SYSTEMATIC REVIEWS

Influence of vitamin D on liver fibrosis in chronic hepatitis C: A systematic review and meta-analysis of the pooled clinical trials data

Alia S Dadabhai, Behnam Saberi, Katie Lobner, Russell T Shinohara, Gerard E Mullin

Alia S Dadabhai, Behnam Saberi, Katie Lobner, Gerard E Mullin, Division of Gastroenterology and Hepatology, the Johns Hopkins University School of Medicine, Baltimore, MD 21224, United States

Russell T Shinohara, Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States

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

Conflict-of-interest statement: All the authors declare that they have no competing interests.

Data sharing statement: Technical appendix, statistical code, and dataset are available from the corresponding author at adadabh1@jhmi.edu.

Manuscript source: Invited manuscript

Correspondence to: Alia S Dadabhai, MD, Assistant Professor, Division of Gastroenterology and Hepatology, the Johns Hopkins University School of Medicine, 4940 Eastern Ave A504, Baltimore, MD 21224, United States. adadabh1@jhmi.edu
Telephone: +1-410-5507857
Fax: +1-410-5507861

Abstract

AIM

To investigate the relationship between vitamin D and liver fibrosis in hepatitis C-monoinfected or hepatitis C virus (HCV)-human immunodeficiency virus (HIV) co-infected patients.

METHODS

Pertinent studies were located by a library literature search in PubMed/Embase/Cochrane/Scopus/LILACS by two individual reviewers. Inclusion criteria: (1) studies with patients with HCV or co-infected HCV/HIV; (2) studies with patients $\geq$ 18 years old; (3) studies that evaluated liver fibrosis stage, only based on liver biopsy; and (4) studies that reported serum or plasma 25(OH)D levels. Studies that included pediatric patients, other etiologies of liver disease, or did not use liver biopsy for fibrosis evaluation, or studies with inadequate data were excluded. Estimated measures of association reported in the literature, as well as corresponding measures of uncertainty, were recorded and corresponding odds ratios with 95%CI were included in a meta-analysis.

RESULTS

The pooled data of this systematic review showed that 9 of the 12 studies correlated advanced liver disease defined as a Metavir value of F3/4 with 25(OH) D level insufficiency. The meta-analysis indicated a significant association across studies.

CONCLUSION
Low vitamin D status is common in chronic Hepatitis C patients and is associated with advanced liver fibrosis.

Key words: Vitamin D; Liver fibrosis; Hepatitis C virus; Chronic hepatitis C

Core tip: Vitamin D levels are associated with more advanced fibrosis in chronic hepatitis C.

INTRODUCTION

Hepatitis C virus (HCV) infection remains one of the most common etiologies of liver disease worldwide. A number of epidemiological papers have addressed the global prevalence of Hepatitis C. Lanini *et al*[1] reported that 100 million people globally have serological evidence of current or past HCV infection causing 700000 deaths annually while others suggest that the actual occurrence is double[2]. HCV remains the most common indication for liver transplantation in the United States[3]. Chronic infection with HCV can lead to liver inflammation, liver fibrosis, cirrhosis, and hepatocellular carcinoma. Liver fibrosis is a result of excessive accumulation of extracellular matrix proteins, as part of the wound healing response to chronic injury and chronic inflammation[4]. Various factors have been associated with progression of fibrosis including duration of infection, age, male sex, diabetes, alcohol consumption and human immunodeficiency virus (HIV) co-infection[5].

Vitamin D is a hormone that has numerous biological properties that influence host physiology by regulating epigenetic regulation of more than 2000 genes throughout the body. Vitamin D is best known for its role in maintaining bone mineralization but has diverse and profound influences which can determine disease development and outcome. Although referred to as a vitamin, this steroid hormone is synthesized in the body by a series of hydroxylation reactions that occur in skin (7-hydroxylation), the liver (25-hydroxylation) and the kidney (1-hydroxylation)[6] (Figure 1). Reduction of the enzymatic conversion of 7-dehydrocholesterol to 1.25 hydroxy vitamin D at any of the three conversion steps can result in suboptimal vitamin D status[7]. Vitamin D has a number of influences on innate and adaptive immunity which are pertinent to study in conditions that are driven by
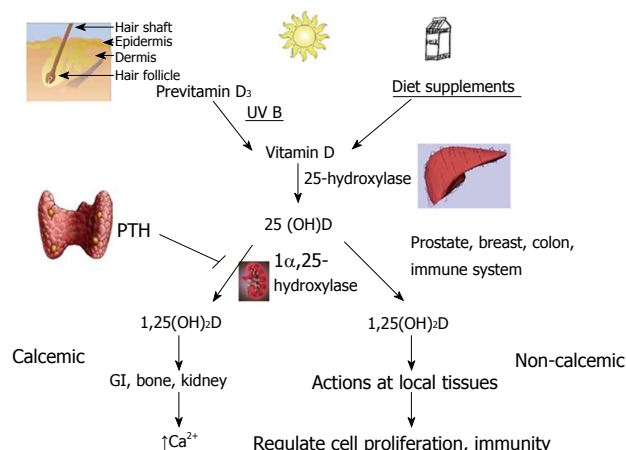
Figure 1 **Vitamin D metabolism.** Vitamin D has diverse influences throughout the body as vitamin D receptors present on virtually every cell. The actions of vitamin D can be subdivided into two larger categories: Calcemic and non-calcemic actions. The non-calcemic actions of vitamin D are legion and have been reviewed elsewhere[6,54-58]. Reproduced with permission[6].
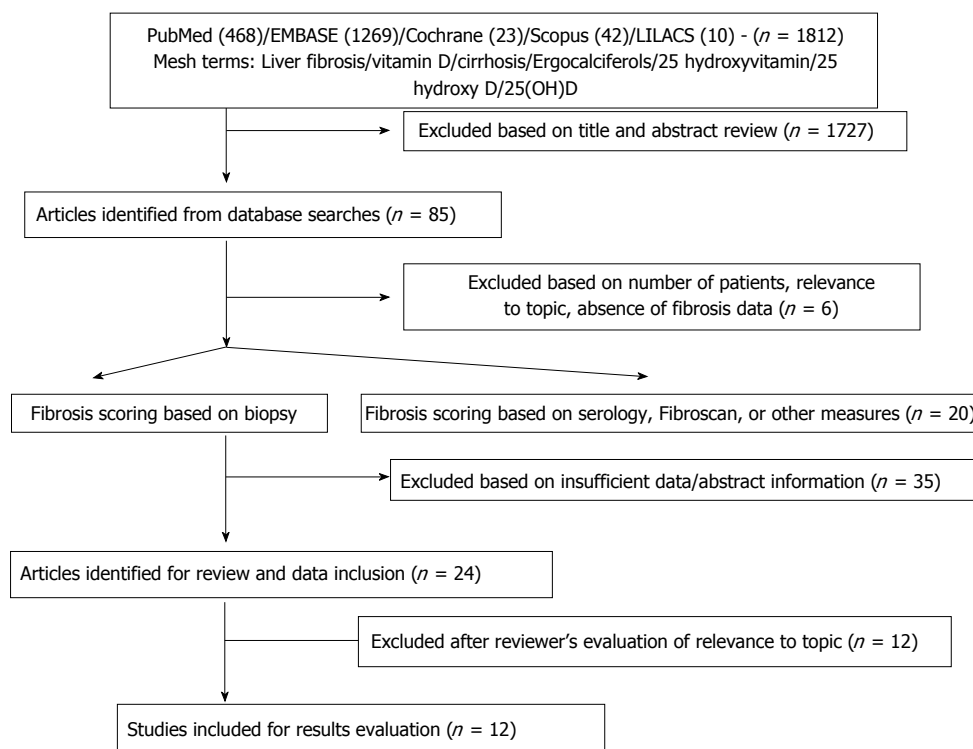
chronic inflammation and maladaptive tissue injury[8,9]. Given the ubiquitous distribution of vitamin D receptors in virtually every cell in the body-suboptimal vitamin D status has been studied for its relationship to numerous diseases[10]. For example, there is substantial evidence that vitamin D benefits rheumatoid arthritis, due to its immunomodulatory effect[11]. The role of vitamin D in various cancers has been established linked to its anti-proliferative action mediated through vitamin D nuclear receptor[12]. There have been numerous reports on lower serum vitamin D levels in patients with chronic liver disease from various etiologies[13]. In chronic HCV, Low vitamin D levels have been reported in 46% to 92% of patients[10] raising suspicion of its potential contribution to disease pathogenesis. There is growing evidence from various groups, that vitamin D levels are inversely correlated with liver inflammation and stage of liver fibrosis in patients with HCV; however, the studies are heterogeneous with occasionally the results are conflicting. Additionally, the methods of reporting liver fibrosis were variable.

