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Vitamin D Expedites The Recovery Of Hepatitis C Infection Patients

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Abstract
Vitamin D deficiency is associated with several adverse health outcomes, and vitamin D appears to have systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illnesses. In addition to dietary recommendations for liver disease, a significant portion of people with the Hepatitis C virus (HCV) take vitamins and herbs to support their liver. Despite this trend, researchers have confirmed that living with chronic Hepatitis C is usually accompanied by a vitamin D deficiency. Worried about the consequences of a vitamin D deficiency, those with the virus may choose to supplement with this vitamin. However, vitamin D is toxic in large doses and taking too much of it could end up being more harmful than not having enough. We analyzed the relationship of vitamin D status with advanced liver fibrosis (ALF) in CHC treatment-native patients and sustained virologic response (SVR) in CHC patients on pegylated interferon alpha plus ribavirin (pegIFNa/ribavirin) therapy. In the present study, 25-hydroxyvitamin D (25-OHD) levels were compared among patients with chronic hepatitis c virus infection, naturally immunized individuals and control individuals. Twenty patients with chronic hepatitis virus infection (group I), 20 naturally immunized individuals (group II) and 20 healthy adults were included in the present study. Markers of hepatitis were measured using commercially available kits based on chemiluminescence assays. Routine biochemical parameters, hepatitis c virus serology, hepatitis B virus 25-OHD and parathyroid hormone levels were measured. Baseline characteristics of the study groups were comparable. Patients in group I had a lower 25-OHD level compared with group II and the control group (7.45±2.19 ng/mL versus 11±7.13 ng/mL and 17±9.18 ng/mL, respectively; P<0.001). In addition, patients in group I had a higher parathyroid hormone level compared with group II and the control group (78.21±31.2 ng/mL versus 55.14±23.4 ng/mL and 64.16±20.15 ng/mL, respectively; P=0.001). In patients infected with hepatitis c virus, diminished 25-OHD levels may be an indicator of the status of viral replication and portends a poor prognosis.

Keywords: hepatitis c, immune system, vitamin D, chronic liver disease

1. Introduction
The key issue in patients with chronic hepatitis C (CHC) is the progression of liver fibrosis as a consequence of various mechanisms of tissue damage caused by viral infection [1] with the ultimate development of cirrhosis and its complications. Other than well known risk factors for fibrosis severity, like liver necroinflammation, older age, consumption of alcohol, duration of infection and viral coinfections [2] metabolic alterations, namely steatosis,[3] insulin resistance (IR) [4] and menopause (in females) [5] can affect the degree of liver fibrosis. In this complex and interesting interplay between liver and metabolic factors, growing evidence also suggest a role of vitamin D status on liver disease severity in patients with chronic hepatitis C. In particular, we firstly reported that fully compensated genotype 1 (G1) CHC patients are characterized by a higher prevalence of 25-hydroxyvitamin D [25(OH) D] deficiency compared to a control population, also showing in this clinical setting an independent inverse relationship between 25(OH)D serum levels and liver fibrosis severity [6]. These clinical data not only were further confirmed by other groups [7, 8] but also their strength was supported by experimental studies showing that vitamin D, acting via its nuclear vitamin D receptor, exerts its protective effect by inhibiting stellate cell proliferation and their profibrogenic activation [9] Vitamin D has therefore a relevant role in patients with CHC, and its metabolism is regulated by several environmental factors, in particular sunlight and diet. In addition, a recent genome-wide association study (GWAS), in a large screening population of about 30 000 European descent individuals divided in a training and a validation set demonstrated that serum concentrations of 25 (OH)-vitamin D are influenced by variants near genes involved in cholesterol synthesis (7-dehydrocholesterol reductase -*DHCR7*), vitamin D hydroxylation (*CYP2R1*) and vitamin D transport (vitamin D-binding protein–*GC*) [10]. With this in mind, in a cohort of biopsy-proven G1 CHC patients, we aimed to assess the association between vitamin serum levels and its genetic determinants, with the severity of liver fibrosis.

