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AIM AND SCOPE

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

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META-ANALYSIS

Vitamin D levels do not predict the stage of hepatic fibrosis in patients with non-alcoholic fatty liver disease: A PRISMA compliant systematic review and meta-analysis of pooled data

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Author contributions: Saberi B, Dadabhai AS and Mullin GE contributed to the data evaluation, manuscript and figure preparation, editing and final submission; Shinohara RT and Wang L provided biostatistics methodology oversight, as well as data extractions, meta-analysis with pooled data figure preparation and manuscript preparation and editing; Nanavati J provided informatics methodology support, conducted the literature search, and reviewed and edited the manuscript.

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Abstract

AIM

To investigate the relationship between 25-hydroxy-vitamin D [25(OH)D] levels and fibrosis stage in patients with non-alcoholic fatty liver disease (NAFLD).

METHODS

Two individual reviewers identified relevant studies using the PubMed, EMBASE, Cochrane, and Scopus databases. Inclusion criteria were as follows: (1)

Studies that evaluated adults with NAFLD and serum or plasma 25(OH)D levels; and (2) assessed fibrosis stage using liver biopsy. A rigorous analysis yielded six articles as having sufficient data to employ in evaluating the association of serum vitamin D levels in patients with NAFLD based on their liver fibrosis stage by histopathological analysis. The lead investigators of each of the six studies were contacted and the data were collected. To meta-analyze vitamin D levels in F0-F2 *vs* F3-F4 fibrosis, a random-effects meta-analysis fit using restricted maximum likelihood was applied. To examine trends across each stage of fibrosis with respect to vitamin D levels, a meta-regression was performed. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 937 subjects from six studies were included in the final analysis to evaluate the association of serum vitamin D levels in patients with NAFLD based on their liver fibrosis stage by histopathological analysis. The lead investigators of each of the six studies were contacted and the data were collected. First, the investigators performed a meta-analysis to compare serum vitamin D levels in patients with NAFLD with stage F0-F2 compared to F3-F4, which did not show significance [meta-estimate of the pooled mean difference = -0.86, P = 0.08 (-4.17, 2.46)]. A meta-regression evaluation of serum vitamin 25 (OH)D levels across the individual stages (F0-F4) of fibrosis did not show an association for the six included studies.

CONCLUSION

Low vitamin D status is not associated with higher stages of liver fibrosis in patients with NAFLD.

Key words: Vitamin D; 25-hydroxyvitamin D; Liver fibrosis; Meta-analysis; Nonalcoholic fatty liver disease; Non-alcoholic steatohepatitis

Core tip: Non-alcoholic fatty liver disease (NAFLD) is a condition that can progress to cirrhosis, hepatic failure, and liver cancer. Vitamin D sufficiency is impaired in the advanced stages of liver disease and in NAFLD. However, our systematic review of the literature and meta-regression confirms that the serum 25-hydroxyvitamin D levels in patients with NAFLD are not associated with the severity of hepatic fibrosis.

Saberi B, Dadabhai AS, Nanavati J, Wang L, Shinohara RT, Mullin GE. Vitamin D levels do not predict the stage of hepatic fibrosis in patients with non-alcoholic fatty liver disease: A PRISMA compliant systematic review and meta-analysis of pooled data. *World J Hepatol* 2018; 10(1): 142-154 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i1/142.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i1.142

INTRODUCTION

Non-Alcoholic fatty liver disease (NAFLD) represents a growing epidemic that requires better understanding in order to develop new therapeutic targets[1]. The definition of NAFLD is based upon the presence of ⩾ 5% hepatic steatosis without having etiologies, such as alcohol[2]. As one of the most prevalent causes of liver disease worldwide, the importance of NAFLD is gaining prominence in the medical literature and in the press. The prevalence of NAFLD is estimated to be 6% to 35% worldwide and 10% to 35% in the United States, increasing parallel to diabetes and obesity[3-5]. Based on these studies, it is estimated that between 75 million to 100 million individuals are at-risk of having NAFLD in the United States. NAFLD is a condition which has a range of manifestations from steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma[1]. The number of adults with NASH on the liver transplant list has grown by a factor of three, and NASH is the 2nd most common etiology of liver disease in patients who are awaiting liver transplantation[6].

Vitamin D is well known for its physiologic role in mineral and skeletal homeostasis[7]. Ultraviolet light from sun exposure transforms 7-dehydrocholesterol, into pre-vitamin D3, which is converted into vitamin D3 (cholecalciferol). Vitamin D controls the expression of genes linked to various processes including immunomodulation which may be highly pertinent to chronic liver disease. Vitamin D has numerous properties that modulate injury, tissue remodeling, fibrogenesis, and chronic inflammation, which may prevent the progression of chronic liver disease[8,9]. Vitamin D has immunomodulatory actions that include the attenuation of interleukin-2, interferon-γ, and interleukin-12, which drive pro-inflammatory T-helper-1 (Th1) response[10] (Figure 1). Vitamin D upregulates anti-inflammatory T-helper-2 (Th2) cytokines and induces regulatory T cells (Tregs)[11].

Vitamin D has a number of potential roles for favorably altering the course of NAFLD (Figure 2), while it also improves the secretion and tissue sensitization to insulin[12]. The adipocyte is felt to be an important contributor to the pathogenesis of NAFLD. Vitamin D deficiency promotes adipocyte proinflammatory cytokines (adipokines), which are elevated in individuals with obesity, metabolic syndrome, and NAFLD, and are felt to contribute to disease[13,14]. Furthermore, vitamin D has been shown to upregulate adiponectin-an adipocyte-derived hormone. Adiponectin improves insulin sensitivity and prevents atherogenesis, which is decreased in those with obesity, metabolic syndrome, and NAFLD[15]. Vitamin D has been shown to inhibit hepatic inflammation and attenuates liver fibrosis in animal models[16]. Thus, the relationship of vitamin D deficiency to NAFLD pathogenesis merits careful analysis.