The aim of this study was to evaluate the relationship between vitamin D status and hepatic fibrosis based on histopathological staging in patients with chronic HCV mono-infection or co-infected HIV-HCV infection, by performing a systematic review of the scientific literature followed by a meta-analysis.

MATERIALS AND METHODS

Search method
Applicable studies were identified by a library literature search in Pubmed/Embase/Cochrane/Scopus/LILACS utilizing the PRISMA checklist[14] "Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated" and the Cochrane review reporting guidelines (6.6.2.2)[15].

```
┌─────────────────────────────────────────────────────────┐
│ PubMed (468)/EMBASE (1269)/Cochrane (23)/Scopus (42)/     │
│ LILACS (10) - (n = 1812)                                  │
│ Mesh terms: Liver fibrosis/vitamin D/cirrhosis/           │
│ Ergocalciferols/25 hydroxyvitamin/25 hydroxy D/25(OH)D    │
└─────────────────────────────────────────────────────────┘
            │
            │        ┌──────────────────────────────────────────────┐
            ├───────▶│ Excluded based on title and abstract review   │
            │        │ (n = 1727)                                     │
            │        └──────────────────────────────────────────────┘
            ▼
┌─────────────────────────────────────────────────────────┐
│ Articles identified from database searches (n = 85)       │
└─────────────────────────────────────────────────────────┘
            │
            │        ┌──────────────────────────────────────────────┐
            ├───────▶│ Excluded based on number of patients,         │
            │        │ relevance to topic, absence of fibrosis data  │
            │        │ (n = 6)                                        │
            │        └──────────────────────────────────────────────┘
            │
      ┌─────┴──────────────────────────────┐
      ▼                                     ▼
┌────────────────────────┐  ┌──────────────────────────────────────┐
│ Fibrosis scoring based │  │ Fibrosis scoring based on serology,   │
│ on biopsy              │  │ Fibroscan, or other measures (n = 20) │
└────────────────────────┘  └──────────────────────────────────────┘
      │
      │        ┌──────────────────────────────────────────────┐
      ├───────▶│ Excluded based on insufficient data/abstract  │
      │        │ information (n = 35)                           │
      │        └──────────────────────────────────────────────┘
      ▼
┌─────────────────────────────────────────────────────────┐
│ Articles identified for review and data inclusion (n = 24)│
└─────────────────────────────────────────────────────────┘
      │
      │        ┌──────────────────────────────────────────────┐
      ├───────▶│ Excluded after reviewer's evaluation of       │
      │        │ relevance to topic (n = 12)                    │
      │        └──────────────────────────────────────────────┘
      ▼
┌─────────────────────────────────────────────────────────┐
│ Studies included for results evaluation (n = 12)          │
└─────────────────────────────────────────────────────────┘
```

Figure 2 Flowchart of study selection process. Eighteen hundred and twelve articles were identified using PubMed (*n* = 468)/EMBASE (*n* = 1269)/Cochrane (*n* = 23)/Scopus (*n* = 42)/LILACS (*n* = 10) search engines. Detailed evaluation of the articles by at least two independent reviewers (total of three) narrowed the studies to twelve (*n* = 2521) based upon inclusion and exclusion criteria as listed in Table 1.

The search terms were as follows: ["Liver cirrhosis" or "liver" and ("cirrhosis" or "fibrosis")] and ["vitamin D" or "Ergocalciferols" or "25 hydroxyvitamin D" or "25 hydroxy vitamin D" or "25 hydroxy D" or "25(OH)D"]. Also, the studies cited by the selected articles were searched for further pertinent studies. The search was performed before July 6, 2016.

Selection criteria
The title and abstract of the studies were carefully reviewed by two individual reviewers, based on the inclusion and exclusion criteria. If there was an agreement between two reviewers, then the study was selected for further analysis. When there was a disagreement, a third reviewer determined if the study qualified for inclusion. Once the articles met the criteria, then the text was reviewed, and data extraction was completed.

Inclusion criteria: (1) studies with patients with HCV or co-infected HCV/HIV; (2) studies with patients ⩾ 18 years old; (3) studies that evaluated liver fibrosis stage, only based on liver biopsy; and (4) studies that reported serum or plasma 25(OH)D levels. Exclusion criteria: (1) liver diseases other than hepatitis C; (2) studies with inadequate data; (3) studies that used non-invasive methods in evaluating liver fibrosis; and (4) age < 18 years.

