

Vitamin D in Standard HCV Regimen (PEG-Interferon Plus Ribavirin), Its Effect on the Early Virologic Response Rate: A Clinical Trial

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Abstract

Background: Patients chronically infected with the hepatitis C virus (HCV) are more likely to have vitamin D deficiency. Recent studies revealed that vitamin D has immunomodulator and antiviral properties and can enhance the effect of interferon on the HCV virus.

Objectives: We aimed to assess the influence of vitamin D supplementation on viral response to PegIFN/RBV therapy.

Patients and Methods: In a randomized-controlled trial 66 patients with HCV (30 with genotype 1 or 4 and 36 with genotype 2 or 3) were randomly divided into two groups in gastroenterology clinic: the study group (n = 34) received oral vitamin D supplementation (1600 IU/day) to maintain serum levels > 30 ng/mL besides the routine treatment of 180 µg PegIFN-α2a plus oral ribavirin. The control group (n = 32) received the same treatment without vitamin D supplementation. The primary outcome was undetectable HCV-RNA at week 12 of treatment, referred to as complete early viral response (cEVR). Real-time polymerase chain reaction (sensitivity: 10 IU/mL) was used to assess HCV RNA. Serum Vitamin D levels were measured at baseline and weeks 4, 8, 12 and 24 of treatment. Spearman's correlation showed that baseline vitamin D correlated with the stage of liver fibrosis in both study and control group (P = 0.04, r = 0.57).

Results: There were no significant differences in baseline characteristics between two groups except serum AST level. Complete EVR rate at week 12 in the vitamin D group was significantly higher than the controls (100% vs 84.4%; P = 0.023) whereas this figure was not significant when genotypes 1 and 4 or 2 and 3 in the test group were compared to those of the control (100% vs 86.7%; P = 0.19 and 100% vs 82.4%; P = 0.22). Serum vitamin D levels were lowest at baseline (22 ± 15 ng/mL), but increased after 12 weeks of vitamin D therapy to a mean level of 52 ± 38 ng/mL (P = 0.02) in study group.

Conclusions: The addition of vitamin D to conventional PegIFN/RBV therapy in HCV patients may significantly improve the viral response.

Keywords: Hepatitis C, Vitamin D, Early Virologic Response

1. Background

Infection with the hepatitis C virus (HCV) is a common cause of hepatic cirrhosis and ultimately hepatic cancer. Approximately 3% of the population are affected chronically, worldwide (1,2). This figure is 0.5% (3,4) in Iran, reaching 52% - 80% amongst its population of IV drug abusers (5,6).

Although more efficient direct acting antiviral medications such as sofosbuvir and ledipasvir have been approved recently, the HCV standard of care (SOC) in Iran is still the administration of pegylated interferon/ribavirin

(PegIFN/RBV) regimen (2). Due to the high cost of such new drugs and the limited number of patients which are able to afford them, there is a strong tendency to improve the efficiency of the SOC (7).

A sustained virologic response (SVR) - defined as undetectable serum levels of HCV RNA at 24 weeks post-therapy - is the primary aim of HCV therapy. In chronic hepatitis C (CHC) with genotypes 2 and 3, the SVR rate is between 60% and 80% using conventional therapy. However, this figure is less than 50% in patients with genotype 1 (8). Most HCV cases in Iran are of genotype 1 (3).

Genotype, HCV RNA level, rapid viral response (RVR)

and early viral response (EVR) are all associated with SVR and independently predict the outcome of antiviral therapy (9). EVR is an important factor during treatment, indicating viral response to PEG/RBV therapy and its stop points.

Recently, in order to improve patient outcomes, there has been a tendency to specifically target HCV using new antiviral therapies such as polymerase or protease inhibitors (10). Yet, not many studies have evaluated the effect of enhancing the role of host factors using immunomodulators (11).

Recently, a significant amount of information has been found regarding the physiologic as well as pathophysiologic role of vitamin D (12). The positive effects of the activation and regulation of both the innate and adaptive immunity by vitamin D have been presented by several studies (13). Vitamin D enhances the effect of innate immunity via the secretion of antibacterial proteins like cathelicidin and beta-defensin. It also increases chemotaxis and macrophage phagocytosis, thereby contributing to the elimination of pathogens (13, 14). In addition, the direct anti-HCV effect of vitamin D has been demonstrated in vitro (15, 16).