Numerous reports have revealed that patients

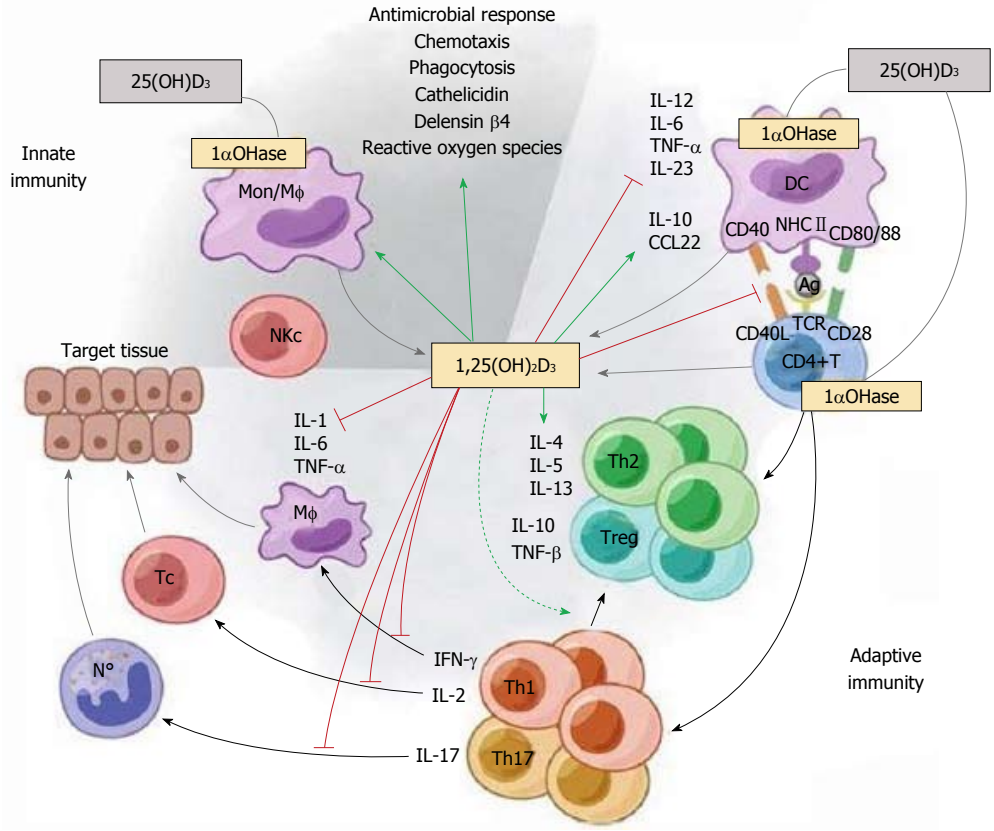

Figure 1 The immunomodulatory effects of 1,25(OH)$_2$D$_3$. 1,25(OH)$_2$D$_3$ targets different players of the innate and adaptive immune compartment. 1,25(OH)$_2$D$_3$ stimulates innate immune responses by enhancing the chemotactic and phagocytotic responses of macrophages, as well as the production of antimicrobial proteins such as cathelicidin. On the other hand, 1,25(OH)$_2$D$_3$ also modulates adaptive immunity. At the level of the APC (like the DC), 1,25(OH)$_2$D$_3$ inhibits the surface expression of the MHC-Ⅱ-complexed antigen and co-stimulatory molecules, in addition to the production of the cytokines IL-12 and IL-23, thereby indirectly shifting the polarization of T cells from a Th1 and Th17 phenotype towards a Th2 phenotype. In addition, 1,25(OH)$_2$D$_3$ directly affects T cell responses, by inhibiting the production of Th1 cytokines (IL-2 and IFN-γ) and Th17 cytokines (IL-17 and IL-21), and by stimulating Th2 cytokine production (IL-4). Moreover, 1,25(OH)$_2$D$_3$ favors Treg cell development *via* modulation of DCs and by directly targeting T cells. Finally, 1,25(OH)$_2$D$_3$ blocks plasma cell differentiation, IgG and IgM production, and B cell proliferation. Reproduced with the permission of the Nature Publishing Group[52].

with chronic liver disease from different etiologies had low vitamin D status[17-21]. In particular, liver diseases heralded by autoimmune or chronic inflammatory states appear to be worsened in the setting of vitamin D deficiency. In a pooled data meta-analysis, we recently showed that in nine of the 12 studies on mono-infected or co-infected patients with chronic hepatitis C, stages three and four fibrosis were associated with profound 25-hydroxyvitamin D deficiency and the associated odds ratio (OR) and the 95% confidence interval (CI) were 1.88 (1.27, 2.77)[22]. The total heterogeneity, I^2, was 66.94%, thus indicating that there was substantial heterogeneity between studies[22].

A recent meta-analysis supports the contention that individuals with NAFLD with and without non-alcoholic steatohepatitis (NASH) are more prone to have hypovitaminosis D[23]. Wang *et al*[23] extracted data from 29 studies and reported that subjects with NAFLD had decreased 25-hydroxyvitamin D and were 1.26 times more likely to be vitamin D deficient. Individuals with inflammatory disease (NASH) have also been reported to have decreased levels of 25(OH)D. In support of our prior findings for chronic hepatitis C, recent studies have suggested that vitamin D levels are further decreased in advanced stages of fibrosis[24-26]. However, limitations have been observed regarding the criterion used to diagnose NAFLD, clinical variation in disease severity among the study groups, and inconsistency in defining vitamin D deficient states[9].

A number of investigations have attempted to link vitamin D status to histological disease activity and fibrosis of NAFLD[26-32]. Jaruvongvanich *et al*[33] systematically reviewed the literature to determine if vitamin D status was associated with NAFLD disease activity or fibrosis score and extracted data from six included studies involving 974 NAFLD subjects[33]. These investigators did not find a difference in the serum 25-hydroxyvitamin D levels among NAFLD patients with high histologic activity *vs* low, nor high fibrosis score *vs* low. In light of these findings, the investigators concluded that vitamin D status was not related to the histologic activity of NAFLD. In their study, Jaruvongvanich *et al*[33] did not assess the association of vitamin D levels across each precise stage of liver fibrosis based on liver biopsy in patients NAFLD.

In the current study, we determined the relationship between serum vitamin D status relative to the precise degree of hepatic fibrosis. Based on the histopath-