Data extraction
A total of 12 studies were included for extraction which was performed by two independent reviewers based on data quality, sufficiency, and relevance. Disagreements were resolved by a third reviewer to reach a consensus. The following data were extracted: Last name of the first author, demographic information of patients, publication year, sample size, HCV genotype, presence or absence of HIV co-infection, pathological fibrosis stage using Metavir score, vitamin D levels, and association of serum vitamin D level and fibrosis stage (Figure 2). The quality of evidence was ascertained by two independent reviewers using The Grading of Recommendations Assessment, Development and Evaluation (GRADE) analysis whereby very low = 1, low = 2, moderate = 3, high = 4[16]. The strength of recommendations were 1 (strong) or 2 (weak)[17]. When there was a disagreement, a third reviewer determined GRADE assessment and strength of recommendations.

Statistical analysis
All statistical computations were conducted in R (Version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria, 2016)[18]. Estimated odds ratios (OR) reported in the literature, as well as 95%CI, were inverted when necessary and included in a meta-analysis. In several studies, the odds ratio for severe fibrosis corresponding to vitamin D deficiency was not reported, but the distribution (mean and standard deviation or inter-quartile range) of vitamin D levels were reported for subjects with and without severe fibrosis separately. To estimate the odds ratio from these studies, a Monte Carlo simulation approach was adopted: For each such study, 1000
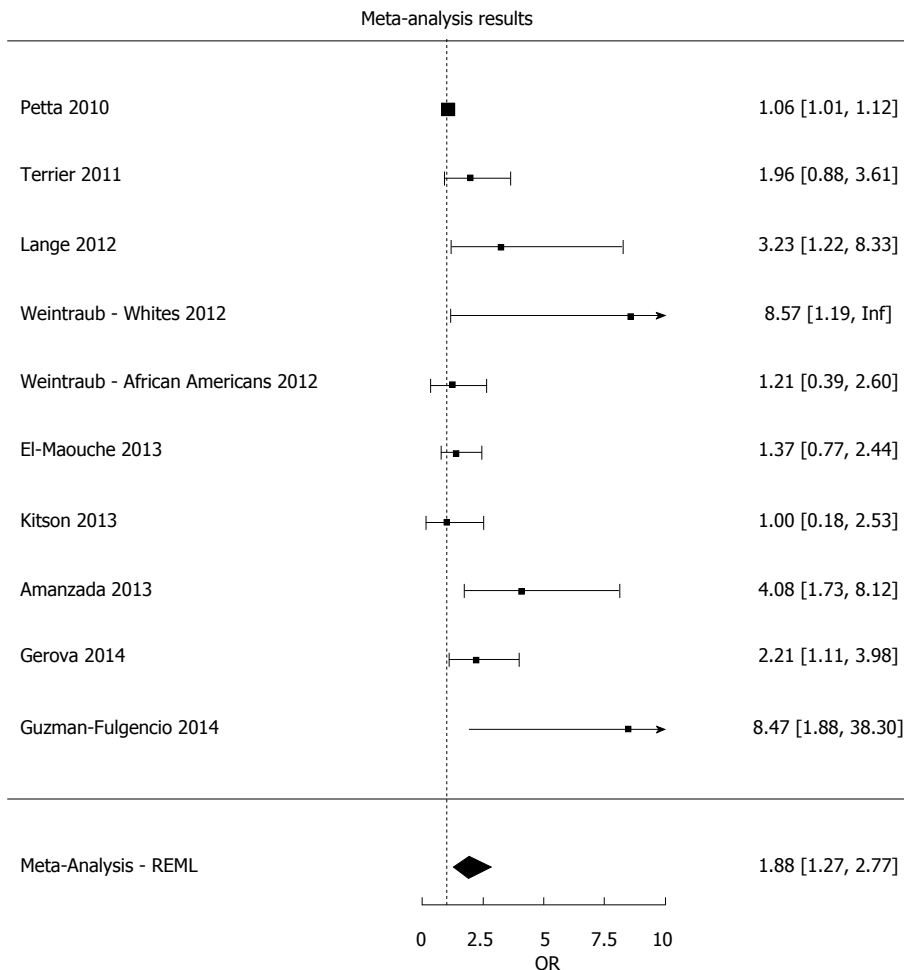
Figure 3 Meta-analysis of the pooled data from the 12 included studies. The odds ratio for severe fibrosis comparing low vitamin D levels was estimated by meta-analyzing studies including a total of 2521 patients. Details concerning the analytic strategy are provided in the Materials and Methods section.

simulated studies were created assuming that vitamin D levels were normally distributed with the reported parameters and the observed number of subjects in each group. The odds ratio for severe fibrosis comparing vitamin D levels with a cutoff of 15 ng/mL was estimated for each simulated dataset. A sensitivity analysis was also conducted by using thresholds of 20 ng/mL and 30 ng/mL. The average odds ratio across simulated datasets were then estimated, and quantile-based confidence intervals were also recorded and included into the meta-analysis. A random-effects meta-analysis fit using restricted maximum likelihood was then fit using the Metafor package in R[19]. $P < 0.05$ was considered statistically significant.

RESULTS

The initial protocol established a series of mesh terms used to identify articles that would evaluate the severity of liver fibrosis in chronic hepatitis C patients with vitamin D levels. Eighteen hundred and twelve articles were found using PubMed (n = 468)/EMBASE (n = 1269)/ Cochrane (n = 23)/Scopus (n = 42)/LILACS (n = 10) search engines. Mesh terms used were liver fibrosis/

vitamin d/cirrhosis/Ergocalciferols/25 hydroxyvitamin/25 hydroxy d/25(OH) D. Detailed evaluation of the articles by at least two independent reviewers (total of three) assessed the sufficiency of data, method of fibrosis qualification, relevance to the topic to narrow the studies to twelve. The data extraction algorithm is summarized in Figure 3. Table 1 reflects the characteristics of the studies relating fibrosis to chronic hepatitis C and vitamin D level. When patients were stratified according to vitamin D status, we found substantial differences between the levels of severity of liver fibrosis. The sensitivity analysis with different cutoffs for the Monte Carlo simulations showed robustness of the result to the choice of cutoff, with significant effects for all thresholds employed.

Definition of vitamin D levels
Vitamin D insufficiency was defined in most studies as below < 30 ng/mL, and deficiency ranged from < 20 ng/mL to 10 ng/mL. While there was some variability in these definitions, there was consistency in the lower limit of normal being < 30 ng/mL. Two of the studies used nmol/L to express 25(OH)D, but were consistent with vitamin D insufficiency below the lower limit of normal < 80 nmol/L.

