducted from 1988 through 1994. This survey was conducted by a complex, stratified and multistage probability sampling design and obtained information using standardized household interviews, physical examinations and testing of biologic samples. Detailed information about design and sampling of the survey is available.[13]

Participants were included in this study if they met the following selection criteria (Fig. 1): age of 20–74 years-old, available clinical and laboratory variables, liver ultrasound data, and mortality follow-up data. Those with heavy alcohol consumption ($\geq$21 drinks/week for men and $\geq$14 drinks/week for women), viral hepatitis (positive serum hepatitis B surface antigen or serum hepatitis C antibody), iron overload (transferrin saturation of $\geq$50%) or pregnancy were excluded ($n = 1,720$). After the selection process, 10,960 participants were included. The original survey was approved by the Centers for Disease Control and Prevention's Institutional Review Board, and all participants provided written informed consent to participate.

Clinical and laboratory data

A wide array of demographic, lifestyle and dietary information as well as anthropometric assessment and comprehensive laboratory data are available in the data set.[14–16] Hypertension was defined as systolic blood pressure of $\geq$140 mmHg or diastolic blood pressure of $\geq$90 mmHg and/or previous use of antihypertensive medication or self-reported hypertension diagnosis. Diabetes mellitus was diagnosed in subjects with history of diabetes diagnosis and/or treatment with a hypoglycemic agent or insulin. Insulin resistance was defined by >3.5 score in the homeostasis model assessment of insulin

resistance (HOMA-IR; or serum glucose x serum insulin/ 22.5).[17] Smoking status was categorized as current, former or never smoker. Education level was treated as binary (below college vs. college or higher), and poverty index was classified with cut-offs of 1.3 and 1.85, according to the poverty guidelines.[18] Metabolic syndrome was defined according to the National Cholesterol Education Program Adult Treatment Panel (commonly known as the NCEP ATP III) definition.[19] History of cardiovascular disease was defined as self-reported history of congestive heart failure, stroke or myocardial infarction. Vitamin D level was measured using Diasorin 25-OH-D assay and deficiency was defined as a level below 20 ng/dL.[20] Serum leptin concentrations were measured by radioimmunoassay at Linco Research, Inc (St Charles, MO, United States)[21] and insulin-like growth factor-1 (IGF-1) was measured by IGF-1 enzyme-linked immunosorbent assay (DSL 10–5600; Diagnostic Systems Laboratories, Webster, TX, United States).[22]

NAFLD

The original NHANES III examination included ultrasonography of the liver and gallbladder at a mobile examination center as a part of the assessment for digestive diseases in adults aged 20 to 74 years-old. Subsequently, the archived gallbladder ultrasound video images were reviewed to assess fatty liver.[23] Three ultrasound reviewers were trained by a board certified radiologist, who was specialized in hepatic imaging. Evaluation of the fatty liver was performed using the following five criteria: 1) parenchymal brightness, 2) liver–to-kidney contrast, 3) deep beam attenuation, 4) bright vessel walls, and 5) gallbladder wall definition. The overall assessment, by an algorithm based on these five criteria, reported normal versus mild, moderate or severe hepatic steatosis. We defined NAFLD as subjects with any degree (mild to severe) of steatosis. In individuals with NAFLD, NAFLD fibrosis score (NFS) was used to assess severity of liver fibrosis.[24]

NFS was calculated as: $-1.675 + 0.037$ X age (years) $+ 0.094$ X body mass index (BMI; kg/m^2) $+ 1.13$ X impaired fasting glycemia or diabetes (yes = 1, no = 0) $+ 0.99$ X aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio $- 0.013$ X platelet (X 10^9/L) $- 0.66$ X albumin (g/dL)

Two cut-off points were selected to categorize subjects with NAFLD into three groups, including those with high probability (NFS > 0.676), intermediate probability (NFS 0.676 to -1.455) and low probability for advanced fibrosis (NFS ≤ -1.455).