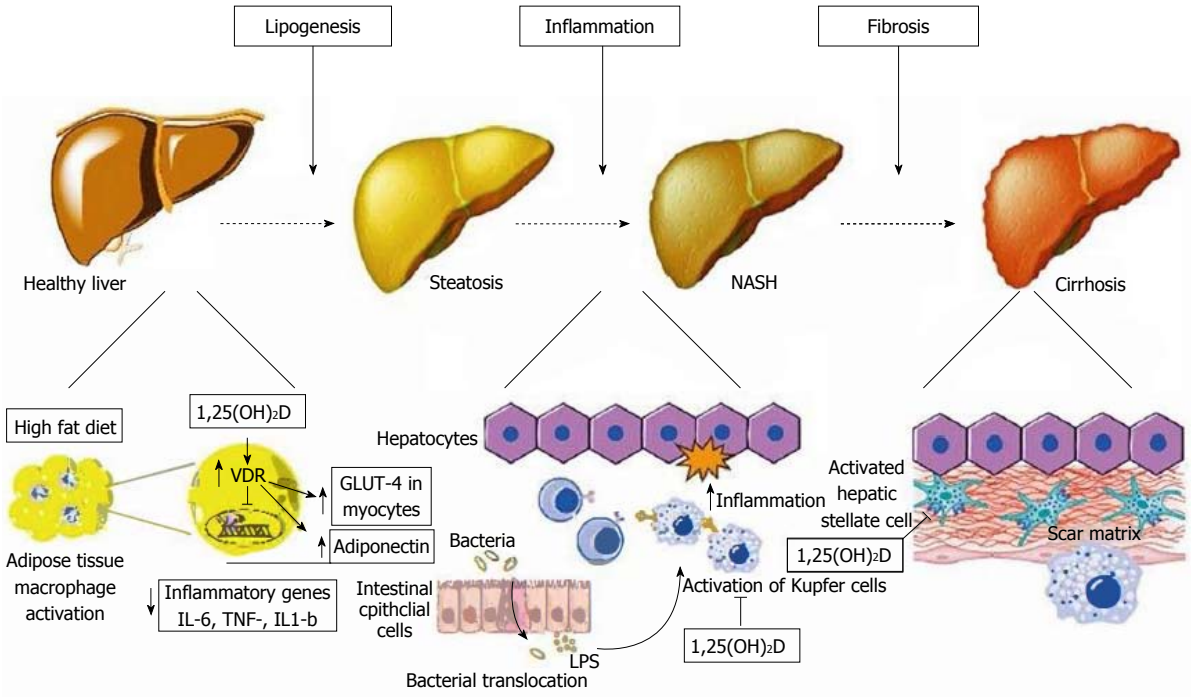

Figure 2 Schematic representation of metabolic, anti-inflammatory, and anti-fibrotic effects of vitamin D on hepatocytes and non-parenchymal hepatic cells (hepatic stellate cells, Kupffer cells) in non-alcoholic fatty liver disease. Left: At the initial stage of lipogenesis, 1,25(OH)D acts on adipocytes and inhibits NF-κB transcription, known as the pro-inflammatory "master switch", and thus inhibits the expression of the inflammatory cytokines IL-6, TNF-α, and IL-1β. It also increases adiponectin secretion from adipocytes and enhances GLUT-4 receptor expression in myocytes, both of which improve insulin resistance; Middle: Increased gut permeability allows the translocation of bacterial pathogens which can activate Toll-like receptors (TLR) on Kupffer cells. 1,25(OH)D downregulates the expression of TLR-2, TLR-4, and TLR-9 in these cells, thus ameliorating inflammation; Right: 1,25(OH)D acts on hepatic stellate cells by binding to VDR, which reduces the proliferation of these cells that play a major role in inducing fibrosis. VDR: Vitamin D receptor; TLR: Toll-like receptor; LPS: Lipopolysaccharide. Reproduced in compliance with Creative Commons in PubMed Central Open Access to Reproduced with the permission of the Baishideng Publishing Group Inc[9].

ological staging in patients with NAFLD, we performed a systematic review and meta-analysis[34].

MATERIALS AND METHODS

Literature search

The present meta-analysis was performed according to the Cochrane Collaboration and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements[35]. Applicable studies were identified by a library literature search using the PubMed, Embase, Cochrane, and Web of Science databases by utilizing the PRISMA checklist from its inception to March 2017, then updated in September 2017. "Present a full electronic search strategy for at least one database, including any limits used, so that it could be repeated" and the Cochrane review reporting guidelines (6.6.2.2). The mesh terms for PubMed were as follows: "Non-alcoholic fatty liver disease", "Vitamin D", and "Liver cirrhosis". Also, the studies cited by the selected articles were searched for further pertinent studies. The details of the search strategy were prepared by the informationist (JN) in collaboration with the authors (Saberi B, Dadabhai AS and Mullin GE), as shown in Table 1.

Study selection

In the first phase, two separate reviewers carefully reviewed the abstract of the studies. When there was an agreement between two reviewers that a study fit the inclusion and exclusion criteria (Table 2), the article was then selected for further assessment. When there was a disagreement between the two reviewers, a third reviewer determined whether the study met the criteria for inclusion. Once the abstracts were included, the text was then carefully reviewed and data extraction was completed by at least two of the reviewers. The flowchart of the included studies is shown in Figure 3.

Data extraction

A total of six studies were included for extraction, which was performed by two independent reviewers (GM, BS) based on data quality, sufficiency, and relevance. Disagreements were resolved by a third reviewer (TS) to reach a consensus. The following data were extracted: last name of the first author, demographic information of patients, publication year, population, sample size, BMI, ALT, study design, method of vitamin D measurement, vitamin D levels in control and subjects, stage of fibrosis based on liver biopsy, and association of serum vitamin D level and fibrosis stage (Table 3). We then contacted the investigators of each study and collected the details of their data regarding serum vitamin D level measurements based on the stages of liver fibrosis (Tables 4 and 5). The

Table 1 Search results of vitamin D and non-alcoholic fatty liver disease

Database/search	Search terms	Search results
EMBASE		
1	("liver cirrhosis"/exp OR cirrhosis: ti, ab OR cirrhoses: ti, ab OR fibrosis: ti, ab OR fibroses: ti, ab)	
2	("vitamin D"/exp OR "25 hydroxyvitamin d"/exp OR "vitamin d": ti, ab OR "ergocalciferols": ti, ab OR "ergocalciferol": ti, ab OR "25 hydroxy vitamin d": ti,ab OR "25 hydroxyvitamin d": ti, ab OR "25 hydroxy d": ti, ab OR "25(OH)D": ti, ab OR "25-hydroxyvitamin d 2": ti, ab)	
3	("nonalcoholic fatty liver"/exp OR "Non-alcoholic Fatty Liver": ti, ab OR "nonalcoholic fatty liver": ti, ab OR "Non-alcoholic Fatty Livers": ti, ab OR "nonalcoholic fatty livers": ti, ab OR "NAFLD": ti, ab OR "NASH": ti, ab OR "nonalcoholic steatohepatitis": ti, ab OR "nonalcoholic steatohepatitides": ti,ab OR "fatty liver"/de OR "fatty liver": ti, ab OR "Steatohepatitis": ti, ab OR "Steatosis of Liver": ti, ab OR "Liver Steatosis": ti, ab OR "Liver Steatoses": ti, ab OR "hepatic steatosis": ti, ab OR "hepatosteatosis": ti, ab)	
4	1 and 2 and 3	199
Web of science		
1	("Non-alcoholic Fatty Liver" OR "nonalcoholic fatty liver" OR "Non-alcoholic Fatty Livers" OR "nonalcoholic fatty livers" OR "NAFLD" OR "NASH" OR "nonalcoholic steatohepatitis" OR "fatty liver" OR Steatohepatitis OR "Steatosis of Liver" OR "Liver Steatosis" OR "Liver Steatoses" OR "hepatic steatosis" OR "hepatosteatosis")	
2	("liver cirrhosis" OR cirrhosis OR cirrhoses OR fibroses OR fibrosis)	
3	("vitamin d" OR "ergocalciferols" OR "ergocalciferol" OR "25 hydroxy vitamin d" OR "25 hydroxyvitamin d" OR "25 hydroxy d" OR "25(OH)D" OR "25-hydroxyvitamin d 2")	
4	1, 2 and 3	69
Cochrane		
1	MeSH descriptor: [Non-alcoholic Fatty Liver Disease] explode all trees	181
2	MeSH descriptor: [Liver Cirrhosis] explode all trees	2462
3	MeSH descriptor: [Vitamin D] explode all trees	2907
4	"Non-alcoholic Fatty Liver" or "nonalcoholic fatty liver" or "Non-alcoholic Fatty Livers" or "nonalcoholic fatty livers" or "NAFLD" or "NASH" or "nonalcoholic steatohepatitis" or "fatty liver" or Steatohepatitis or "Steatosis of Liver" or "Liver Steatosis" or "Liver Steatoses" or "hepatic steatosis" or"hepatosteatosis": ti, ab, kw	1470
5	"liver cirrhosis" or cirrhosis or cirrhosis or fibrosis or fibroses: ti,ab,kw	13273
6	"vitamin d" or "ergocalciferols" or "ergocalciferol" or "25 hydroxy vitamin d" or "25 hydroxyvitamin d" or "25 hydroxy d" or "25(OH)D" or "25-hydroxyvitamin d 2": ti,ab,kw	6061
7	1 or 4	1470
8	2 or 5	13273
9	3 or 6	6722
10	7 and 8 and 9	13
PubMed		
1	(("Non-alcoholic Fatty Liver Disease"[Mesh] OR "Non-alcoholic Fatty Liver"[tw] OR "nonalcoholic fatty liver"[tw] OR "Non-alcoholic Fatty Livers"[tw] OR "nonalcoholic fatty livers"[tw] OR "NAFLD"[tw] OR "NASH"[tw] OR "nonalcoholic steatohepatitis"[tw] AND "Fatty Liver"[Mesh: noexp] OR "fatty liver"[tw] OR Steatohepatitis[tw] OR "Steatosis of Liver"[tw] OR "Liver Steatosis"[tw] OR "Liver Steatoses"[tw] OR "hepatic steatosis"[tw] OR "hepatosteatosis"[tw])	
2	("vitamin d"[mh] OR "vitamin d"[tw] OR "ergocalciferols"[tw] OR "ergocalciferol"[tw] OR "25 hydroxy vitamin d"[tw] OR "25 hydroxyvitamin d"[tw] OR "25 hydroxy d"[tw] OR "25(OH)D"[tw] OR "25-hydroxyvitamin d 2"[tw])	
3	("liver cirrhosis"[mh] OR cirrhosis[tw] OR cirrhoses[tw] OR fibrosis[tw] OR fibroses[tw])	
4	1 and 2 and 3	56
		Search results
	Total	337
	Duplicated	101
	Final total	236