Table 1 Pooled data of vitamin D levels and liver fibrosis from the 12 included studies

Year	Author	Country	Design	n	HCV GT	HIV	Definition of vitamin D insufficiency (I)/deficiency (D)	Outcome (serum vitamin D and liver fibrosis)	P value/OR 95%CI	GRADE quality of evidence very low = 1, low = 2, moderate = 3, high = 4 and strength of recommendation: 2 = strong 1 = weak
2010	Petta	Italy	Prospective	197	1	No	< 30 ng/mL for low vitamin D level	Low 25(OH)D associated with severe fibrosis (F3/F4)	0.942 [0.893, 0.994] P = 0.009	GRADE 3 Strong
2011	Terrier	France	Prospective	189	1,-4 other	Yes	< 10 ng/mL D, 10-30 ng/mL (I)	Low 25(OH)D correlate with severe fibrosis (F3/F4)	P = 0.04	GRADE 3 Strong
2012	Lange	Sweden	Retrospective	496	1, 4	No	< 10 ng/mL D, < 20 ng/mL (I)	Advanced fibrosis stage F2-F4 *vs* F0-F1 associated with low 25(OH)D	0.31 [0.12, 0.82] P = 0.018	GRADE 2 Weak
2012	Weintraub	United States	Cross-sectional	171	1	No	< 20 ng/mL or < 30 ng/mL (I)	Higher 25(OH)D predictive of milder fibrosis (F0-F2) in white patients but not in African Americans	P = 0.007	GRADE 2 Weak
2012	Baur	Switzerland	Cohort	251	1, 3	No	< 20 ng/mL (I)	(1) 25(OH)D lower in more advanced fibrosis (F2 *vs* F0-1); (2) low 25-OH vitamin D associated with rapid fibrosis progression rate.	P = 0.005, P = 0.013	GRADE 3 Strong
2013	El-Maouche	United States	Prospective	116	-	Yes	< 15 ng/mL (D)	(1) The prevalence of significant fibrosis (F2 ≥ 2) was similar among those with and without low Vitamin D; (2) low 25(OH)D not associated with significant fibrosis after adjusting for other confounders	P = 0.43 1.37 [0.77, 2.44]	GRADE 3
2013	Mandorfer	Austria	Prospective	65	1, 4	Yes	< 10 ng/mL D, 10-30 ng/mL (I)	Patients with D-DEF displayed a higher prevalence of advanced liver Fibrosis than patients with D-NORM	P = 0.009	Strong GRADE 3
2013	Kitson	Australia and New Zealand	Prospective	274	1	No	< 50 nmol/L D < 75 nmol/L (I)	Baseline 25(OH)D level did not vary with fibrosis stage (F3/4 *vs* F0-2)	P = 0.18	Strong GRADE 3
2013	Amanzada	Germany	Prospective	191	1	Yes	< 30 ng/mL (I)	Low 25(OH)D associated with advance fibrosis (F0-2 *vs* F3/4)	P = 0.02	Strong GRADE 3
2014	Gerova	Bulgaria	Retrospective	296	1, 4	No	< 25 nmol/L (D), 25-50 nmol/L for profound (I), 50–80 nmol/L for mild (I)	Lower 25OHD levels were registered in cases with advanced fibrosis compared to those with mild or absent fibrosis	P >0.05	Strong GRADE 2
2014	Guzman-Fulgencio	Spain	Retrospective	174	1, 4	Yes	< 10 ng/mL (D), 10-30 ng/mL (I)	Low 25(OH)D deficiency associated with advanced fibrosis (F3/4 *vs* F0-2)	P = 0.005	Weak GRADE 2
2015	Esmat	Egypt	Prospective	101	4	No	< 20 ng/mL (D), 20-30 (I)	No correlation found between vitamin D levels and stage of liver fibrosis	P = 0.26	Weak GRADE 3

HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; GRADE: Grading of Recommendations Assessment, Development and Evaluation.

Table 2 Selection criteria for inclusion and exclusion

Inclusion criteria
    Age ⩾ 18 yr
    Studies including mono-infected HCV or co-infected HCV/HIV
    Studies that evaluated liver fibrosis stage, only based on liver histology
    Studies that reported serum or plasma 25(OH)D levels
Exclusion criteria
    Age < 18 yr
    Other etiologies of liver disease, other than hepatitis C
    Studies that used non-invasive methods in evaluating liver fibrosis
    Inadequate data

HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

Association between vitamin D deficiency and severity of liver disease

Among the articles used for data extraction, there were seven prospective studies, three retrospective studies, one cross-sectional analysis, and one cohort study (Table 1). In a review of the results, nine studies demonstrated a significant association between plasma levels of vitamin D and degree of HCV-related hepatic fibrosis. Three studies showed no correlation was found between vitamin D levels and stage of liver fibrosis. Patient characteristics between these studies were all similar and could not account for the variability of the findings between the three negative studies and the nine positive studies. Only one of the three negative studies was conducted in the northern hemisphere. Overall, hepatitis C genotypes were not different among the negative studies, although El-Maouche *et al*[20] did not identify which genotype(s) were included. The forest plot of the data used in this systematic review showed that advanced liver disease defined as a Metavir value of F3/4 was associated with severe 25(OH)D insufficiency as follows; OR (95%CI): 1.88 (1.27, 2.77), and I^2 (total heterogeneity/total variability): 66.94% indicated substatial heterogeneity between studies.

Plasma vitamin D levels and seasonal variation

Notably there were several latitudes identified in the studies which can affect Vitamin D levels, however, the scope of this difference in this analysis's outcome was not assessed. In the article by Guzmán-Fulgencio *et al*[21] significant seasonal variation of plasma 25(OH)D levels was observed with the subjects in the first semester (winter/spring) having lower plasma 25(OH)D levels than patients evaluated in the second semester (summer/autumn) ($P < 0.001$). A higher percentage of patients with vitamin D deficiency (25(OH)D < 25 nmol/L) was found in the first semester (winter/spring) ($P < 0.001$). Since not all the studies identified the time frame of vitamin D levels and biopsy procurement, we were unable to qualify the significance of this on the study results.

DISCUSSION

The results of our systematic analysis of the literature demonstrated an association between advanced liver fibrosis (defined as Metavir F3/F4) in chronic hepatitis C (CHC) with vitamin D status as reflected by 25-hydroxyvitamin D [25(OH)D] serum levels. In nine[21-29] of twelve studies (75%) that qualified for data extraction (Tables 1 and 2) the final analysis demonstrated an overall association between low vitamin D status as defined as serum 25(OH)D < 15 ng/mL with advanced liver fibrosis (F3/F4 stage disease) in CHC as proven by biopsy analysis for fibrosis stage. These data are highly consistent with prior reports, and the expected pathophysiological interference of 25-hydroxylation of vitamin D as liver fibrosis increases and functional hepatic capacity decreases over the course of hepatitis C disease progression[6].