Recent studies have introduced vitamin D as a SVR predictor; low serum 25-OH-vitamin D3 (25(OH)D3) levels (< 20 ng/mL) before therapy are strongly associated with a poor response to antiviral treatment (11, 17).

Moreover, a recent meta-analysis on 8321 patients with CHC from different countries reported that low serum levels of vitamin D may negatively affect the degree of hepatic fibrosis (18).

In several recent clinical trials the co-administration of vitamin D and PegIFN/RBV has led to a better viral response and higher SVR rate in both genotypes 1, 4 (19, 20) and 2, 3 (11). However, other studies have reported conflicting results and vitamin D supplementation did not affect the response to HCV treatment (21).

Typically, patients with chronic liver disease lack sufficient amounts of vitamin D, with more than a third recently found to have severely deficient levels (22). Despite limited evidence and conflicting reports regarding vitamin D supplementation in viral hepatitis and associated liver diseases, there is still great interest in the application of this adjuvant therapy for improving the treatment response. This was the first study in Iran to assess this hypothesis.

2. Objectives

Given the high cost of new oral drugs including sofosbuvir and ledasprevir in Iran until now, our aim was to evaluate the possible effects of adding vitamin D to the

PegIFN/RBV regimen on the cEVR rate in patients infected with HCV genotype 1, 2, 3 or 4.

3. Patients and Methods

3.1. Patients

In this randomized-controlled clinical trial 80 chronic HCV patients presenting to the gastroenterology clinic at Imam Reza Hospital, Mashhad, Iran were recruited from February 2012 to April 2015.

The adult patients included in the study had the following inclusion criteria: adult patients with chronic HCV infection (> 6 months) and detectable serum levels of HCV RNA (genotype 1, 2, 3 or 4) with compensated liver disease fulfilling the following criteria of an absolute neutrophil count above 1,500 per mm³, a platelet count above 90,000 per mm³, and a normal hemoglobin level.

Patients with co-infection with hepatitis B virus or HIV, decompensated liver disease (Child-Pugh classification B or C), autoimmune or metabolic liver disease, hepatocellular carcinoma, a history of anti-HCV therapy or use of medications which alter vitamin D3 levels or metabolism (calcium, vitamin D supplementation, estrogen, alendronate, isoniazid, anticonvulsants, and orlistat), or a history of diarrhea or malabsorption syndromes like celiac and chronic pancreatitis or those with renal or parathyroid diseases were excluded from the study.

The study procedure was fully supported by the research council ethics committee of Mashhad University of Medical Sciences (IRCT2013112915581N1) and all participants were required to fill out informed consent sheets prior to study entrance.

3.2. Study Design (Sample Size)

This was a randomized-controlled, two-group assignment study. Using concealed envelopes marked in advance, study participants were randomized in a 1:1 ratio by simple method randomization following screening, fulfilling the inclusion criteria, and signing an informed consent. In total, 68 patients were selected and randomly allocated into a study and a control group with 34 and 34 patients respectively (Figure 1).

A thorough medical history was obtained from each participant at study entrance and a complete physical examination was conducted. Prior to the start of treatment, in order to detect HCV-RNA a quantitative reverse transcription-polymerase chain reaction was performed using Abbott Real-Time HCV kit with a detection limit of 12 IU/mL at weeks 0, 4 and 12. In the study group vitamin D levels were measured at weeks 0, 4, 8, 12 and 24, during treatment; the serum 25(OH)D level was measured with a