Mortality

All participants of NHANES III over 17 years of age were passively followed for mortality through December 31, 2011, by linking NHANES III participants with National Death Index records through a probabilistic match, a well-established matching method. The NHANES III-Linked Mortality File uses the Underlying Cause of Death 113 (UCOD_113) code to classify all deaths according to the International Classification of Diseases, 9th Revision (ICD-9) for deaths prior to 1998 and to ICD-10 for those between 1999 and 2011.[25] Overall mortality and the following leading cause-specific mortalities were assessed: (1) cardiovascular-related (UCOD_113 54–64); (2) malignancy-related (UCOD_113 19–43); (3) cerebrovascular-related (UCOD_113 70); (4) lung-related (UCOD_113 82–86, 76–78); (5) kidney-related (UCOD_113 97–101);
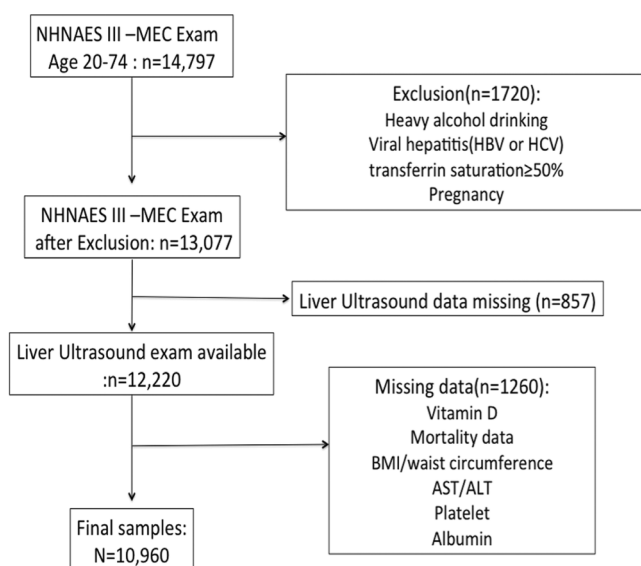

Fig. 1. Consort diagram of patient selection. Adopted from Kim *et al*.[23]

(6) diabetic-related (UCOD_113 046), and (7) Alzheimer's disease-related (UCOD_113 052). Liver-related mortality could not be investigated since it was classified as 'others' in the public version of mortality data.

Statistical analysis

All data was analyzed using the 'survey' package in R software version 3.2.2.[26] We analyzed frequencies of categorical variables and means ± standard errors (SEs) of continuous variables. Baseline characteristics across groups were compared using the chi-square test for categorical variables and the two sample *t*-test or ANOVA for continuous variables. The trending of degree of NAFLD was tested by treating steatosis status and NFS category as an ordinal variable. To calculate the hazard ratios (HRs), Cox proportional hazards regression analysis was used for survival analysis, including overall and disease-specific mortality.

In the analysis of association of vitamin D and degree of NAFLD and fibrosis, both unadjusted and adjusted models were used. Factors included in the limited model were age, sex, race, census region and season, while other metabolic disorder-related factors (hypertension, diabetes, dyslipidemia, BMI, metabolic syndrome, HOMA-IR, leptin, IGF-1) were added to our full model. For survival analyses, HRs were calculated by adjusting for age, sex and race. *p*-values were two-sided, and a *p*-value of <0.05 was considered to indicate statistical significance. All analyses utilized sample weights that accounted for unequal selection probabilities and non-response. All variance calculations accounted for the design effects of the survey using Taylor series linearization.[27]

Results

Demographics

From 1988 to 1994, there were 10,960 NHANES participants who met our selection criteria (Fig. 1). Among them, 4,991 individuals were classified as having vitamin D deficiency, corresponding to a weighted prevalence of 30.8% (95% confidence interval (CI): 28.2–33.4) (Table 1). The vitamin D-deficient population tended to be older, more female predominant, of non-white ethnicities, current smokers, had blood drawn in winter, and lived in the South and West regions. People with vitamin D deficiency had higher BMI, waist circumference, waist-to-hip ratio and HOMA-IR score as well as higher levels of glycated hemoglobin (HbA1c), triglycerides, r-GT, platelets and leptin. They also had a higher incidence of hypertension, diabetes, insulin resistance and metabolic syndrome. Vitamin D deficiency was associated with low albumin and IGF-1.