methodologies utilized by the authors to assess the severity of fibrosis score are shown in Tables 6 and 7.

Statistical analysis

Statistical computations were conducted in R (Version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria, 2016)[36] and RevMan 5.3 (The Cochrane Collaboration, 2014). In several studies, the mean and variance of vitamin D levels in the combined F0-F2 and F3-F4 fibrosis stage groups were unavailable in combined form despite multiple attempts from the authors; hence, the vitamin D levels were estimated using Monte Carlo simulations assuming vitamin D levels were normally distributed with the reported parameters for each fibrosis stage. For the meta-analysis of the comparisons between low fibrosis (F0-F2) *vs* high fibrosis (F3-F4), a random-effects meta-analysis fit using a restricted maximum likelihood (REML) was then fit using the metafor package in R. To assess associations across each fibrosis level, a meta-regression fit *via* REML was conducted using the metan and metareg functions in RevMan 5.3. $P < 0.05$ was considered statistically significant[36]. The risk of publication bias across the included studies for all outcome measures was assessed by the construction of funnel plots.

RESULTS

Study selection

The search strategy utilized medical subject headings (MeSH) terms used to identify articles that evaluated

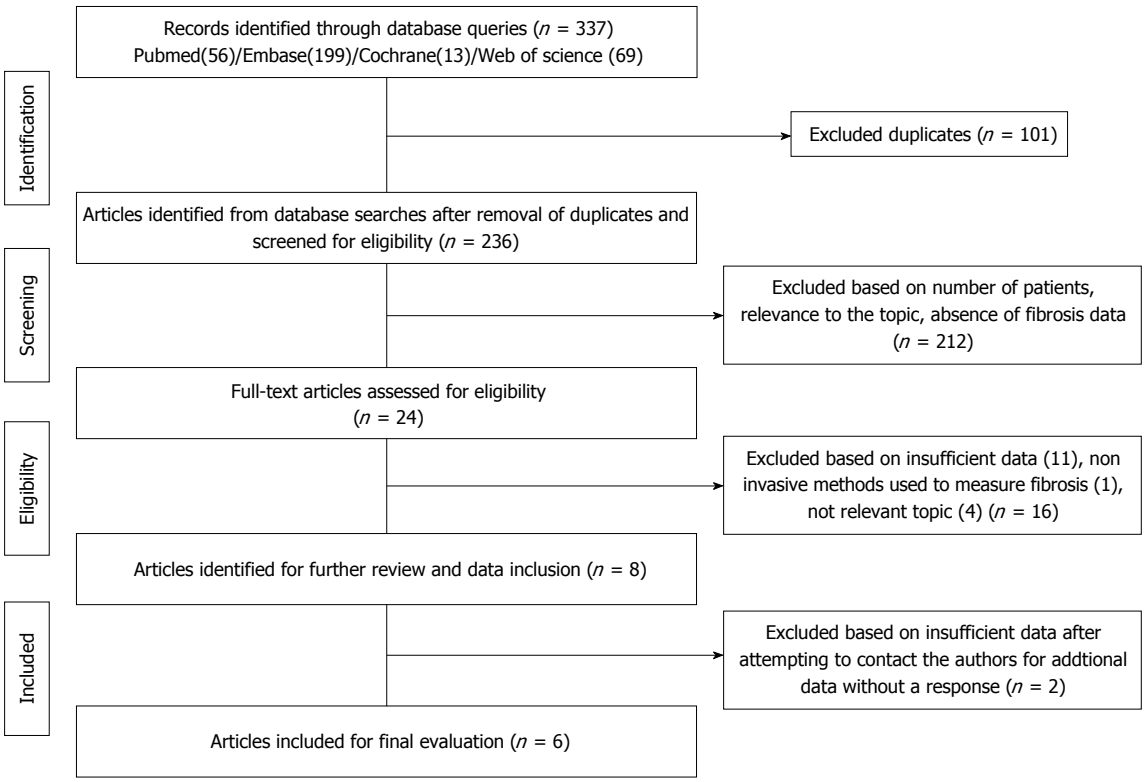

Figure 3 Flowchart illustrating the process for the selection of the included articles. Three hundred and thirty-seven articles were identified using PubMed (*n* = 56)/EMBASE (*n* = 199)/Cochrane (*n* = 13)/Web of Science (*n* = 69) search engines. A detailed evaluation of the articles by at least two independent reviewers (total of three) assessed the sufficiency of data, the method of fibrosis qualification, and relevance to the topic in order to narrow the studies to six.