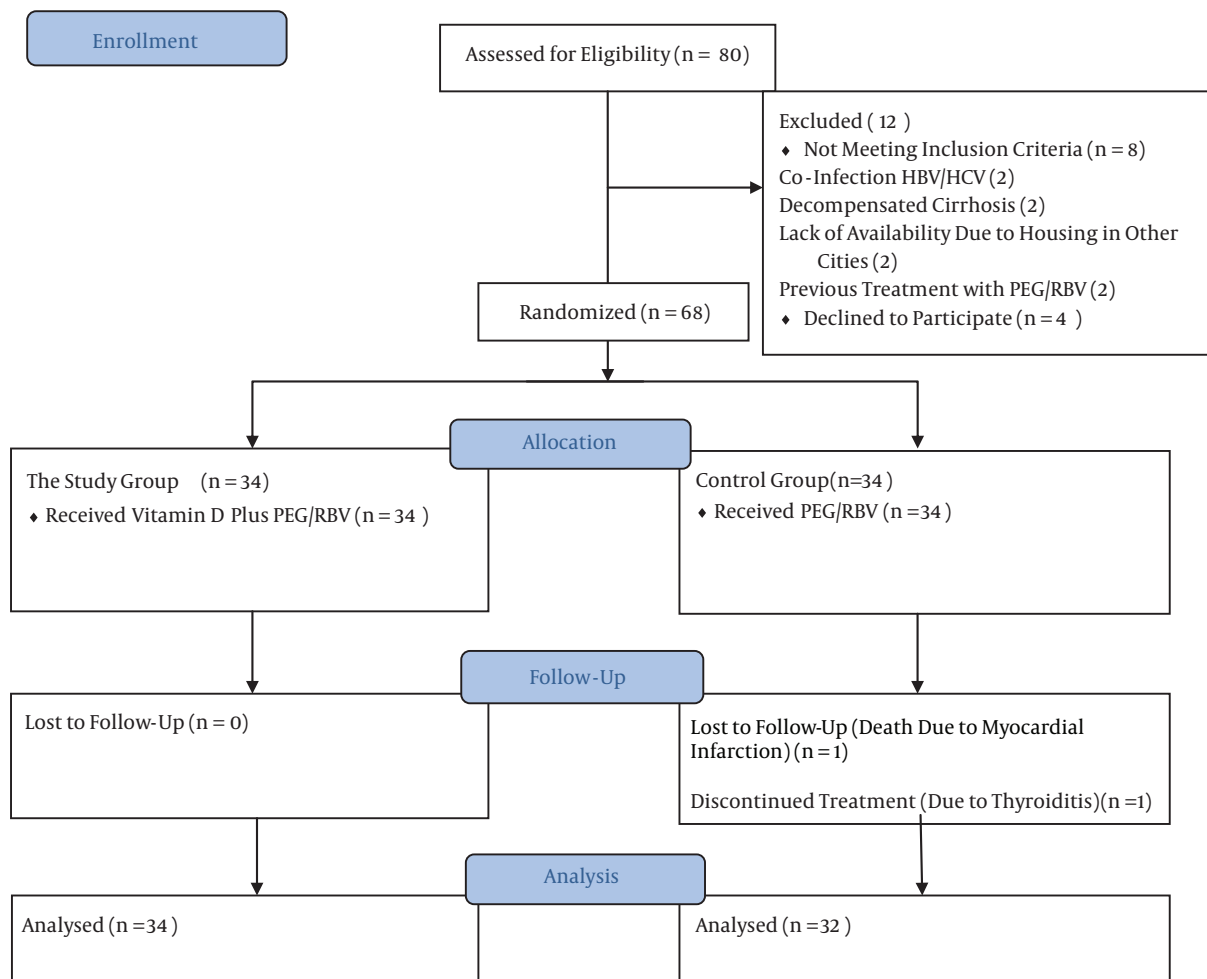


Figure 1. Study Design

commercial ELISA kit (immunodiagnostic system, UK) in accordance to the manual by the manufacturer. Vitamin D levels < 20 ng/mL are regarded as deficient, with levels 20 - 30 ng/mL and > 30 ng/mL considered insufficient and normal, respectively (7).

Complete EVR referred to undetectable HCV viral load 12 weeks after initiation of therapy.

All patients were visited monthly for six months in the Gastroenterology clinic by an expert gastroenterologist. Every effort was made to improve patient compliance, such as free doctor visits and free HCV PCR tests during treatment.

Yet, two patients were excluded during follow-up from the control group following their development of myocardial infarction and thyroiditis.