2. Review
The vitamin D receptor (VDR) is widely expressed in the liver and its expression is negatively associated with the severity of liver histology in CHC patients. 8 Moreover, vitamin D has antiproliferative and antifibrotic effects on the liver, and may have potential therapeutic value.9 Vitamin D is produced naturally during exposure to ultraviolet B radiation[2]. It is then metabolized in the liver forming 25-hydroxyvitamin D (25(OH) D), which is later metabolized to the active form, 1, 25-dihydroxyvitamin D (1, 25(OH) 2D), in the kidneys. In the blood, 25(OH) D is the main circulating form of vitamin D, and its concentration in plasma is the most reliable indicator of vitamin D status.10 There is consensus that levels of 25(OH) D below 25 mmol/L (10 ng/mL) are qualified as deficient, and that over 75 nmol/L (30 ng/mL) may be required for optimal health [11]. In HCV infection, vitamin D status has been associated with CHC-related outcomes such as liver fibrosis progression in treatment-native patients and SVR in patients on peg

IFNa/ribavirin therapy [10]. This evidence suggests the potential for vitamin D supplementation as a preventive and/or early treatment strategy for CHC. Recently, a many publications have reported on vitamin D status and CHC, but some conflicting conclusions have been reached [8]. To our knowledge, only one meta-analysis has been published to date about the association between vitamin D and SVR, [12] but it only involved a literature search through March 2012. Treatment of hepatitis C virus (HCV) infection is usually carried out using pegylated interferon (PEG-IFN) and ribavirin (RBV) for 24 wk for HCV genotypes 2 or 3, or 48 wk for HCV genotype 1 and the main objective of HCV therapy is a sustained virologic response (SVR), defined as an undetectable serum HCV-RNA level at 24 wk after the end of therapy. Rates of SVR range from 60%-70% in chronic hepatitis C (CHC) patients with genotypes 2 and 3, but is less than 50% in patients with genotype 1 using conventional therapy. Recently, studies were conducted to analyze the influence of genetic and metabolic factors in antiviral response [5] recent reviews showed that vitamin D levels can influence HCV treatment. Vitamin D itself is considered biologically inactive and is hydroxylated to 25-hydroxyvitamin D [25(OH) D] in the liver [9]. 25(OH) D is the main circulating vitamin D metabolite and is used for classification of the vitamin D status. In the kidney, 25(OH) D is converted to 1, 25 dihydroxyvitamin D [1, 25(OH) D] by 1-alpha-hydroxylase, however, it has been demonstrated that this conversion can occur in many extra-renal tissues including the liver. Finally, 25(OH) D or 1, 25(OH) [2D] bind to the ubiquitously expressed vitamin D receptor (VDR), which regulates approximately 3% of the human genome. In this context, vitamin D deficiency has been associated with an increased risk of cancer [7, 11] cardiovascular, autoimmune [14, 5] and infectious diseases [6, 10]. Due to these facts, there is great research interest in the role of vitamin D status in various infectious diseases. Some studies have shown that high levels of serum vitamin D level are an independent predictor of SVR following anti-viral therapy, and higher SVR is achieved with vitamin D supplementation in CHC individuals. However, Lange *et al.* [8] found that vitamin D deficiency was associated with a lower SVR rate only in CHC genotype 2/3 patients (treated with PEG-IFN and RBV for 24 wk), but not in CHC genotype 1 patients. Moreover, Jazwinski *et al.* found no association between vitamin D levels and SVR in 82 African American genotype 1 CHC-naïve patients, treated with PEG-IFN and RBV [7, 10]. As vitamin D has an uncertain clinical value in HCV infected individuals and taking into consideration the limitations of previous reviews, we conducted an updated systematic review and meta-analysis to comprehensively assess vitamin D deficiency regarding antiviral therapy and the influence of vitamin D supplementation on SVR.

3. Materials and methods

Thirty-five patients who had been followed in the outpatient clinic of the infection diseases department due to chronic hepatitis B (HBsAg positive, anti-HBs negative for at least six months), who had normal liver enzyme levels and had not received antiviral treatment (group I; mean (± SD) age 32.5±9.8 years; 22 men), and 30 naturally immunized individuals (HBsAg negative, anti-HBs and anti-HBc-IgG positive) (group II; mean age 31.1±5.5 years; 18 men) [9] were included in the present study. Thirty age matched healthy adult subjects were also included as a control group (mean age 32.4±8.4 years; 17 men). Because the level of 25-hydroxyvitamin D (25-OHD) fluctuates according to seasonal changes (effects of sunlight), the study was initiated in the winter season and continued to the end of March. Patients with chronic renal failure, chronic liver disease, cardiac failure (ejection fraction <50%), bone disorders, thyroid disorders, previous gastrectomy or having intestinal malabsorption and taking calcium, vitamin D or antidepressant drugs, hepatitis C, hepatitis D, HIV infection, and systemic bacterial or fungal infection, and other causes of liver disease, such as alcohol consumption and autoimmune hepatitis, were excluded from the present study [11].