Table 2 Inclusion and exclusion criteria of studies on vitamin D in non-alcoholic fatty liver disease

Inclusion criteria
Patients ⩾ 18 yr
Studies that evaluated vitamin D in NAFLD
Studies that evaluated the liver fibrosis stage, only based on liver biopsy
Studies that reported serum or plasma 25(OH)D levels
Exclusion criteria
Age < 18 yr
Liver diseases other than NAFLD
Studies that used non-invasive methods to evaluate liver fibrosis
Studies with inadequate data

25-OH(D): 25-hydroxyvitamin D; NAFLD: Nonalcoholic fatty liver disease.

serum vitamin D levels in patients with NAFLD based on the severity of liver fibrosis stage. Three hundred and thirty-seven articles were identified by PubMed (*n* = 56), EMBASE (*n* = 199), Cochrane (*n* = 13), and Web of Science (*n* = 69) search engines and one hundred and one duplicates were removed. Two independent reviewers provided a detailed evaluation of the articles assessed. This evaluation included data adequacy, criterion used to measure fibrosis, and overall pertinence to streamline for qualitative synthesis (Figure 3). All studies were cross-sectional. Table 2 summarizes the baseline characteristics, including the year of study, country, gender, population, BMI, Mean ALT (IU/L), vitamin D levels in NAFLD, and control patients. We then contacted investigators for the included studies and collected detailed data on vitamin D levels (Median or interquartile ranges; IQRs) based on the stage of fibrosis 4 (F0-F4). Out of the eight studies eligible for quantitative synthesis, we were able to gather and assemble vitamin D levels for each fibrosis stage category in a total of six studies (Tables 3-5). Based on this information on serum vitamin D levels, we then performed a quantitative synthesis across the six studies by performing a meta-analysis comparing F0-F2 (low fibrosis) *vs* F3-F4 (high fibrosis) groups and a meta-regression for the five categories of liver fibrosis (F0-F4).

Definition of vitamin D levels

Vitamin D status is based upon serum 25(OH)D values but this remains controversial. The most stable and plentiful metabolite of vitamin D in human serum, 25(OH)D, has a half-life of about 3 wk, making it the most suitable indicator of vitamin D status[37]. The lower limit of normal was defined as being less than 30 ng/mL, thus serum 25(OH)D lower than 30 ng/mL defined insufficiency. Deficient serum vitamin D was defined by some investigators as 25(OH)D < 20 ng/mL while others used < 10 ng/mL as the cutoff. During the data extraction, we discovered that two of the studies did not use ng/mL to express serum 25(OH)D. Instead, the unit used was nmol/L to express serum

Table 3 Characteristics of patients' studies for vitamin D status in non-alcoholic fatty liver disease

Citation	Patel *et al*[32]	Luger *et al*[30]	Barchetta *et al*[28]	Anty *et al*[27]	Dasarthy *et al*[24]	Bril *et al*[29]	Nelson *et al*[31]	Targher *et al*[26]
Year	2016	2016	2012	2016	2014	2015	2016	2007
Country	United States	Austria	Italy	France	United States	United States	United States	Italy
Subjects (M, F)	293 (195, 98)	50 (10, 40)	45 (22, 23)	398 (64, 334)	187 (51, 136)	239 (204, 35)	190 (89, 101)	120 (80, 40)
Population	Suspected NAFLD undergoing liver biopsy	Gastric bypass patients	Suspected NAFLD	Morbidly obese referred for bariatric surgery	Biopsy proven NAFLD, normal controls	Overweight patients	Biopsy proven NAFLD	Biopsy proven NAFLD
Mean BMI	36.1 ± 7.8	43.8 ± 4.3	30.5 ± 5.5	42.8 ± 5.0	35.7 ± 7.0	34.6 ± 0.4	35.6 ± 10.8	26.3 ± 2.0
Subjects	NAFLD	All	NASH	All	NAFLD	NASH	NAFLD	NAFLD
Mean ± SD ALT IU/L	66.5 ± 51.2	36.4 ± 20.8	87.5 ± 46.6	35.2 ± 24.5	45.9 ± 30.0	64.0 ± 4.0	77.0 ± 48.2	105 ± 42.0
Subjects	NAFLD	All	NASH	Morbidly Obese	NAFLD	NASH	NAFLD	NAFLD
Study design	Cross sectional	Cross sectional	Cross sectional	Cross sectional	Cross sectional	Cross sectional	Cross sectional	Cross sectional
Vitamin D analysis	CLIA	Not described	CLIA	CLIA	CLIA	CLIA	GC-MS	CLIA
Mean/SD 25(OH)D (ng/mL), (*n*) subjects	27.6 ± 11.8	15.6 ± 5.6	22.0 ± 12.4	19.2 ± 9.0	21.2 ± 10.4	21.8 ± 1.0	20.9 ± 4.0	20.4 ± 8.8
Mean/SD 25(OH)D (ng/mL) Non-NAFLD Controls	27.9 ± 12.8	NA	52.9 ± 11.02	21.5 ± 10/2	35.7 ± 6.0	24.5 ± 2.1	NA	30.0 ± 6.0
P value; NAFLD *vs* controls	0.878	NA	Not significant	0.13	< 0.01	0.18	NA	< 0.001

n: Number of subjects; 25-OH(D): 25-hydroxylvitamin D; SD: Standard deviation; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; BMI: Body mass index; CLIA: Chemiluminescence; GC-MS: Gas chromatography mass spectroscopy; M: Male; F: Female.

Table 4 Relationship of vitamin D to liver fibrosis in non-alcoholic fatty liver disease

Author/year/Ref	(*n*), 25-OH(D) Mean ± SD F0	(*n*), 25-OH(D) Mean ± SD F1	(*n*), 25-OH(D) Mean ± SD F2	(*n*), 25-OH(D) Mean ± SD F3	(*n*), 25-OH(D) Mean ± SD F4	*P* value
Patel *et al*[32], 2016	(39) 24.4 ± 10.4	(78) 26.5 ± 8.9	(55) 29.1 ± 12.5	(63) 30.7 ± 14.1	(9) 20.2 ± 20.2	0.028
Targher *et al*[26], 2007	(16) 20.8 ± 8.4	(10) 14.4 ± 9.2	(7) 10.0 ± 10.0	(6) 6.0 ± 10.8	0	0.01
Anty *et al*[27], 2016	(50) 20.04 ± 7.81	(233) 19.91 ± 9.12	(98) 18.28 ± 9.58	(15) 16.71 ± 9.86	(2) 25 ± 10.18	0.01
Luger *et al*[30], 2016	(2) 15.6 ± 5.2	(30) 15.2 ± 6.0	(8) 15.6 ± 4.4	(4) 17.6 ± 7.6	(2) 20.4 ± 4.4	0.792
Bril *et al*[29], 2015	(61) 20.5 ± 10.4	(75) 24.2 ± 15.1	(22) 20.8 ± 12.1	(22) 25.5 ± 12.2	(5) 21.1 ± 6.9	0.27
Barchetta *et al*[28], 2012	(1) 20.5	(10) 23.5 ± 14.4	(7) 16.25 ± 6.1	(6) 28.8 ± 14.9	(1) 17.3	0.56

n: Number of subjects; 25-OH(D): 25-hydroxylvitamin D; SD: Standard deviation; F0-F4: Severity score of hepatic fibrosis.