3.3. Study Medications

In the study group (consisting of eighteen patients with HCV genotype 1 or 4 and 16 with genotype 2 or 3), those with baseline vitamin D levels < 30 ng/mL were given vitamin D₃ (Cholecalciferol) pearls, 50000 IU once weekly. Once sufficient vitamin D levels (> 30 ng/mL) were reached, antiviral therapy was initiated with Peg/RBV. Vitamin D levels were kept above 30 ng/mL throughout antiviral therapy with monthly doses of 50,000 IU (1,600 IU/day) of vitamin D.

Routine anti-HCV treatment including weekly subcutaneous injections of Peg-INF- α 2a at the standard dose of 180 μ g plus RBV (Rebetol, MSD), dosage determined based on patient's weight and genotype, was administered for 48 weeks in patients with genotypes 1 and 4 and for 24 weeks in those with genotypes 2 and 3.

During the course of treatment, serum vitamin D lev-

els were checked monthly and vitamin D supplementation was stopped when levels exceeded 75 ng/mL (23).

In the control group (consisting of 15 HCV patients with a genotype 1 or 4 and 17 with a genotype 2 or 3) only Peg-IFN- α 2a plus RBV was administered with the same dosage as the study group and serum vitamin D level was checked at baseline.

3.4. Sample Size

The sample size was calculated based on the study by Abu-Mouch et al. (19), in terms of type I error (or $\alpha = 0.05$). Also, to have 90% power for comparing the effect of adding vitamin D to the PegIFN/RBV regimen on the cEVR rate in patients infected with HCV, we had the sample size of 30 patients in each group. To account for potential inadequacies in our assumptions and some loss to follow-up, the sample size was increased by 20%.

3.5. Statistical Analysis

The data were per protocol analyzed using SPSS ver. 16 (Statistical Package for Scientific Studies).

For the normally distributed variables, a t-test was used for comparisons. For qualitative variables the Chi-square test or Fisher's exact test were applied.

Spearman's correlation was used for comparison of two non-normally distributed quantitative variables. A P value < 0.05 was regarded as statistically significant.

4. Results

Overall, sixty-six patients, eighty-seven percent of whom were male were studied in two groups of 34 (test) and 32 (control) subjects. The mean age was 42.45 ± 10.7 years. The two groups showed no significant differences concerning age, gender, BMI, and baseline laboratory values except aspartate aminotransferase, which was higher in the control group (Table 1).

Major risk factors for infection with HCV included IV drug abuse (69%), history of imprisonment (32.8%), blood transfusion (8%) and unprotected sexual contact (13.6%). The mean serum level of vitamin D in all patients was 22.5 ± 16.2 ng/mL, with vitamin D deficiency (< 20 ng/mL) occurring in 68.6%, of which 25.7% had severely deficient levels (< 12 ng/mL).

In study group Following 12 weeks of vitamin D therapy, serum levels of vitamin D increased from their lowest of 22 ± 15 ng/mL at baseline to a mean level of 52 ± 38 ng/mL ($P = 0.02$). At this time, 15.4% of the studied cases had vitamin D deficiency and 84.6% had normal vitamin D levels, showing a meaningful difference based on Wilcoxon signed-rank test ($P = 0.002$), as demonstrated in Figure 2.

Table 1. Baseline Characteristics for the Studied Population^a

	Vitamin D Group (n = 34)	Control Group (n = 32)	P Value
Age, y	41.7 $\pm$ 9.48	43.21 $\pm$ 12.08	0.57
Sex	29 $\pm$ 34	29 $\pm$ 32	> 0.99
Male			
Percent	87.9	90	
BMI, kg/m ²	23.89 $\pm$ 3.2	25.51 $\pm$ 6.3	0.37
Iv drug abuser	23 $\pm$ 34	18 $\pm$ 32	0.9
Percent	69.7	56	
Genotype			0.8
1 and 4	18 $\pm$ 52	15 $\pm$ 46	
2 and 3	16 $\pm$ 47	17 $\pm$ 53	
Stage of fibrosis > F2 in genotype 1 and 4	6 $\pm$ 15	5 $\pm$ 15	0.23
Percent	40	73	
AST, IU/L	56.07 $\pm$ 29.63	89.96 $\pm$ 59.42	0.01
ALT, IU/L	86 $\pm$ 59.84	107.04 $\pm$ 110.7	0.38
ESR	6.46 $\pm$ 9.6	3.5 $\pm$ 1.7	0.58
HCV RNA, IU/mL	$2.7 \times 10^6 \pm 8.2 \times 10^5$	$3.03 \times 10^6 \pm 2.6 \times 10^6$	0.85
Serum Vitamin D baseline, ng/mL	22 $\pm$ 15	23 $\pm$ 13	0.65

^aValues are expressed as mean $\pm$ SD.