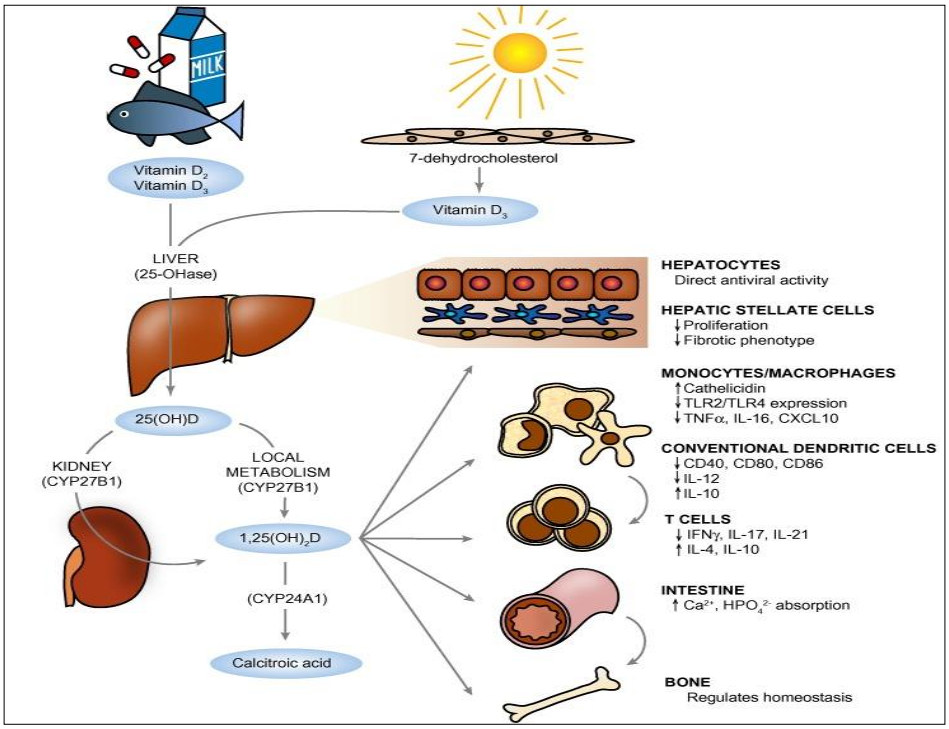

Fig 1

4. Laboratory Tests

Serum parathyroid measurements were performed using an electrochemiluminescence-based method on an E 170 Modular Analytic System (Roche, USA) device. 25-OHD levels were measured using a 25OH-Vitamin D3-Ria-CT Kit (Biosource Europe, Belgium). Reference ranges for 25-OHD were 10 ng/mL to 50 ng/mL for the winter season and 20 ng/mL to 120 ng/mL for the summer season.Hepatitis markers were determined using commercially available kits based on chemiluminescence assays. HBV DNA was quantified using the PCR Cobas Taqman 48 system (Roche, USA) [8].We measured the vitamin D levels in people with chronic liver disease. Of those evaluated, 85 percent of the study participants had chronic Hepatitis C. After dividing every vitamin D deficiency into three categories (mild, moderate and severe), the investigators found the following:

- 92.4 percent of those with chronic liver disease had some degree of vitamin D deficiency
- At least 33 percent of participants were severely deficient in vitamin D
- Severe vitamin D deficiency was more common among those with cirrhosis