25(OH)D. Vitamin D insufficiency was defined as below the lower limit of normal (< 80 nmol/L).

Association between vitamin D deficiency and the severity of liver disease

Six included studies were cross-sectional analyses. A meta-analysis was conducted to compare the 25(OH) serum levels in patients with NAFLD according to the fibrosis stage (F0-F2 *vs* F3-F4) using a random-effects model. The results are shown in the Forest plot in Figure 4. We found no difference in the serum vitamin D levels according to high *vs* low severity of hepatic fibrosis in subjects with NAFLD [(meta estimate mean difference = -0.86 (-4.17, 2.46)], I^2 (total heterogeneity /total variability): 50.0%, χ^2 = 9.95, df = 5, *P* value = 0.08]. The forest plot (Figure 4 and Supplemental Figure 1) also demonstrates heterogeneity among the six studies. The funnel plot in Figure 5 shows some asymmetry, thereby suggesting a limited publication bias within the studies. The NAFLD subjects in two of the eight relevant studies from the qualitative synthesis had significantly lower serum 25(OH)D in controls when compared to those with NAFLD[24,26].

We then further categorized the patients into five groups based on the stage of their fibrosis from F0-F4 (Table 4) and conducted a meta-regression, and found no association (*P* = 0.86, Supplementary Figure 2) between fibrosis stage and vitamin D levels across the six studies.

DISCUSSION

We examined the peer-reviewed literature of reports of NAFLD patients for an association of serum vitamin D with the stage of liver fibrosis by conducting a systematic review and meta-analysis. A total of eight cross-sectional studies underwent a full article review and were included for qualitative synthesis. We contacted the investigators of each study and collected details of their data regarding serum vitamin D level measurements based on the specific stage of liver fibrosis. Investigators from six of the eight included studies provided sufficient data to perform a quantitative analysis on a total of 937 subjects with the diagnosis of NAFLD. First, we performed a meta-

Table 5 Relationship of vitamin D to liver fibrosis in non-alcoholic fatty liver disease by high *vs* low fibrosis score

Author	(*n*), 25-OH(D) Mean ± SD F0-F2	(*n*), 25-OH(D) Mean ± SD F3-F4
Patel *et al*[32], 2016	(172) 26.9 ± 10.7	(72) 29.4 ± 15.4
Targher *et al*[26], 2007	(33) 16.6 ± 10.0	(6) 6.0 ± 10.8
Anty *et al*[27], 2016	(381) 19.5 ± 9.1	(17) 17.7 ± 10.0
Luger *et al*[30], 2016	(40) 15.2 ± 5.6	(6) 18.6 ± 6.4
Bril *et al*[29], 2015	(158) 22.3 ± 13.2	(27) 24.7 ± 11.5
Barchetta *et al*[28], 2012	(7) 20.2 ± 11.07	(18) 26.7 ± 14.2

n: Number of subjects; 25-OH(D): 25-hydroxylvitamin D; SD: Standard deviation; F0-F4: Severity score of hepatic fibrosis.

Table 6 Levels of sIL-2R, ALT, and HBV DNA in the sera of patients with chronic HBV infection (mean ± SD)

Study	Fibrosis stage used
NASH Clinical Research Network Scoring System Definition	Seven stages:
Kleiner *et al*[53], 2005	F0: No fibrosis F1a: Mild zone 3 sinusoidal fibrosis F1b: Moderated zone 3 sinusoidal fibrosis F1c: Peri-portal sinusoidal fibrosis F2: Zone 3 sinusoidal fibrosis and peri-portal sinusoidal fibrosis F3: Bridging fibrosis F4: Cirrhosis
Brunt *et al*[54], 1999	Stage 1: Zone 3 perisinusoidal/pericellular fibrosis; focally or extensively present Stage 2: Zone 3 perisinusoidal/pericellular fibrosis with focal or extensive periportal fibrosis Stage 3: Zone 3 perisinusoidal/pericellular fibrosis and portal fibrosis with focal or extensive bridging fibrosis Stage 4: Cirrhosis

NASH: Nonalcoholic steatohepatitis.

analysis comparing 25(OH)D levels in subjects with high *vs* low stages of fibrosis (F0-F2 *vs* F3-F4). This association was not statistically significant [meta-estimate pooled mean difference= -0.86, P = 0.08 (-4.17, 2.46)]. These results were consistent with the findings by Jaruvongvanich *et al*[33] who reported that there was no difference in serum 25-hydroxyvitamin D levels among 974 NAFLD subjects across the same six studies. In their study, Jaruvongvanich *et al*[33] compared the high *vs* low histologic activity of NAFLD [pooled mean difference =-0.93 (-2.45, 0.58), I^2 = 0%], and likewise, for the high *vs* low fibrosis score [pooled mean difference=0.88 (-2.65, 4.42), I^2=64%][33]. They concluded that vitamin D status was not related to the histologic activity of NAFLD. We also conducted a meta-regression to determine whether there was an association between serum vitamin D levels and stage of liver fibrosis (F0-F4) in NAFLD. As shown in Table 4, there are conflicting reports with three studies demonstrating significance

Table 7 Methodology for grading of hepatic fibrosis utilized by the authors of the six included studies

Study	Fibrosis stage used
Anty *et al*[27], 2016	Kleiner *et al*[53], 2005
Barchetta *et al*[28], 2012	Brunt *et al*[54], 1999
Bril *et al*[29], 2015	Kleiner *et al*[53], 2005
Luger *et al*[30], 2016	Kleiner *et al*[53], 2005
Patel *et al*[32], 2016	Kleiner *et al*[53], 2005
Targher *et al*[26], 2007	Brunt *et al*[54], 1999

($P < 0.05$)[26,27,32] and three finding no association ($P > 0.05$)[28-30]. Our meta-regression did not find an association between vitamin D level and fibrosis stage across the studies.

As mentioned earlier, NAFLD encompasses a histological spectrum that encompasses a wide range of pathology. Hepatic steatosis, inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma are all possible consequences of NAFLD, and can even coexist in the same patient. It is well documented that a proportion of patients with NASH with liver inflammation will develop fibrosis, with this stage progressing over time from F0 to F4[38]. In a meta-analysis of patients with NAFLD, the proportion of fibrosis for stage 0 (35.8%), stage 1 (32.5%), stage 2 (16.7%), 3 (9.3%), and 4 (5.7%) respectively[39]. Patients with NASH and baseline F0 fibrosis had an estimated annual fibrosis progression rate of 0.14 stages (95%CI, 0.07-0.21 stages), corresponding to 1 stage progression over 7.1 years for patients with NASH (95%CI, 4.8-14.3)[39]. It is well known that the major risk factors for NAFLD include obesity, insulin resistance, dyslipidemia, diabetes mellitus, and metabolic syndrome[38].