The mean serum level of vitamin D in the control group was 23 ± 13 ng/mL.

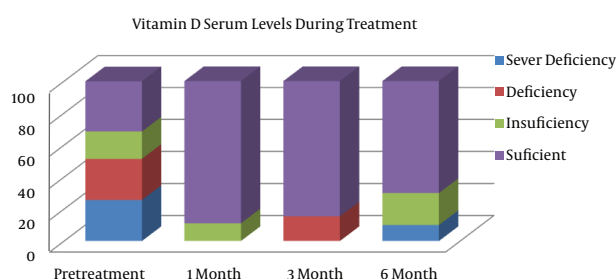
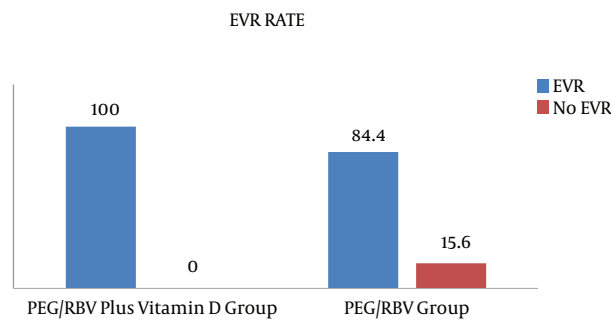


Figure 2. Classification of Serum [25(OH) D] Levels in Vitamin D Group Patients Throughout the Study Period

The treatment outcome at week 12 (EVR rate) is presented in Figure 3. Complete EVR was obtained in 34/34 (100%) patients in the test group, a remarkably higher ratio than that of the control (27/32; 84.4%) ($P = 0.023$). However, between subtypes of the vitamin D group (genotypes 1 versus 4 and 2 versus 3) the complete EVR rate showed no

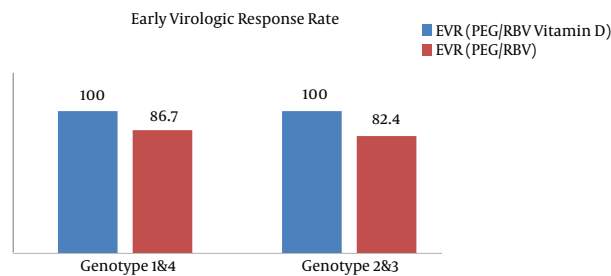
meaningful difference between the two groups (100% versus 86.7% ($P=0.19$) and 100% versus 82.4% ($P=0.22$), respectively) (Figure 4).

Figure 3. Treatment Outcomes at Week 24 (EVR).



EVR, early virologic response.

Figure 4. Treatment Outcomes at Week 24 (EVR) in Different hcv Genotypes



EVR, early virological response.

At baseline, levels of 25(OH)D were found to have a positive correlation with the stage of liver fibrosis ($r=0.57$, $P=0.04$). However, there was not association with AST and ALT levels, BMI and baseline viral load ($P=0.82$, $r=0.04$; $P=0.6$, $r=-0.09$; $P=0.52$, $r=-0.134$; $P=0.21$, $r=-0.22$ respectively).

5. Discussion

It may be suggested by the findings from this study that the EVR rate in HCV-infected patients without prior therapy improves measurably following the co-administration of vitamin D with the current standard treatment regimen as compared to standard therapy alone.

In the present study the EVR rate in the control group (84.4%) was similar to that of previous reports (24, 25). The overall EVR rate reported in the study by Berak et al. from Iran was 88% for genotype 1 patients (26). The same value

was 71.3% in 115 patients with genotype 1 and 85.2% in 61 patients with genotype 2 or 3 in the study by Alavian et al. (27). In another study from Iran, the EVR rate for genotypes 1 and 4 was 88% and for genotypes 2 and 3 was 90%, with the overall cEVR at 89% (2).