5. Observation

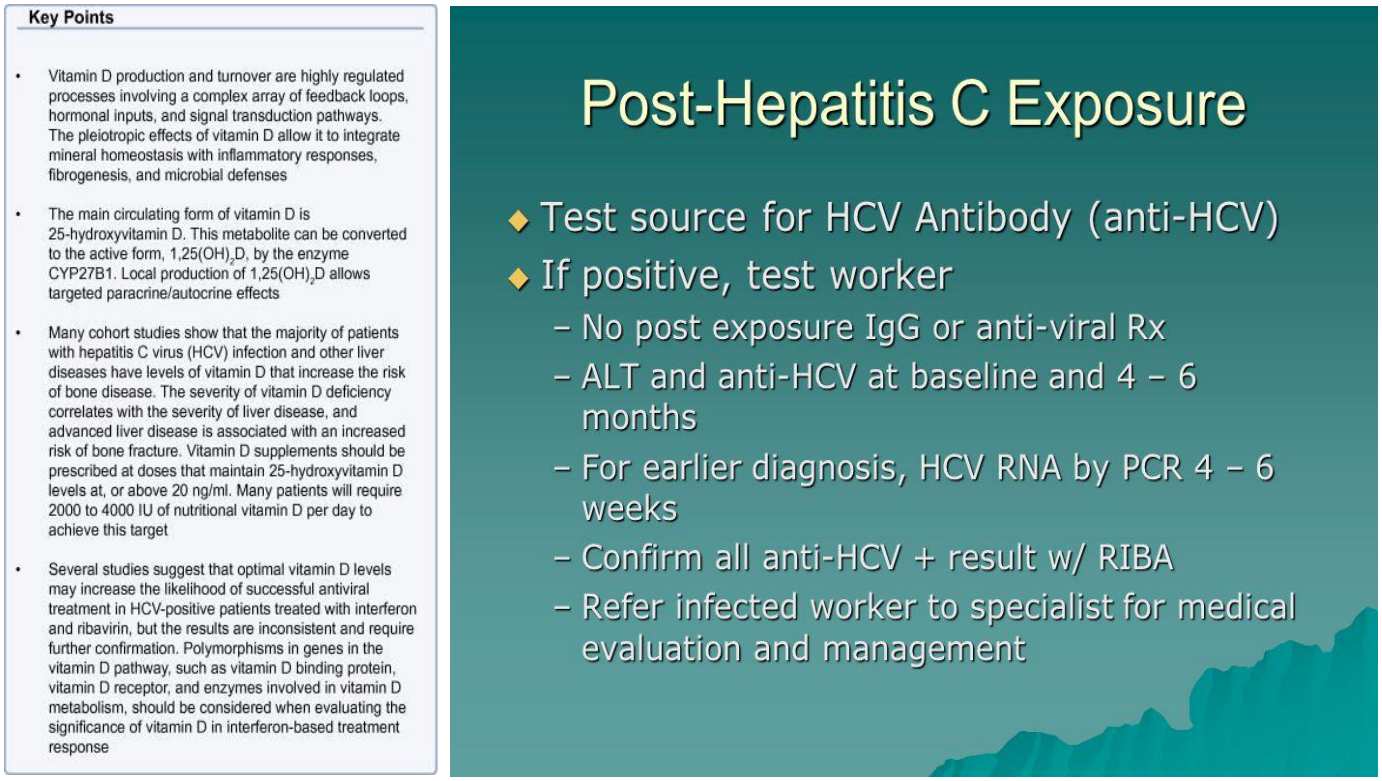

Fig 2

Recent studies have revealed functions of vitamin D in addition to those in bone metabolism. It has been found to be involved in autoimmune disorders such as inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, psoriasis, diabetes, certain cancer types, hypertension, heart failure, atherosclerosis, peripheral artery disease and several infectious diseases [10]. Vitamin D directly leads to the expression of vitamin D receptor and CYP27B1 in vascular smooth muscle cells and in endothelial cells [11]. Recently, it has been recognized that vitamin D has other functions in addition to its role in bone metabolism. It has been demonstrated that vitamin D deficiency may play a role in the development of autoimmune diseases, inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis, diabetes, certain cancer types, cardiac failure, stroke and infectious diseases such as tuberculosis and pneumonia, and that vitamin D supplementation is efficacious in these patients [11, 12]. There is evidence that vitamin D may have a protective role in influenza and other viral diseases and may decrease the risk of developing AIDS in HIV-positive patients, hepatitis and other viral infections [8, 9]. Sabetta *et al.*, [12] demonstrated that maintenance of a vitamin D serum concentration of 38 ng/mL or higher could significantly reduce the incidence of acute viral respiratory tract infections, including influenza, at least during the fall and winter in temperate zones. In Indian children younger than five years of age, subclinical vitamin D deficiency was a significant risk factor for severe acute lower respiratory tract infections [1, 2]. Chronicity of hepatitis B infection is also influenced by mutations in the vitamin D receptor gene, with polymorphisms being associated with higher viral load and increased disease progression and severity. Of note, the t allele is associated with enhanced Th1 cellular immunity and promotes more efficient clearance of several viral infections, including hepatitis B and dengue virus [13, 12]. One study involving patients with hepatitis C virus demonstrated that vitamin D inhibits viral RNA replication, reportedly by inducing oxidative stress in a manner similar to the action of cyclosporine [15]. Petta *et al.* [6] demonstrated that low serum 25-OHD levels were associated with risk of severe fibrosis and low sustained viral response to interferon treatment in patients chronically infected with genotype 1 hepatitis C