Vitamin D level by degree of NAFLD on liver ultrasound finding

Table 2 and Fig. 2A show the relationship between vitamin D levels by the degree of fatty liver based on liver ultrasound findings (normal, mild, moderate or severe). Among the 10,960 people in our cohort, 4,015 (34.3%) were identified with NAFLD. There were 1,491 people with mild, 1,707 with moderate and 817 with severe steatosis. In the total population, vitamin D level had a significant inverse relationship with the degree of fatty liver disease (for normal, mild, moderate and severe steatosis, levels were 25.1 ± 0.29 ng/mL,

24.7 ± 0.42 ng/mL, 23.7 ± 0.37 ng/mL, 23.6 ± 0.60 ng/mL, respectively (trend *p* < 0.001)). The relationship remained significant after adjusting for known demographic factors including age, sex, race, census region and season of the study (limited model). However, the significance disappeared after adjusting for metabolic risk factors such as hypertension, diabetes, BMI, metabolic syndrome, HOMA-IR, leptin and IGF-1 levels (full model), suggesting that vitamin D may be placed on the same causal pathway with these factors and highly correlated. Table S1 showed that vitamin D level significantly correlated with metabolic risk factors, with leptin having the highest correlation coefficient (*r*=−0.24). Similar relationship trends were observed among both sexes and all racial groups, with the exception of the non-Hispanic black group.

Vitamin D level by degree of NFS

Table 3 and Fig. 2B show the relationship between the vitamin D level and NFS category. There were 2,354 people with low NFS (≤ −1.455), 1,379 people with intermediate NFS, and 282 people with high NFS (>0.676). In the total population, vitamin D level was inversely related to the NFS category (24.7 ± 0.38 ng/mL, 23.4 ± 0.42 ng/mL, and 21.5 ± 0.57 ng/mL for low, intermediate and high NFS, respectively (trend *p* < 0.001)). This statistical significance remained after known demographic variables were taken into account (limited model), while it disappeared after adjusting for other metabolic risk factors of NAFLD (full model). In the subgroup analyses, a similar trend was only observed in the female group (23.7 ± 0.49 ng/mL, 21.5 ± 0.54 ng/mL and 19.2 ± 0.75 ng/mL for low, intermediate and high NFS, respectively (trend *p* < 0.001)) and non-Hispanic whites. An inverse association between vitamin D levels and NFS group existed in the Hispanic group, but the association was not robust enough to be significant after adjustment.

Mortality by vitamin D status in NAFLD patients

In NAFLD patients, there were a total of 1,090 deaths during the follow-up period (median of 18.8 years). Cardiovascular-related mortality was the leading cause of death (*n*=253), followed by malignancy (*n*=256), lung disease (*n*=76), diabetes (*n*=66), cerebrovascular disease (*n*=62), Alzheimer's disease (*n*=22), and kidney disease (*n*=15). Vitamin D deficiency was borderline significant for age-, race- and sex-adjusted all-cause mortality (HR: 1.16, 95%CI: 0.99–1.36, *p* = 0.06). Vitamin D deficiency was prognostic in diabetes- and Alzheimer's disease-related mortality (HR: 3.64, 95%CI: 1.51–8.82 for diabetes, and HR: 4.80, 95%CI: 1.53–15.1 for Alzheimer's disease). There was no statistically significant association between vitamin D deficiency and other leading causes of death. The vitamin D association with diabetes- and Alzheimer's disease-related mortality were not observed in non-NAFLD participants (Table S2).

Discussion

There is growing research interest in vitamin D's role in metabolic syndrome, along with the skeletal benefits of vitamin D. Since NAFLD is associated with metabolic syndrome and obesity, we investigated the association of vitamin D and NAFLD severity as well as the prognostic value of vitamin D in NAFLD. Our study suggests that vitamin D level is inversely