A recent systematic review of the literature by Abbasi *et al*[30] studied the relationship between low vitamin D status [< 20 ng/mL 25 OH(D)] and the severity of the CLD. A comparatively abridged search strategy yielded 641 articles for consideration and ultimately 19 articles and 4895 study patients with CLD for data extraction showing that almost 80% of patients with chronic liver disease had severe vitamin D deficiency. García-Álvarez *et al*[31] conducted a systematic review evaluating the relationship of vitamin D status to advanced liver fibrosis in CHC-naïve patients and sustained virological response (SVR) to therapy using pegylated interferon/ribavirin (Peg-IFN/RBV). Seven of fourteen papers utilized for their extraction evaluated advanced liver fibrosis (1083 patients) and eleven for SVR (2672 patients). Approximately 70% of CHC patients had low 25(OH)D whereby the definition of insufficiency varied (20 or 30 ng/mL), and 50% of the HCV-infected patients had 25(OH)D levels < 10 or 20 ng/mL. Overall, low vitamin D status was related to a diagnosis of advanced stage of liver disease. Luo et al utilized a search methodology restricted to PubMed and Embase databases before October 2013 included studies that analyzed the association between serum vitamin D status and the severity of liver fibrosis in 8231 CHC patients without other restrictions yielding six global studies for data extraction[13]. One study recruited 6567 participants as part of the Swiss Hepatitis C Cohort Study[23] raising concerns for skewing of the extracted data. The mean data from extracted studies suggested that lower serum vitamin D is a risk factor for progressive liver fibrosis in CHC patients. However, there was a high heterogeneity and inconsistencies depending upon data set utilized (OR data studies *vs* mean data extracted). Our search methodology instead included 2521 patients which incorporated the 2012 study by Lange *et al*[32] which evaluated 468 HCV patients treated with alpha interferon-based regimens for vitamin D status and advanced disease demonstrating that fibrosis stages F2-F4 *vs* F0-F1 associated with low 25(OH)D.

The nine studies showing a positive association between low vitamin D with an advanced stage of fibrosis had variations in their definition of vitamin D status which challenged our ability to Meta-analyze the data. Low vitamin D was stratified according to by either

insufficient (I) or deficient (D) (Table 1) in eight[21-27,29] of the nine studies. Gerova *et al*[28] used three categories; mild insufficiency, profound insufficiency, and deficiency. Overall, of the twelve papers in our final analysis, two[28,33] utilized nmol/L to measure serum 25(OH) vitamin D status. Insufficiency was defined as < 30 ng/mL in seven with another two using equivalent levels in nmol/L[28,34], < 20 ng/mL in two[23,25] while El-Maouche studied only deficient patients (< 15 ng/mL)[20]. The definition of "deficiency" was utilized by all but two[20,34] of the studies as < 10 ng/mL 25(OH) vitamin D. The prevalence of vitamin D deficiency in a population depends on upon the definition used [< 20 or < 30 ng/mL (50 or 75 nmol/L)]. In the National Health and Nutrition Examination Survey (NHANES), 41.6 percent of United States adults had (25[OH]D) levels < 20 ng/mL (50 nmol/L)[35]. The Institute of Medicine recommends the attainment of the serum 25(OH)D levels of > 20 < 40 ng/mL (50 to 100 nmol/L), however, many define sufficient vitamin D status as 25(OH)D > 30 and < 50 ng/mL (75 to 125 nmol/L)[36,37].

Hepatitis C genotype (1-6) did not change the outcome of analyses between advanced fibrosis in CHC with vitamin D status[20,33,34]. The geographical latitudes of study site and variable seasonal fluctuations have provided challenges to vitamin D status, but did not appear to influence the outcome of the negative outcome studies[20,33,34] Esmat *et al*[34] conducted a open-labelled RCT of 101 HVC4 Egyptian patients undergoing standard of care (SOC) Peg-IFN/RBV plus/minus 15000 IU vitamin D$_3$ (cholecalciferol). The fibrosis stage (F1-F3) at baseline was not different according to 25(OH) vitamin D levels. El-Maouche *et al*[20] evaluated HIV-HCV co-infected patients for histological fibrosis using the Metavir system [0 (no fibrosis) to 4 (cirrhosis)] and used banked serum as a source for vitamin D determination. Similar to Esmat *et al*[34], the prevalence of significant fibrosis (F2 $\geqslant$ 2) was similar among those with and without low vitamin D while low 25(OH)D status was not associated with significant fibrosis after adjusting for other confounders. Finally, Kitson *et al*[38] from Australia evaluated pre-treatment 25-hydroxyvitamin D [25(OH)D] level in a cohort of 274 treatment-naive patients with HCV-1 to evaluate the association between vitamin D status, virological response, and liver histology after 48 wk of pegylated interferon alfa-2a plus ribavirin therapy. Baseline 25(OH)D level did not vary with fibrosis stage (F3/4 *vs* F0-2).

The manner by which vitamin D may influence the course of CHC may be due to effects on viral clearance, immune modulation, cell differentiation and proliferation and inflammation regulation. Vitamin D is not only involved in calcium homeostasis but has also has been associated with the mechanism of cellular proliferation, and immuno-modulation[39]. Several studies have shown that vitamin D levels are inversely correlated with stage of liver fibrosis in patients with CHC. Nine[21-29] of the twelve studies that we included for data extraction reported the inverse correlation of vitamin D levels with the stage of liver fibrosis in patients with CHC. Vitamin D has anti-inflammatory, anti-proliferative and anti-fibrotic effects that dampen inflammatory cell recruitment to the liver and mitigate hepatic fibrosis progression[40]. HCV may also have its own direct actions that impair vitamin D activity and status. It has been hypothesized that HCV affects 25-hydroxylation of vitamin D through cytokine induction or oxidative stress or through disruption in lipid metabolism where HCV suppress 25(OH)D levels due to a decrease in the production of vitamin D precursor, 7-dehydrocholesterol[10].

The profound relationship of vitamin D to immunity and inflammation, and our findings raise questions about how vitamin D status may impact the outcome of the many non-HCV chronic liver diseases. Individuals with chronic liver disease have significant global prevalence, morbidity, poor quality of life and mortality. Prior works have demonstrated adverse survival outcomes in patients with lowered vitamin D status[41,42]. In our analyses, we excluded papers reporting the analysis of vitamin D in chronic liver diseases other than HCV including chronic hepatitis B (CHB) which has a higher global prevalence of approximately 300 million infected individuals. Yu *et al*[43] evaluated the potential association between serum vitamin D level and liver histology or virological parameters in treatment-naïve patients with chronic hepatitis B infection in Southern China. They reported that patients infected with genotype B had a higher prevalence of vitamin D insufficiency than individuals with CHC. Furthermore, in chronic hepatitis B patients, serum 25(OH) D was not correlated with viral load or fibrosis. Mi *et al*[44] reported that vitamin D status was not different among Asians with non-cirrhotic CHB and CHC.