virus. Another study also showed that vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C [3] Vitamin D is linked not only to liver fibrosis but also to liver cirrhosis. A significant correlation exists between polymorphisms in the vitamin D receptor gene and the occurrence of hepatocellular carcinoma in patients with liver cirrhosis; this association is even more prominent in alcoholic patients [12, 11].

In the present study, vitamin D levels were examined in patients with chronic HBV infection and naturally immunized individuals. Vitamin D levels were found to be lower in the chronic hepatitis C patients compared with naturally immunized individuals and control individuals (P<0.001).When the three groups were compared in our study, 25-OHD levels of the patients with chronic hepatitis B were significantly lower than the other groups (P<0.001) [3]. In addition, we found a relationship between vitamin D levels and viral load (HBV-DNA). Our present data show that vitamin D deficiency may be related to increased viral replication in patients with HBV infection. Kaleli *et al.* [9]. Showed that neopterin levels, as a marker for immune activation, were higher in replicative HBV carriers. Vitamin D is known to suppress proinflammatory cytokines and cause an increase in interleukin-10 levels [9]. Because of these effects, it is believed that vitamin D deficiency may be related to the development of increased viral replication. In our study, when the three groups were compared, levels of parathyroid hormone in the replicative HBV patients were significantly higher than those of the nonreplicative patients and controls (P=0.001). As a result, our study revealed a relationship between vitamin D deficiency and viral replication in patients with chronic HBV infection [4]. However, 25-OHD levels were found to be similar in the group with previous HBV infection (the naturally immunized group) and the control group. This suggests that vitamin D deficiency may increase viral replication and vitamin D supplementation may be useful in patients with chronic HBV infection. The most important limitation of our study was the small number of patients. There is a need for large-scale research into this issue.

6. Discussion

In this study, we have shown that, in a cohort of patients with biopsy-proven G1 CHC, DHCR7 GG genotype, other than being associated with lower vitamin D serum levels, was also independently linked to the severity of liver fibrosis, together with well-known risk factors for fibrosis, including lower vitamin D serum levels. Different lines of evidence showed a relevant role of vitamin D status in patients with CHC patients, and with neoplastic and cardiometabolic disorders [6, 7] prompting genetic, clinical and experimental research on vitamin D metabolism and actions [6].

In our study we showed that, in G1 CHC patients, lower levels of serum 25(OH) D were independently linked to the DHCR7 GG genotype. Our data are in agreement with the GWAS study of Wong and colleagues on a cohort of about 30 000 subjects that identified the DHCR7 gene as able to affect vitamin D serum levels, with the lowest values in GG patients [10]. In addition, the presence of lower vitamin D serum levels in patients with the DHCR7 GG genotype was also recently reported in a large cohort of Caucasian patients with chronic liver diseases due to different aetiologies [8]. In our study, we did not identify a link between CYP2R1 and GC SNPs, also linked to vitamin D deficiency in the above quoted GWAS study [10] and vitamin D serum levels. This issue could be related to the demographic, clinical and biochemical characteristics of our studied population, like the very high prevalence of vitamin D deficiency, as well as to the relative low number of included patients [7].

This study offers the first evidence that the DHCR7 GG genotype, together with lower 25(OH)D serum levels, and with other known risk factors for fibrosis severity, such as older age, low cholesterol and high triglycerides levels, moderate–severe steatosis and high necroinflammatory activity, is independently associated with the presence of severe liver fibrosis in G1 CHC patients. Grünhage and colleagues,[18] in a cohort of more than seven hundred patients with mostly clinically diagnosed chronic liver disease due to different aetiologies (60% HCV related), showed that, among subgroups of patients with liver stiffness measurement (LSM) lower than 7 kPa and lower than 9.5 kPa, the DHCR7 GG genotype was associated with higher LSM values. These data therefore suggested, with limits related to LSM use as surrogate marker of fibrosis, and to subgroups analyses, a potential association between severity of liver disease and the DHCR7 GG genotype. In this line, our study added further and relevant evidence about this issue, demonstrating the association between the DHCR7 GG genotype and severity of histological liver fibrosis, in a cohort of compensated, homogeneous and fully characterized biopsy-proven G1 CHC patients. Another relevant finding of our study is that both the DHCR7 genotype and vitamin D levels were independently linked to the presence of severe liver fibrosis. This issue suggests that the association between the DHCR7 GG genotype and liver fibrosis is far complex. In fact, on the one hand, it is plausible that DHCR leads to fibrosis via lowering vitamin D serum levels. [10] Experimental evidence in fact suggests that vitamin D, via interaction with VDR, is able to inhibit stellate cell proliferation and their profibrogenic activation. [9]. On the other hand, our results suggest that DHCR7 genotype could prompt fibrogenesis also via other direct/indirect mechanisms. However, literature data do not provide us with further help on this issue, and therefore, additional experimental work is needed.