Table 1. Demographic characteristics

	Vitamin D deficiency (*n* = 4,991) 30.8% [95%CI: 28.2–33.4]	Without vitamin D deficiency (*n* = 5,969) 69.2% [95%CI: 66.6–71.8]	Adjusted *p*-value*
Age in years	44.1 ± 0.30	42.7 ± 0.16	<0.001
Sex as % male	36.5 ± 1.23	52.3 ± 0.54	<0.001
Race as %			
Non-Hispanic white	55.2 ± 1.97	85.9 ± 0.96	<0.001
Non-Hispanic black	24.1 ± 1.37	4.1 ± 0.35	
Hispanic	8.0 ± 0.68	4.0 ± 0.39	
Others	12.7 ± 1.28	6.0 ± 0.82	
Education			
Below college level	61.1 ± 1.51	54.8 ± 1.67	0.92
≥College level	38.9 ± 1.51	45.2 ± 1.67	
Poverty index[#]			
≤1.3	22.4 ± 1.69	14.4 ± 0.91	0.71
1.3–1.85	11.8 ± 0.71	9.64 ± 0.93	
≥1.85	65.7 ± 1.84	75.8 ± 1.24	
Smoking status as %			
Never	47.3 ± 1.28	45.1 ± 1.19	<0.001
Current	29.6 ± 1.07	26.6 ± 1.06	
Former	23.0 ± 0.90	28.3 ± 0.83	
Alcohol consumption as drinks/week	3.95 ± 0.17	4.42 ± 0.14	0.271
Season of screening date			
Spring	27.7 ± 0.42	26.5 ± 0.43	<0.001
Summer	19.8 ± 0.36	33.9 ± 0.50	
Fall	31.0 ± 0.43	28.8 ± 0.47	
Winter	21.5 ± 0.39	10.8 ± 0.24	
Census region			
Northeast	16.5 ± 1.62	22.4 ± 1.58	<0.001
Midwest	18.3 ± 1.58	25.9 ± 2.29	
South	40.0 ± 3.36	32.6 ± 2.72	
West	25.2 ± 3.70	19.0 ± 3.40	
BMI in kg/m^2	28.3 ± 0.20	26.1 ± 0.10	<0.001
Waist circumference in cm	94.7 ± 0.43	91.1 ± 0.26	<0.001
Waist to hip ratio	0.91 ± 0.002	0.91 ± 0.002	<0.001
Hypertension as %	25.8 ± 1.03	20.3 ± 0.82	<0.05
Diabetes as %	6.8 ± 0.64	4.2 ± 0.35	<0.05
Insulin resistance as %	25.4 ± 1.33	15.1 ± 1.02	<0.001
HOMA-IR	3.59 ± 0.18	2.64 ± 0.93	<0.01
HbA1c as %	5.5 ± 0.02	5.3 ± 0.02	<0.001
Hyperlipidemia as %	37.9 ± 1.72	34.7 ± 1.23	0.19
Total cholesterol in mg/dL	206.1 ± 1.13	203.5 ± 0.92	0.43
LDL in mg/dL	127.0 ± 0.97	125.7 ± 0.97	0.55
HDL in mg/dL	50.1 ± 0.52	49.7 ± 0.41	0.19
TG in mg/dL	147.3 ± 2.89	143.4 ± 2.91	<0.05
AST in IU/L	20.8 ± 0.25	20.8 ± 0.19	0.727

(*continued*)

Table 1. (*continued*)

	Vitamin D deficiency (n = 4,991) 30.8% [95%CI: 28.2–33.4]	Without vitamin D deficiency (n = 5,969) 69.2% [95%CI: 66.6–71.8]	Adjusted p-value*
ALT in IU/L	17.5 ± 0.45	17.5 ± 0.44	0.074
r-GT in IU/L	30.6 ± 0.99	26.6 ± 0.69	<0.05
Albumin in g/dL	4.1 ± 0.02	4.2 ± 0.02	<0.05
Platelet as × 10⁹/L	282 ± 2.9	267 ± 2.1	<0.001
Leptin	15.3 ± 0.52	10.4 ± 0.30	<0.001
IGF-1	256 ± 4.18	280 ± 3.55	<0.05
Vitamin D in mg/dL	15.1 ± 0.10	29.1 ± 0.17	<0.001
CRP	0.45 ± 0.01	0.38 ± 0.01	0.06
Metabolic syndrome as %	37.3 ± 1.72	28.2 ± 1.33	<0.001

*adjusted by age, sex, and race.
#Poverty index was classified with cut-offs of 1.3 and 1.85 according to the poverty guidelines.

related to the severity of NAFLD and liver fibrosis. Moreover, vitamin D deficiency is significantly related to diabetes- and Alzheimer's disease-related mortality in NAFLD patients and has borderline significance in overall mortality.