Low vitamin D status is associated with the risk of progression and the severity of hepatic inflammation in patients with non-alcoholic fatty liver disease[45,46]. Primary biliary cirrhosis has been extensively analyzed for correlations of vitamin D status predicting the outcome to ursodeoxycholic acid (UCDA) therapy and the influence of vitamin D supplementation to UCDA intervention[47-49]. Autoimmune hepatitis (AIH) has also been studied for the potential influence of vitamin D given the epidemiological association of this hormone with a number of diseases with autoimmunity[50,51]. However, there are not sufficient studies to draw meaningful conclusions of serum 25(OH)D and AIH at this time.

Altered vitamin D physiology *via* resistance from genetic polymorphisms of the vitamin D receptor (VDR) could also influence the outcome of CHC. Baur *et al*[25] demonstrated that low 25(OH)D plasma levels and VDR bAt[CCA]haplotype were associated with rapid fibrosis progression in CHC, separately and synergistic when co-present. Petta *et al*[52] reported that low hepatic VDR expression was inversely related to the severity of advanced liver fibrosis in patients with genotype 1 cCHC patients. Grunhage reported that a single nucleotide polymorphism (SNP) linked to the *DHCR7* gene coding vitamin D precursor dehydrocholesterol was related to altered serum 25(OH)D in chronic liver disease patients

with no or mild fibrosis[53].

CHC with severely low vitamin D status is accompanied by advanced liver fibrosis. Interventional trials aimed to normalize vitamin D status in early stages of CHC may shed light on whether correction of vitamin D status in this patient population should become the standard of care.

COMMENTS

Background
Hepatitis C remains a global health burden affecting over 100 million people worldwide. There is growing evidence that vitamin D is inversely associated with liver inflammation and fibrosis in patients with chronic hepatitis C.

Research frontiers
Currently hepatitis C is being dramatically eradicated with DAA therapy. Possible augmentation of DAA therapy by vitamin D in those patients who already have fibrosis may decrease long term damage in the liver parenchyma.

Innovations and breakthroughs
The pooled data of this systematic review showed that 9 of the 12 studies correlated advanced liver disease defined as a Metavir value of F3/4 with 25(OH) D level insufficiency. The meta-analysis indicated a significant association across studies. Low vitamin D status is common in chronic Hepatitis C patients and is associated with advanced liver fibrosis.

Applications
Augmentation of standard hepatitis C therapy of direct acting antiviral meds with vitamin D may assist with long term decrease in liver fibrosis.

Peer-review
This is a very interesting and informative paper, and it deserves publication.

REFERENCES

1   **Lanini S**, Easterbrook PJ, Zumla A, Ippolito G. Hepatitis C: global epidemiology and strategies for control. *Clin Microbiol Infect* 2016; **22**: 833-838 [PMID: 27521803 DOI: 10.1016/j.cmi.2016.07.035]

2   **Shin EC**, Sung PS, Park SH. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat Rev Immunol* 2016; **16**: 509-523 [PMID: 27374637 DOI: 10.1038/nri.2016.69]

3   **Kim WR,** Smith JM, Skeans MA, Schladt DP, Schnitzler MA, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2012 Annual Data Report: liver. *Am J Transplant* 2014; **14** Suppl 1: 69-96 [PMID: 24373168 DOI: 10.1111/ajt.12581]

4   **Sebastiani G**, Gkouvatsos K, Pantopoulos K. Chronic hepatitis C and liver fibrosis. *World J Gastroenterol* 2014; **20**: 11033-11053 [PMID: 25170193 DOI: 10.3748/wjg.v20.i32.11033]

5   **Poynard T**, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; **362** (9401): 2095-2100 [PMID: 14697814]

6   **Mullin GE**, Dobs A. Vitamin d and its role in cancer and immunity: a prescription for sunlight. *Nutr Clin Pract* 2007; **22**: 305-322 [PMID: 17507731]

7   **DeLuca HF**. Vitamin D: Historical Overview. *Vitam Horm* 2016; **100**: 1-20 [PMID: 26827946 DOI: 10.1016/bs.vh.2015.11.001]

8   **Pludowski P**, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, Shoenfeld Y, Lerchbaum E, Llewellyn DJ, Kienreich K, Soni M. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. *Autoimmun Rev* 2013; **12**: 976-989 [PMID: 23542507 DOI: 10.1016/j.autrev.2013.02.004]

9   **Gatti D**, Idolazzi L, Fassio A. Vitamin D: not just bone, but also immunity. *Minerva Med* 2016; **107**: 452-460 [PMID: 27441391]

10   **Iruzubieta P**, Teran A, Crespo J, Fabrega E. Vitamin D deficiency in chronic liver disease. *World J Hepatol* 2014; **6**: 901-915 [PMID: 25544877 DOI: 10.4254/wjh.v6.i12.901]

11   **Peterlik M,** Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. *Eur J Clin Invest* 2005; **35**: 290-304 [PMID: 15860041 DOI: 10.1111/j.1365-2362.2005.01487.x]

12   **Feldman D**, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer* 2014; **14**: 342-357 [PMID: 24705652 DOI: 10.1038/nrc3691]

13   **Luo YQ,** Wu XX, Ling ZX, Cheng YW, Yuan L, Xiang C. Association between serum vitamin D and severity of liver fibrosis in chronic hepatitis C patients: a systematic meta-analysis. *Zhejing Daxue Xuebao Zirankexueban* 2014; **15**: 900-906 [DOI: 10.1631/jzus.B1400073]

14   **Hutton B**, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JP, Straus S, Thorlund K, Jansen JP, Mulrow C, Catalá-López F, Gøtzsche PC, Dickersin K, Boutron I, Altman DG, Moher D. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med* 2015; **162**: 777-784 [PMID: 26030634 DOI: 10.7326/M14-2385]

15   Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. In: Higgins JPT GSe, ed: The Cochrane Collaboration; 2011. Available from: URL: http://handbook.cochrane.org

16   **Balshem H**, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, Vist GE, Falck-Ytter Y, Meerpohl J, Norris S, Guyatt GH. GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol* 2011; **64**: 401-406 [PMID: 21208779 DOI: 10.1016/j.jclinepi.2010.07.015]

17   **McClave SA,** DiBaise JK, Mullin GE, Martindale RG. ACG Clinical Guideline: Nutrition Therapy in the Adult Hospitalized Patient. *Am J Gastroenterol* 2016; **111**: 315-334; quiz 335 [PMID: 26952578 DOI: 10.1038/ajg.2016.28]

18   **Team RC**. A language and environment for statistical computing. R Foundation for Statistical Computing 2016 [DOI: 10.1007/s10985-007-9065-x]

19   **Viechtbauer W**. Conducting meta-analyses in R with the metafor package. *J Statistical Software* 2010; **36**: 1-48 [DOI: 10.18637/jss.v036.i03]