In our study, the virologic response after 12 weeks was observed to have a significant increase. The EVR rate was with vitamin D supplementation higher than without it (100% vs 84.4%). However, the difference in genotype subgroups was not statistically significant, which may be consequent to the small number of patients in the subgroups.

Many studies have shown remarkable outcomes regarding the likely beneficial effect of vitamin D supplementation on the outcome of SOC therapy in HCV infection (19, 20).

According to the study performed by Abu-mouch et al. on 72 patients, the co-administration of vitamin D and Peg-a-2b/ribavirin to patients with chronic HCV genotype 1 infection substantially improved the viral response (94% vs 48%) (19).

Another study was performed on 84 HCV-infected patients with genotype 1b and multivariate analysis showed a significant decrease in viral load at 24 weeks (compared to that of week 8) in those receiving vitamin D in addition to antiviral therapy (20).

Esmat et al. reported different results in a study of 101 chronic HCV genotype 4 patients. Vitamin D deficiency was diagnosed in 95% of these cases, whereas vitamin D supplementation did not significantly affect their response to treatment (21).

In a recent study by Terrier et al previously null-responders chronically infected with HCV genotypes 1 or 4 had no improvement in their EVR rate when vitamin D was co-administered with PegIFN/RBV (24, 28).

A meta-analysis conducted in 2014 on 2605 chronic HCV patients concluded that SVR to PEG-IFN/RBV therapy is not significantly associated with baseline 25(OH)D level, regardless of genotype (25).

To date, the precise mechanism behind an enhanced RVR, EVR, and SVR caused by vitamin D is not understood. The role that the immune system plays in acquiring an EVR and SVR is paramount (29). This may be due to the multiple interconnections between vitamin D, the immune response, and the inflammatory status (18).

Vitamin D is converted into its active form (1, 25-dihydroxy-vitamin D3) in the liver (30, 31). Therefore the production of active vitamin D metabolites, may be negatively affected in individuals with chronic liver disease (21). 1, 25 vitamin D3 seems to have a modulatory role in the immune system, mainly by regulating the function of T-cells (32). Furthermore, most cells involved in the function of the immune system express the vitamin D receptor (VDR)

(33). Increasing information show that vitamin D provides better immunity by protecting the host from pathogens and the deleterious effects of prolonged inflammatory responses (34).

Sabry et al. reported reduced IL-6, visfatin, and hyaluronic acid levels in genotype 4a HCV patients during treatment with VitaminD and SOC, which indicates decreased inflammation (35). Therefore, it has been suggested that low vitamin D concentrations prior to initiation of therapy correlate with a poor response to antiviral therapy (11, 36, 37).

Furthermore, recently it has been proposed that the response to regimens containing IFN- α in hepatitis C patients may be increased with vitamin D enhanced inhibition of HCV replication (16).

Several mechanistic studies have reported that calcitriol and IFN- α stimulate an inhibitory effect caused by the interaction between inactive VDR and Stat1. As a consequence, IFN- α -induced binding of phosphorylated Stat1 to its DNA target sequences was enhanced by calcitriol. Thus, VDR silencing results in an elevated response to IFN- α by the hepatocytes. Recently it has been found that, the effect of IFN- α is suppressed by VDR via the Jak-STAT pathway (38).

However, in another study calcitriol was demonstrated to strengthen the anti-HCV properties of miR-130a in Con1b replicon and J6/JFH1 culture systems independent of type I interferon signaling pathway (39).

In another study, the response to antiviral therapy in patients with chronic hepatitis C seemed to be influenced by vitamin D with underlying polymorphic genes such as CYP27B1, CYP24A1 and VDR controlling the mechanism (29).

According to several studies, the prevalence of vitamin D deficiency is high in the Middle East, including Iran, ranging from 20% to 80% depending on the country (40, 41).