Our findings can be supported by prior studies[7,8,28] that reported a similar inverse relationship of vitamin D and the degree of NAFLD. Nonetheless, the role of vitamin D in the pathogenesis of NAFLD is still controversial. Since vitamin D is associated with known NAFLD risk factors, such as insulin resistance, diabetes and obesity, it is plausible that vitamin D may play a role in the development of NAFLD.[1–4] Dasarathy *et al.*[7] showed that 148 patients with biopsy-proven NAFLD had significantly lower vitamin D levels, when compared to controls, and that these lower concentrations were associated with significantly higher levels of steatosis and fibrosis in NAFLD patients. Additionally, Targher *et al.*[9] also reported that in 60 consecutive patients with biopsy-proven NAFLD,

vitamin D deficiency was closely associated with the histological severity of hepatic steatosis, necroinflammation and fibrosis, independent of HOMA-IR and metabolic syndrome.

Our findings also suggest that vitamin D's association with the degree of NAFLD and liver fibrosis is more prominent in female sex and the non-Hispanic white group (Tables 2 and 3). A possible explanation of vitamin D's prominent role in females could be from the fact that vitamin D level has an inverse relationship to the level of progesterone and estradiol, which are thought to increase histologic severity in NAFLD patients.[29,30] However, recently, Bril *et al.*[31] reported no association of vitamin D levels with insulin resistance or severity of NASH, when matched for clinical parameters (BMI, total adiposity, and prevalence of pre-diabetes/type 2 diabetes).

Our findings also demonstrate that the statistically significant association disappeared after adjusting for known NAFLD risk factors in the full model (Tables 2 and 3). However, this

Table 2. Vitamin D level by degree of NAFLD on liver ultrasound findings

	No NAFLD (n = 6,945)	NAFLD			Unadjusted p-value	Limited model p-value*	Full model p-value#
		Mild (n = 1,491)	Moderate (n = 1,707)	Severe (n = 817)			
Total							
Vitamin D in mg/dL	25.1 ± 0.29	24.7 ± 0.42	23.7 ± 0.37	23.6 ± 0.60	<0.001	<0.01	0.189
Sex							
Male	26.6 ± 0.33	25.7 ± 0.45	25.0 ± 0.50	25.6 ± 0.74	<0.05	<0.05	0.216
Female	23.9 ± 0.34	23.9 ± 0.50	22.3 ± 0.49	20.9 ± 0.68	<0.001	<0.01	0.514
Racial group							
White	26.9 ± 0.31	26.4 ± 0.44	25.1 ± 0.44	25.0 ± 0.69	<0.001	<0.01	0.256
Black	16.9 ± 0.39	16.7 ± 0.47	16.4 ± 0.49	16.4 ± 0.76	0.296	0.112	0.144
Hispanic	21.9 ± 0.34	21.1 ± 0.47	20.4 ± 0.53	20.5 ± 0.48	<0.001	<0.001	0.339