The main limitation of this study lies in the low number of patients carrying the at-risk DHCR7 GG genotype. This issue could affect the interpretation of our results. However, the similar low prevalence of DHCR7 GG genotype reported in other studies [10, 11] then our biologically plausible results [9, 10] and the presence in the literature of similar results [10, 11] makes us confident about the accuracy of our data, which obviously needs further validation in large cohort studies. Another limitation of our study is its cross-sectional nature and its inability to dissect the temporal relation between DHCR7 genotype, 25 (OH) D and fibrosis. A further methodological drawback is the potentially limited external validity of the results for different populations and settings. Another limitation of this study is the lack of data on the potential confounders that may influence the levels of vitamin D, such as exposure to sunshine, dietary intake and the prevalence of osteoporosis. However, all of the subjects involved in this study lived in Sicily, where sunshine is abundant.

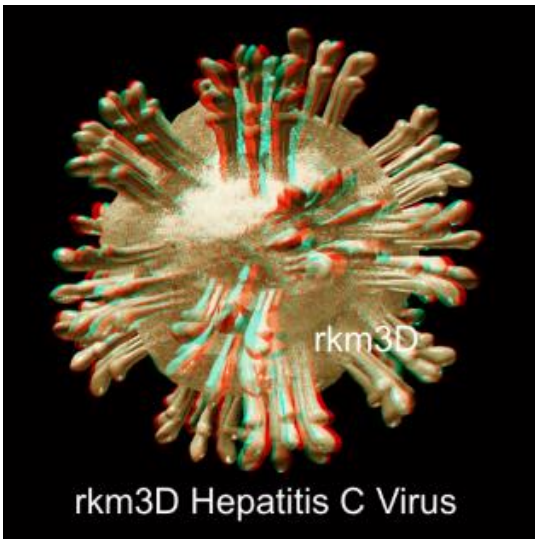

Fig 3

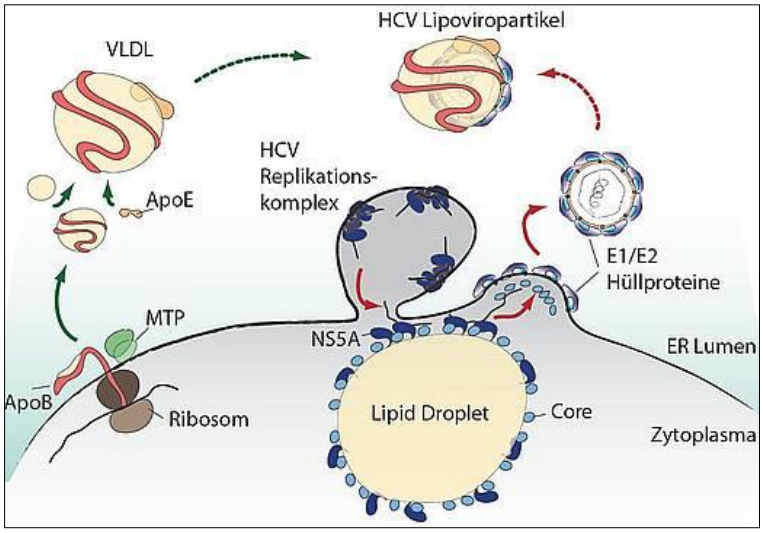

Fig 4

Table 1

	Group A (n = 31)	Group B (n = 29)	Total (n = 60)	Control (n = 28)	P value
ALT (IU/L)	75.46 ± 18.18	87.96 ± 14.11	81.71 ± 18.25	21.35 ± 6.21	0.148
AST (IU/L)	75.5 ± 24.95	104 ± 11.204	89.75 ± 23.41	35.74 ± 4.92	0.24
Calcium (mg/dL)	6.733 ± 1.52	6.657 ± 1.24	6.69 ± 1.38	8.2 ± 0.91	0.83
Phosphorus (mg/dL)	4.24 ± 0.42	4.21 ± 0.39	4.25 ± 0.41	3.1 ± 0.65	0.68
Alkaline phos (IU/L)	140.97 ± 10.98	99.12 ± 24.94	120.05 ± 27.66	78.48 ± 21.10	0.20
HCV RNA (IU/mL)	1393290 ± 52580	967371 ± 56534	1180334 ± 79910	10.21 ± 0.74	0.001 [1]
Vitamin D (nmol/L)	65.26 ± 22.71	57.9 ± 16.17	61.58 ± 17.05	98.31 ± 3.50	0.05 [1]
PTH (pg/mL)	179.10 ± 10.25	188.03 ± 14.96	186.56 ± 25.67	65.71 ± 12.05	0.01 [1]

7. Summary & Conclusion

Vitamin D is increasingly becoming recognized as an important physiological regulator with pleiotropic effects. A growing body of experimental and clinical evidence suggests that vitamin D deficiency is a risk factor in HCV-infected patients and that vitamin D supplementation might protect against liver disease progression and improve responses to treatment. There is still a lack of consensus on optimal 25(OH) D target levels and dosing strategies. The existing evidence highlights the need for additional well-designed clinical trials to evaluate the effects of vitamin D supplementation. The