20   **El-Maouche D**, Mehta SH, Sutcliffe CG, Higgins Y, Torbenson MS, Moore RD, Thomas DL, Sulkowski MS, Brown TT. Vitamin D deficiency and its relation to bone mineral density and liver fibrosis in HIV-HCV coinfection. *Antiv Ther* 2013; **18**: 237-242 [DOI: 10.3851/IMP2264]

21   **Guzmán-Fulgencio M**, García-Álvarez M, Berenguer J, Jiménez-Sousa MÁ, Cosín J, Pineda-Tenor D, Carrero A, Aldámiz T, Alvarez E, López JC, Resino S. Vitamin D deficiency is associated with severity of liver disease in HIV/HCV coinfected patients. *J Infect* 2014; **68**: 176-184 [PMID: 24184809 DOI: 10.1016/j.jinf.2013.10.011]

22   **Terrier B**, Carrat F, Geri G, Pol S, Piroth L, Halfon P, Poynard T, Souberbielle JC, Cacoub P. Low 25-OH vitamin D serum levels correlate with severe fibrosis in HIV-HCV co-infected patients with chronic hepatitis. *J Hepatol* 2011; **55**: 756-761 [PMID: 21334402 DOI: 10.1016/j.jhep.2011.01.041]

23   **Lange CM**, Bibert S, Kutalik Z, Burgisser P, Cerny A, Dufour JF, Geier A, Gerlach TJ, Heim MH, Malinverni R, Negro F, Regenass S, Badenhoop K, Bojunga J, Sarrazin C, Zeuzem S, Müller T, Berg T, Bochud PY, Moradpour D. A genetic validation study reveals a role of vitamin D metabolism in the response to interferon-alfa-based therapy of chronic hepatitis C. *PLoS One* 2012; **7**: e40159 [PMID: 22808108 DOI: 10.1371/journal.pone.0040159]

24   **Weintraub SJ**, Fleckenstein JF, Marion TN, Madey MA, Mahmoudi TM, Schechtman KB. Vitamin D and the racial difference in the genotype 1 chronic hepatitis C treatment response. *Am J Clin Nutr* 2012; **96**: 1025-1031 [PMID: 23015322 DOI: 10.3945/ajcn.112.039974]

25   **Baur K**, Mertens JC, Schmitt J, Iwata R, Stieger B, Eloranta JJ, Frei P,

Stickel F, Dill MT, Seifert B, Ferrari HA, von Eckardstein A, Bochud PY, Müllhaupt B, Geier A. Combined effect of 25-OH vitamin D plasma levels and genetic vitamin D receptor (NR 1I1) variants on fibrosis progression rate in HCV patients. *Liver Int* 2012; **32**: 635-643 [PMID: 22151003 DOI: 10.1111/j.1478-3231.2011.02674.x]

26   **Mandorfer M**, Reiberger T, Payer BA, Ferlitsch A, Breitenecker F, Aichelburg MC, Obermayer-Pietsch B, Rieger A, Trauner M, Peck-Radosavljevic M. Low vitamin D levels are associated with impaired virologic response to PEGIFN+RBV therapy in HIV-hepatitis C virus coinfected patients. *AIDS* 2013; **27**: 227-232 [PMID: 23238552 DOI: 10.1097/QAD.0b013e32835aa161]

27   **Amanzada A**, Goralczyk AD, Moriconi F, van Thiel DH, Ramadori G, Mihm S. Vitamin D status and serum ferritin concentration in chronic hepatitis C virus type 1 infection. *J Med Virol* 2013; **85**: 1534-1541 [PMID: 23852677 DOI: 10.1002/jmv.23632]

28   **Gerova DI**, Galunska BT, Ivanova II, Kotzev IA, Tchervenkov TG, Balev SP, Svinarov DA. Prevalence of vitamin D deficiency and insufficiency in Bulgarian patients with chronic hepatitis C viral infection. *Scand J Clin Lab Invest* 2014; **74**: 665-672 [PMID: 25005344 DOI: 10.3109/00365513.2014.930710]

29   **Petta S**, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, Cabibi D, Licata G, Porcasi R, Marchesini G, Craxí A. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* 2010; **51**: 1158-1167 [PMID: 20162613 DOI: 10.1002/hep.23489]

30   **Abbasi HA**, Mozaffari HM, Esmaeilzadeh A, Mosannen mozaffari HM, Bahari A, Rezayat KA, Ghanaei O, Ganji A, Mokhtarifar A, Goshayeshi L. Association between Vitamin D deficiency and the severity of chronic liver disease and liver cirrhosis: Systematic literature review. *Govaresh* 2016; **21**: 64-71

31   **García-Álvarez M**, Pineda-Tenor D, Jiménez-Sousa MA, Fernández-Rodríguez A, Guzmán-Fulgencio M, Resino S. Relationship of vitamin D status with advanced liver fibrosis and response to hepatitis C virus therapy: a meta-analysis. *Hepatology* 2014; **60**: 1541-1550 [PMID: 24975775 DOI: 10.1002/hep.27281]

32   **Lange CM**, Bibert S, Kutalik Z. A large-scale genetic validation study coupled with in-vitro analyses reveal a role of vitamin d-signaling in the pathogenesis and treatment of chronic hepatitis C. *J Hepatol* 2011; **54**: S537

33   **Kitson MT**, Button P, Roberts SK. Reply to: "Vitamin D status does not predict sustained virologic response or fibrosis stage in chronic hepatitis C genotype 1 infection". *J Hepatology* 2013; **59**: 194-195 [DOI: 10.20524/aog.2016.0037]

34   **Esmat G**, El Raziky M, Elsharkawy A, Sabry D, Hassany M, Ahmed A, Assem N, El Kassas M, Doss W. Impact of vitamin D supplementation on sustained virological response in chronic hepatitis C genotype 4 patients treated by pegylated interferon/ribavirin. *J Interferon Cytokine Res* 2015; **35**: 49-54 [PMID: 25061714 DOI: 10.1089/jir.2014.0060]

35   **Forrest KY**, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res* 2011; **31**: 48-54 [PMID: 21310306 DOI: 10.1016/j.nutres.2010.12.001]

36   **Ross AC**, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA. The 2011 Dietary Reference Intakes for Calcium and Vitamin D: what dietetics practitioners need to know. *J Am Diet Assoc* 2011; **111**: 524-527 [PMID: 21443983 DOI: 10.1016/j.jada.2011.01.004]

37   **Ross AC**. The 2011 report on dietary reference intakes for calcium and vitamin D. *Public Health Nutr* 2011; **14**: 938-939 [PMID: 21492489 DOI: 10.1017/S1368980011000565]