*adjusted for age, sex, race, census region, season.
#adjusted for age, sex, race, census region, season, hypertension, diabetes, dyslipidemia, BMI, metabolic syndrome, HOMA-IR, leptin, IGF-1.
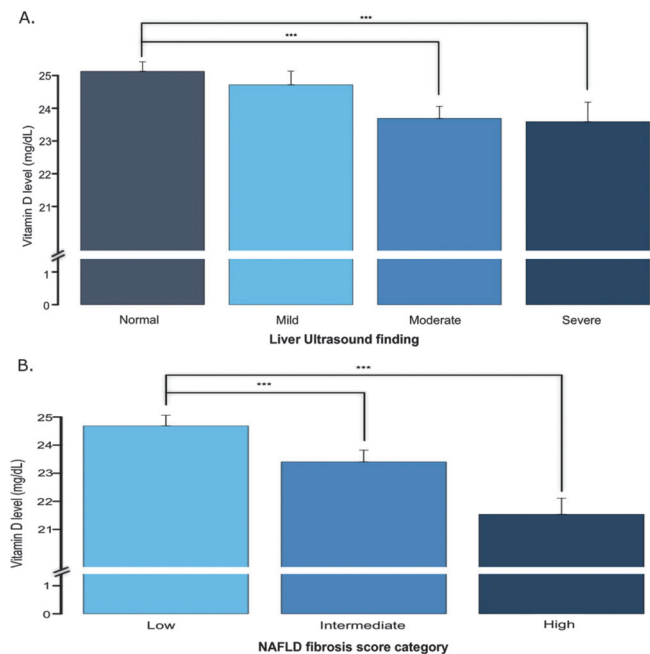
Fig. 2. Vitamin D level by the degree of NAFLD. (A) Vitamin D level by the degree of NAFLD on liver ultrasound findings; **(B)** Vitamin D level by NFS category. ***Unadjusted *p*-value <0.001.

finding may not necessarily imply that vitamin D does not play a role in NAFLD, but rather is confounded by other variables in the same causal pathway in the pathogenesis given vitamin D's role in the development of obesity, diabetes, insulin resistance and metabolic syndrome.[1-4] Table S1 also showed high correlation between vitamin D and metabolic risk factors. Nelson *et al.*[8] recently suggested a possible mechanism by which vitamin D deficiency may be involved in pro-inflammatory pathways, such as MAPK and NF-kappa B in NASH pathogenesis. However, only a long-term follow-up study or clinical trials in NAFLD patients with vitamin D supplements will disclose the true effect of vitamin D in NAFLD pathogenesis. Thus, further investigation is warranted to

disclose a more concrete causal pathway between vitamin D and other risk factors in the pathogenesis of NAFLD.

Our study also suggests that vitamin D deficiency may be associated with mortality in NAFLD, especially related to diabetes and Alzheimer's disease. Several studies reported the mortality patterns of NAFLD,[11,23,32,33] indicating cardiovascular complications as the most common cause of death followed by malignancy and liver disease, as compared to the general population. In our study, we focused on survival analyses only in NAFLD patients in terms of overall and top leading causes of death (cardiovascular, malignancy, cerebrovascular, lung, kidney, diabetes, and Alzheimer's disease). To our knowledge, this is the first study to report vitamin D's prognostic role in NAFLD. In the general population, a study suggested that low vitamin D level is associated with increased overall mortality and cardiovascular disease using NHANES III with follow-up mortality data through 2004.[34]

Our study, with a longer follow-up period (until 2011), also confirmed similar results in our whole participants (Table S3). Furthermore, the Ludwigshafen Risk and Cardiovascular Health study showed that in subjects with metabolic syndrome, vitamin D deficiency is associated with higher all-cause and cardiovascular disease-related mortality.[35] Even though vitamin D deficiency did not reach a statistically significant level in overall morality in NAFLD patients (*p* = 0.06, Table 4), there was still a trend that was consistent with the findings in the general population and in people with metabolic syndrome. A reason for failing to reach significance could be due to the low power of this study.

Even though vitamin D levels have been found to be inversely related to the risk of cardiovascular disease[1], vitamin D deficiency was not prognostic in cardiovascular disease-related mortality in NAFLD participants. Notably, the significant relationship to diabetes-related mortality was only observed in those participants with NAFLD (Table S2). Furthermore, it was interesting to find that vitamin D has a prognostic role in Alzheimer's disease-related mortality in NAFLD. This association could be explained in part by the facts that vitamin D deficiency is linked to cognitive decline[36] and falls[37] among the elderly, and Alzheimer's disease can be induced by NAFLD, insulin resistance, and metabolic syndrome.[38,39]