outcomes should include effects on the fibrosis progression rate (in patients with ongoing HCV replication and in patients who achieve a SVR), the incidence of hepatocellular carcinoma, and the incidence of bone fractures. In light of data showing protective effects of vitamin D supplementation in preventing influenza virus infection [46] studies of vitamin D supplementation in HCV patients should also examine vaccine responses and susceptibility to infectious diseases. Given that the relationships between vitamin D and chronic inflammation and progressive hepatic fibrosis are not unique to HCV infection, and that vitamin D deficiency may also be a factor in other liver diseases [7], clinical trials to study the effects of vitamin D supplementation in HCV patients are likely to be broadly relevant to the field of hepatology. Although dose-response data are limited, many liver disease patients will likely require relatively high doses of nutritional vitamin D to achieve 25(OH) D levels above 20 ng/ml. Until clinical data are available, 4000 IU/day is a reasonable daily dose for patients with baseline 25(OH) D levels below 10 ng/ml and 2000 IU/day is an appropriate starting dose for patients with levels between 10 and 20 ng/ml.

Table 2: Comparison of clinical and biochemical features of HCV patients and controls.

	Group I (n=35)	Group II (n=30)	Control (n=30)	P value
Age (years)	32.5 ± 9.8	31.1 ± 5.5	32.4 ± 8.4	NS
Sex (males) (N, %)	22 (55%)	18 (60%)	17 (56.6%)	NS
Body mass index (kg/m^2)	22.96 ± 3.35	22.51 ± 2.85	23.49 ± 4.39	NS
Creatinine(mg/dl)	0.89 ± 0.9	0.78 ± 0.8	0.75 ± 0.8	NS
Hemoglobin(g/dl)	14.1 ± 1.4	13.1 ± 1.3	13.9 ± 1.3	NS
AST(mg/dl)	29.17 ± 3.18	26.7 ± 2.15	27.8 ± 3.4	NS
ALT(mg/dl)	31.25 ± 3.9	33.16 ± 2.3	31.9 ± 3.14	NS
TSH(mcIU/ml)	1.35 ± 1.1	1.22 ± 1.09	1.52 ± 1.45	NS
Parathormone (pg/ml)	88.21 ± 34.2	74.16 ± 20.15	75.14 ± 23.4	0.001
25OHvitaminD(ng/ml)	7.65 ± 4.19	14.17 ± 9.18	12.1 ± 7.13	<0.001

In this meta-analysis, the levels of vitamin D were also associated with SVR, although different methods of vitamin D determination were used. Lai *et al.* [8] demonstrated bias and variability in 25 (OH) D measurements between laboratories and between different assays [quimioluminescence and liquid chromatography-tandem mass spectrometry (LC-MS/MS)] which can significantly affect clinical decision-making. In this situation, the adoption of common standards to allow assay calibration is urgently required.

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