Vitamin D receptors (VDR) are expressed abundantly in the liver had have diverse consequences on metabolism which include the regulation of genes involved in glucose and lipid metabolism, and immunomodulation[40]. Low vitamin D has been reported to be strongly associated with insulin resistance[41]. Previous studies have estimated links between vitamin D and the development of NAFLD through various mechanisms that were recently reviewed by Eliades *et al*[9]. Vitamin D action on adipocytes and downregulates inflammatory cytokines IL-6, TNF-α and IL-1β through NF-κB pathway. Vitamin D also enhances the GLUT-4 receptor expression in myocytes, and also improves insulin utilization by increasing adiponectin secretion from adipocytes. Vitamin D downregulates the expression of various toll receptors in kupffer cells, thereby lessening inflammation caused by bacterial translocation (Figure 2)[9].

Also, researchers have noted vitamin D to have antifibrotic properties, as well as its involvement in the pathophysiology of liver fibrosis. The main cell involved in development of fibrosis in NAFLD is hepatic stellate cell (HSC). The HSCs become activated by losing their characteristic vitamin A droplets. Activated

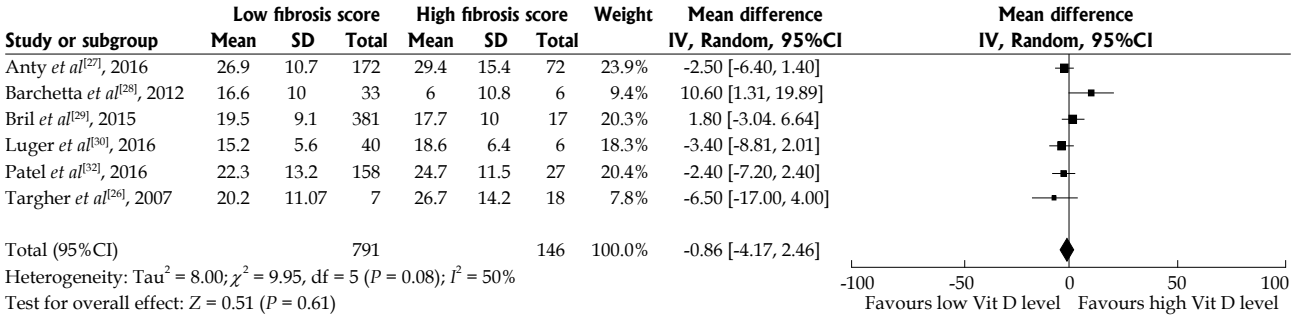

Figure 4 Random effects pooled the mean difference of 25-hydroxyvitamin D levels in nonalcoholic fatty liver disease patients with high and low fibrosis scores. A meta-analysis of the pooled data of the six included studies according to fibrosis scores of low F0-2 *vs* high F3-4. Figure 4 illustrates the forest plot of the results of the six included studies, with 95%CI, and the overall effect (under the random-effects model) with 95%CI are illustrated in this forest plot. The six included studies[26-30,32] assessed the association of 25-hydroxyvitamin D among patients with nonalcoholic fatty liver disease (NAFLD). We used a random-effects model to assess the pooled data in a meta-analysis as previously described[36]. The statistical heterogeneity was not significant with I^2 of 37.8% ($P_{heterogeneity}$ = 0.0766); however, we observed a trend towards high heterogeneity. We found no difference in 25-hydroxyvitamin D among NAFLD patients with high (F3-4) *vs* low (F0-2) fibrosis, with the summary effect size of 0.95 representing mean differences between F0-2 and F3-4 NAFLD patients. Overall, our analysis confirmed that there was no association between serum 25-hydroxyvitamin D and low *vs* high fibrosis score in NAFLD patients from the six included studies.

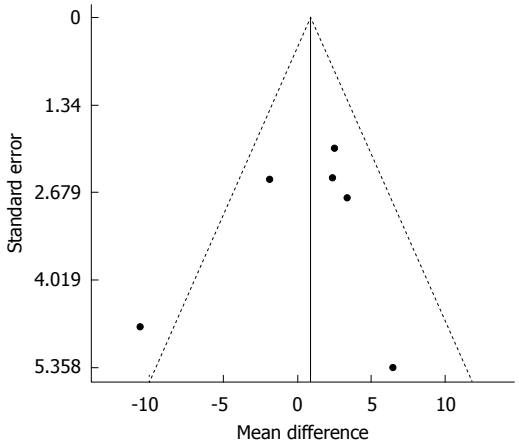

Figure 5 Funnel plot of standard error by differences in Means for 25(OH)D. We analyzed the data for a possible publication bias. The circles represent observed published studies. The funnel plot was asymmetric, thereby suggesting a possible publication bias.

HSCs then produce an extracellular matrix, which leads to fibrosis and cirrhosis[42]. It is thought that the effect of vitamin D on the liver is complex but by binding to HSC VDR it reduces proliferation of these cells which play a major role in inducing fibrosis. It is known that liver nonparenchymal cells, including HSCs, express fully functional VDR, which has led many researchers to consider the vitamin D pathway as a possible modulator of liver fibrosis[43,44]. Ding *et al*[45] demonstrated that administration of the synthetic VDR agonist Calcipotriol ameliorated liver fibrosis in a standard mouse model of a Carbon Tetrachloride (CCL4) hepatic injury. Interestingly, they also showed that liver fibrosis was discovered in mice who have a genetic deletion of VDR, which strongly supports its role in hepatic homeostasis. Furthermore, activation of VDR signaling interferes with a wide range of transforming growth factor-beta (TGFβ)/SMAD)-dependent transcriptional responses on pro-fibrotic genes in HSCs[45].

In addition to the suggested mechanistic link between vitamin D and NAFLD, various clinical cohorts have shown the association of vitamin D and fibrosis in fatty liver disease patients. In a study by Nelso *et al*[31] 190 biopsy-proven NASH adults in the Non-alcoholic Steatohepatitis Clinical Research Network (NASHCRN) cohort were reviewed. The results demonstrate an independent association between serum 25-hydroxyvitamin D, increased NASH histological activity, and the presence of fibrosis. Although epidemiologic studies are promising in showing the association between low vitamin D levels and chronic liver disease, such as NAFLD, this study suggests that the current literature has a dearth of evidence to establish causality between vitamin D and the histopathologic stage of liver fibrosis[8]. Some of the recent studies raised doubts regarding a causal link between vitamin D deficiency and non-skeletal health outcomes reviewing prospective studies and clinical trials, thereby suggesting that having a vitamin D deficiency is a predictor rather than the cause of the disease[46]. Well-designed prospective randomized clinical trials are needed to better understand the influence the oral intake (food and supplement) of vitamin D to the point of sufficiency on disease progression in NAFLD patients.

A few clinical trials using small numbers of study subjects have evaluated the effect of vitamin D supplementation in patients with NAFLD. These studies should be interpreted with caution, given the small sample sizes and short course of follow up. In a small double-blind, placebo-control trial study, NAFLD patients were randomly assigned to receive vitamin D (50000 IU every 14 d for 4 mo) *vs* placebo[47]. The period of 4 mo was used as the benchmark for analysis of results. The authors reported that the serum levels of liver chemistries, homeostatic model assessment for insulin resistance (HOMA-IR), or grades of hepatic steatosis as measured by ultrasound, were not at variance (vitamin D *vs* placebo)[47].