Table 3. Vitamin D level by degree of NAFLD fibrosis score

	Low NFS (*n* = 2,354)	Intermediate NFS (*n* = 1,379)	High NFS (*n* = 282)	Unadjusted *p*-value	Limited model* (*p*-value)	Full model# (*p*-value)
Total						
Vitamin D in mg/dL	24.7 ± 0.38	23.4 ± 0.42	21.5 ± 0.57	<0.001	<0.05	0.595
Sex						
Male	25.7 ± 0.42	25.1 ± 0.45	24.4 ± 0.79	0.155	0.247	0.520
Female	23.7 ± 0.49	21.5 ± 0.54	19.2 ± 0.75	<0.001	<0.05	0.391
Racial group						
White	26.6 ± 0.41	24.6 ± 0.46	22.9 ± 0.73	<0.001	<0.05	0.369
Black	16.2 ± 0.40	16.6 ± 0.60	16.9 ± 0.97	0.236	0.329	0.908
Hispanic	21.1 ± 0.46	20.2 ± 0.47	17.1 ± 1.04	<0.05	0.261	0.369

*adjusted for age, sex, race, census region, season.
#adjusted for age, sex, race, census region, season, hypertension, diabetes, dyslipidemia, BMI, metabolic syndrome, HOMA-IR, leptin, IGF-1.

Table 4. Mortality by vitamin D level in NAFLD patients

Variable	No. of deaths	Age, sex, race adjusted model	
		HR (95% CI)	*p*-value
All-cause mortality			
Vitamin D ≥20 mg/dL	573	REF	0.06
Vitamin D <20 mg/dL	517	1.16 (0.99–1.36)	
Cardiovascular disease			
Vitamin D ≥20 mg/dL	131	REF	0.29
Vitamin D <20 mg/dL	122	1.28 (0.81–2.03)	
Malignancy			
Vitamin D ≥20 mg/dL	131	REF	0.89
Vitamin D <20 mg/dL	125	1.03 (0.70–1.50)	
Cerebrovascular disease			
Vitamin D ≥20 mg/dL	34	REF	0.64
Vitamin D <20 mg/dL	28	1.26 (0.48–3.34)	
Lung disease			
Vitamin D ≥20 mg/dL	46	REF	0.33
Vitamin D <20 mg/dL	30	1.52 (0.66–3.52)	
Kidney disease			
Vitamin D ≥20 mg/dL	6	REF	0.91
Vitamin D <20 mg/dL	9	1.11 (0.19–6.37)	
Diabetes			
Vitamin D ≥20 mg/dL	36	REF	<0.01
Vitamin D <20 mg/dL	30	3.64 (1.51–8.82)	
Alzheimer's disease			
Vitamin D ≥20 mg/dL	11	REF	<0.01
Vitamin D <20 mg/dL	11	4.80 (1.53–15.1)	

The strength of this study was that our findings were based on nationally representative data that was linked with mortality information with median follow-up of over 18 years. This allowed us to investigate the long-term effects of vitamin D deficiency and assess the mortality outcomes in NAFLD. Limitations of this study included that the NAFLD was not confirmed by liver biopsy. However, previous studies reported good accuracy of screening by liver ultrasound,[23,40] and a non-invasive diagnostic tool may be more appropriate in a large epidemiological study. Due to data restrictions on less common causes of death, liver-related mortality information could not be obtained in the public version of NHANES mortality data. However, since liver-related deaths were even less common than that of kidney-related deaths, we may assume that the liver-related mortality analysis would likely be underpowered. Additionally, participants with other liver diseases, such as autoimmune hepatitis or primary biliary cholangitis, could not be excluded. Lastly, given the cross-sectional study design, a causal relationship of vitamin D in NAFLD could not be disclosed.

In conclusion, vitamin D level was inversely related to the degree of liver steatosis and fibrosis. Moreover, vitamin D deficiency was associated with an increased risk of diabetes- and Alzheimer's disease-related mortality in NAFLD patients.

Further prospective cohort or randomized trials are warranted to confirm our findings and to gain in-depth insight on vitamin D's role with other metabolic risk factors in NAFLD pathogenesis.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study and design, statistical analysis, administrative, technical, or material support, and study supervision (HSK, SHK, NP), acquisition, analysis or interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content (HSK, LR, NK, SHK, NP), full

access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis (HSK). All authors approved the final version of the manuscript.

Disclaimer

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