38   **Kitson MT**, Dore GJ, George J, Button P, McCaughan GW, Crawford DH, Sievert W, Weltman MD, Cheng WS, Roberts SK. Vitamin D status does not predict sustained virologic response or fibrosis stage in chronic hepatitis C genotype 1 infection. *J Hepatol* 2013; **58**: 467-472 [PMID: 23183524 DOI: 10.1016/j.jhep.2012.11.017]

39   **Nagpal S**, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* 2005; **26**: 662-687 [PMID: 15798098 DOI: 10.1210/er.2004-0002]

40   **Rahman AH**, Branch AD. Vitamin D for your patients with chronic hepatitis C? *J Hepatol* 2013; **58**: 184-189 [PMID: 22871501 DOI: 10.1016/j.jhep.2012.07.026]

41   **Finkelmeier F**, Kronenberger B, Zeuzem S, Piiper A, Waidmann O. Severe 25-hydroxyvitamin D deficiency is associated with infections and mortality in cirrhosis. *J Hepatol* 2015; **62**: S377-S378

42   **Grünhage F,** Mahler M, Reichel C, Lammert F. Extremely low vitamin d levels are associated with increased mortality in patients with liver cirrhosis. *J Hepatol* 2012; **56**: S247 [PMID: 24236541 DOI: 10.1111/eci.12205]

43   **Yu R**, Sun J, Zheng Z, Chen J, Fan R, Liang X, Zhu Y, Liu Y, Shen S, Hou J. Association between vitamin D level and viral load or fibrosis stage in chronic hepatitis B patients from Southern China. *J Gastroenterol Hepatol* 2015; **30**: 566-574 [PMID: 25238258 DOI: 10.1111/jgh.12783]

44   **Mi LJ,** Rincon-Bejarano LA, Babbar R. Vitamin d deficiency in Asian-American patients with chronic hepatitis B in New York downtown hospital. *Hepatology* 2011; **54**: 614A

45   **Nelson JE**, Roth CL, Wilson LA, Yates KP, Aouizerat B, Morgan-Stevenson V, Whalen E, Hoofnagle A, Mason M, Gersuk V, Yeh MM, Kowdley KV. Vitamin D Deficiency Is Associated with Increased Risk of Non-alcoholic Steatohepatitis in Adults with Non-alcoholic Fatty Liver Disease: Possible Role for MAPK and NF-kappaB? *Am J Gastroenterol* 2016; **111**: 852-863 [PMID: 27002799 DOI: 10.1038/ajg.2016.51]

46   **Eliades M**, Spyrou E. Vitamin D: A new player in non-alcoholic fatty liver disease? *World J Gastroenterol* 2015; **21**: 1718-1727 [PMID: 25684936 DOI: 10.3748/wjg.v21.i6.1718]

47   **Zhou X**, Guo G, Wang L, Shi Y, Han Y. Vitamin D supplementation therapy for primary biliary cirrhosis: A retrospective clinical study. *Hepatol Inter* 2016; **10**: S492-S493 [DOI: 10.1016/S0168-8278(16)01219-8]

48   **Guo GY**, Shi YQ, Wang L, Ren X, Han ZY, Guo CC, Cui LN, Wang JB, Zhu J, Wang N, Zhang J, Cai Y, Han Y, Zhou XM, Fan DM. Serum vitamin D level is associated with disease severity and response to ursodeoxycholic acid in primary biliary cirrhosis. *Aliment Pharmacol Ther* 2015; **42**: 221-230 [PMID: 25982180 DOI: 10.1111/apt.13244]

49   **Agmon-Levin N**, Kopilov R, Selmi C, Nussinovitch U, Sánchez-Castañón M, López-Hoyos M, Amital H, Kivity S, Gershwin EM, Shoenfeld Y. Vitamin D in primary biliary cirrhosis, a plausible marker of advanced disease. *Immunol Res* 2015; **61**: 141-146 [PMID: 25424577 DOI: 10.1007/s12026-014-8594-0]

50   **Efe C**, Kav T, Aydin C, Cengiz M, Imga NN, Purnak T, Smyk DS, Torgutalp M, Turhan T, Ozenirler S, Ozaslan E, Bogdanos DP. Low serum vitamin D levels are associated with severe histological features and poor response to therapy in patients with autoimmune hepatitis. *Hepatol Inter* 2015; **9**: S130

51   **Efe C**, Kav T, Aydin C, Kav T, Aydin C, Cengiz M, Imga NN, Purnak T, Smyk DS, Torgutalp M, Turhan T, Ozenirler S, Ozaslan E, Bogdanos DP. Low serum vitamin D levels are associated with severe histological features and poor response to therapy in patients with autoimmune hepatitis. *Dig Dis Sci* 2014; **59**: 3035-3042 [PMID: 25002309 DOI: 10.1007/s10620-014-3267-3]

52   **Petta S**, Grimaudo S, Tripodo C, Cabibi D, Calvaruso M, Di Cristina A, Guarnotta C, Macaluso FS, Minissale MG, Marchesini G, Craxì A. The hepatic expression of vitamin D receptor is inversely associated with the severity of liver damage in genotype 1 chronic hepatitis C patients. *J Clin Endocrinol Metab* 2013; **100**: 193-200 [PMID: 25268393 DOI: 10.1210/jc.2014-2741]

53   **Grünhage F**, Hochrath K, Krawczyk M, Höblinger A, Obermayer-Pietsch B, Geisel J, Trauner M, Sauerbruch T, Lammert F. Common genetic variation in vitamin D metabolism is associated with liver stiffness. *Hepatology* 2012; **56**: 1883-1891 [PMID: 22576297 DOI: 10.1002/hep.25830]

54   **Parian AM**, Limketkai BN, Shah ND, Mullin GE. Nutraceutical Supplements for Inflammatory Bowel Disease. *Nutr Clin Pract* 2015; **30**: 551-558 [PMID: 26024677 DOI: 10.1177/0884533615586598]

55   **Mullin GE.** Micronutrients and inflammatory bowel disease. *Nutr Clin Pract* 2012; **27**: 136-137 [PMID: 22223669 DOI: 10.1177/0884533611433436]

56   **Mullin GE**, Turnbull LK, Kines K. Vitamin D: a D-lightful health supplement: part II. *Nutr Clin Pract* 2009; **24**: 738-740 [PMID: 19955553 DOI: 10.1177/0884533609351534]

57   **Mullin GE**, Turnbull L, Kines K. Vitamin D: a D-lightful health supplement. *Nutr Clin Pract* 2009; **24**: 642-644 [PMID: 19841251 DOI: 10.1177/0884533609343938]

58   **Clarke JO**, Mullin GE. A review of complementary and alternative approaches to immunomodulation. *Nutr Clin Pract* 2008; **23**: 49-62 [PMID: 18203964 DOI: 10.1177/011542650802300149]