In a more recent study, a 12-wk, randomized, controlled, double-blind trial was conducted on 120 NAFLD patients randomly assigned to three groups. Each patient received 25 μg calcitriol (n = 37), 500 mg calcium carbonate, plus 25 μg calcitriol (n = 37) or placebo (n = 36) every day following a weight-loss program. Serum insulin and HOMA-IIR significantly reduced in subjects who received vitamin D compared to control group. Adjusting to the baseline measurements, the patients who received vitamin D showed a significant decrease in ALT and stage of fat, as evaluated by liver ultrasound following 12 wk of intervention[48]. In another small, clinical, double-blind, placebo-controlled trial on patients with NAFLD and type 2 diabetes from Italy, there was no significant difference found between patients who received 24 weeks of vitamin D *vs* placebo in terms of primary endpoint, hepatic fat fraction (HFF) measured by MRI, nor hepatic outcomes, such as liver enzymes, CK18, and Fatty Liver Index (FLI)[49]. Most of these studies evaluated markers of inflammation and degree of fat, but not the degree of fibrosis, except for the clinical trial by Corte *et al*[50] which studied 41 pediatrics patients who were enrolled to receive docosahexanoic acid (DHA) and vitamin D *vs* placebo. All patients had a liver biopsy diagnosing NAFLD at the beginning of the study. Furthermore, patients on the treatment arm also received liver biopsy at completion. The combination of vitamin D and docosahexaenoic acid treatment reduced the nonalcoholic fatty liver disease activity score (NAS) in the treatment group[50]. These investigators reported a reduction of the activation of HSC and fibril-forming collagen but not fibrosis score in the treatment group. Moreover, the ALT and HOMA-IR were all decreased with treatment[50]. A meta-analysis of seven clinical trials of vitamin D supplementation with 452 participants concluded that Vitamin D supplementation did not affect a number of markers associated with insulin resistance such as triglycerides, total-, LDL-cholesterol, FPG, insulin, HOMA-IR, AST, ALT, and BMI[51].

Finally, hepatic inflammatory processes, such as NASH, are known to deplete 25(OH)D levels and promote oxidative stress and other mediators, which contribute to progressive fibrogenesis and resistance to supplementation with vitamin D[51,52].

There are a number of noteworthy limitations to this meta-analysis. The included studies in the meta-analysis are all cross-sectional studies. Observational research is not enough to conclude a causal link between vitamin D and severity of liver disease. Randomized controlled trials will provide complementary evidence concerning such an association. If the benefits are not reproduced in randomized trials, then the relationship between vitamin D and NAFLD is probably the result of confounding or physiological events involved in these disorders[46]. There was heterogeneity among the included patient population in the studies. The BMI was variable among the studies, and particularity patients included in the study by Targher had a mean BMI of 26.3 that was significantly lower than others[26]. The evaluation of the stage of fibrosis is usually made through NASH clinical trial research network scoring system (Table 6)[53]. In two of the six included studies in the meta-analysis, Tragher and Barchetta used the liver fibrosis staging system developed by Brunt *et al*[54], which is slightly different from the NASH clinical trial research network scoring system (Tables 6 and 7). Our study was not adjusted for other confounders of metabolic syndrome, such as diabetes, obesity, and insulin resistance. Moreover, our study did not evaluate other factors that can affect vitamin D levels such as diet, circadian rhythm and season. Studies have shown that serum vitamin D levels are higher in individuals who use diet high in: dairy products, fatty fish and vitamin D supplementation. Vitamin D is directly associated with sun exposure and the serum levels of vitamin D is lower in winters[55].

In summary, prior studies have illustrated that vitamin D may be involved in the pathogenesis of NAFLD. However, in this meta-analysis, we found no evidence that the progression of fibrosis in subjects with NALFD is linked to low vitamin D status. These data are consistent with the aforementioned failure of clinical trials using vitamin D supplementation to improve NAFLD.

ARTICLE HIGHLIGHTS

Research background

Vitamin D is a hormone and a vitamin combined that appears to effects cells throughout the body and impart abundant health benefits. There are many studies on its potential role in modifying chronic liver disease. Given the escalating prevalence of non-alcoholic fatty liver disease (NAFLD) worldwide, we studied the literature for the association of vitamin D serum levels and progression of scar tissue formation in NAFLD.

Research motivation

The goal of a systematic review is to pull together the peer-reviewed literature and then apply standardized guidelines to extract the papers that used proper methodology. In this instance, we sorted through 337 papers to find relevant peer-reviewed manuscripts of sufficient quality to provide scientific evidence about the association between vitamin D level and hepatic fibrosis.

Research objectives

The primary objective was to determine whether there was an association of serum vitamin D and the degree of scar tissue in the liver.

Research methods

We followed international guidelines for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses in systematically analyzing the 337 articles with duplicate screening and extraction by the authors. The authors contacted investigators of previous papers to report crucial data not stated in their manuscripts. An expert biostatistician assisted with data analysis by using Cochrane RevMan 5 software.

Research results

We discovered that only six of the 337 studies presented sufficient data to be included in the meta-analysis. We did not find an association of serum vitamin D with the degree of liver scarring in NAFLD.

Research conclusions

We applied advanced methodologies to determine the relationship between stages of liver scarring and serum vitamin D levels. We observed that serum vitamin D was not associated with liver scar tissue accumulation irrespective of the phase of hepatic injury. In February 2017, we reported in *World Journal of Hepatology* that there was an association between the degrees of scar tissue formation in chronic Hepatitis C with the serum level of vitamin D. Given that vitamin D appears to have a strong influence on immunity and wound healing, it is still possible that supplemental vitamin D to normal levels could help prevent liver disease progression in NAFLD. Interventional trials would be best suited to explore this possibility. This study further elucidated that serum vitamin D does not appear to be associated with the stage of liver scar tissue accumulation. Application of meta-regression permits an analysis of the individual phases of liver scar tissue formation in association with the serum levels of vitamin D. This meta-analysis utilized data synthesis and statistical inquiry to study whether the degree of liver scarring is associated with serum vitamin D status, and found no association. Clinicians should bear in mind that many patients with nonalcoholic fatty liver disease are obese and have lower serum vitamin D levels than non-obese subjects due to sequestration into adipose tissues. Thus, supplementation with vitamin D3 to sufficient levels should be considered.

Research perspectives

When conducting a meta-regression, there may be crucial data that is unavailable that does require proactive investigation by researchers for analysis. Careful meta-analyses can help the scientific community to integrate evidence across studies. Systematic reviews and synthesis, as in our paper, should employ vigilance in data extraction and make efforts to contact the authors of relevant prior works to obtain further information about missing data, statistical analysis, and to clarify methods. As in our paper, acknowledgment of authors who cooperate with the provision of information for systematic review and synthesis should be noted in the resulting manuscript.

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