IV drug abusers and patients with chronic hepatitis especially chronic hepatitis C are especially at risk for vitamin D deficiency (42). Almost 93% of chronic HCV patients experience insufficient levels of vitamin D with one-third demonstrating severe deficiency (21, 37). In our study, these figures were 68.6% for deficient levels (< 30 ng/dL) and 25.7% for severely deficient levels (< 12 ng/dL). HCV infection is thought to induce cytokines and oxidative stress, which in turn influence direct and indirect 25-hydroxylation, ultimately leading to vitamin D deficiency. It is also believed that altered metabolism of lipids induced by the virus suppresses 25(OH)D levels (37). Recently, HCV was associated with a decreased level of 7-dehydrocholesterol, the endogenous vitamin D precursor (43). In general, the main risk factor for infection with the hepatitis C virus in Iran is IV drug abuse. Accordingly, sixty-nine percent of our patients had a history of current or past

IV drug abuse. However, vitamin D deficiency in a significant proportion of IV drug users (IDUs) is assumed to be the result of nutritional factors (44). Therefore, routine laboratory testing of vitamin D levels in patients with liver disease has been recommended by certain studies (13).

In another recently conducted study, 398 HCV infected patients with genotype 1 were evaluated prospectively. While patients received antiviral treatment, fluctuations in levels of 25-OH-vitamin D exceeding 5 ng/mL (10) were observed in 66 patients (39%) (45). In the current study, 25(OH)D serum levels of patients in the study group also varied considerably during treatment. Despite receiving vitamin D supplementation in the first month, only 88.9% of patients had sufficient vitamin D levels (> 30 ng/dL), a figure which decreased to 70% after six months of treatment. This major change may be either due to the suggested synergistic influence on IFN-gene expression and HCV replication by 1,25(OH) $_2$ D $_3$ and IFN- α or because of seasonal variation in vitamin D consumption and production.

Finally, the main limitations of our study were the small number of patients for each genotype, and the short follow-up period. Moreover, neither was the study placebo-controlled nor were the patients blinded to who took vitamin D supplements. Another limitation was the fact that no information was available regarding determinants of the viral response, e.g. polymorphisms of the IL28B gene and the VDR. However this was the first study in Iran in which vitamin deficiency was very common.

Taken together, in order to better evaluate the effect of vitamin D on achieving a SVR and clarify the mechanism of vitamin D supplementation in such patients, further research is warranted with long-term follow-up on a larger population.

5.1. Conclusions

Vitamin D deficiency is prevalent among chronic hepatitis C cases and the combination of vitamin D plus Peg/RBV significantly increases the rate of rapid, early viral responses in treatment-naïve patients who are infected with HCV genotype 1, 2, 3 or 4. Therefore, vitamin D supplementation may indeed be a potential therapeutic choice in this group of patients who cannot be provided with or may have contraindications to the use of specifically targeted novel antiviral medications. Moreover, vitamin D is a low cost complementary treatment resulting in improved viral response for patients in countries like Iran where new anti-HCV drugs are expensive or unavailable until now, but may be available and affordable in near future.

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Footnotes

Authors' Contribution: Saeid Amel Jamehdar carried out RNA extractions and real-time PCR. Ladan Goshayeshi collected patient samples, performed clinical registrations, participated in the design of the study and its revising and editing, carried out the manuscript writing, and submitted the article as the corresponding author. Hasan Vosoghnia, Azita Ganji, Seyed Mousal-Reza Hossein, Ali Bahari, and Omid Ghanaei collected patient samples, and participated in the design of the study and editing. Maryam Sahebbari participated in the design of the study and the statistical analysis of the manuscript. Farnood Rajabzadeh performed liver ultrasonography and Kamran Ghaffarzadehgan reviewed liver biopsies. All authors had access to the study data and reviewed and approved the final manuscript.

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References

1. Neal KR, Trent Hepatitis CG, Ramsay S, Thomson BJ, Irving WL. Excess mortality rates in a cohort of patients infected with the hepatitis C virus: a prospective study. *Gut*. 2007;**56**(8):1098-104. doi: [10.1136/gut.2006.113217](#). [PubMed: [17344277](#)].
2. Jabbari H, Bayatian A, Sharifi AH, Zaer-Rezaee H, Fakharzadeh E, Asadi R, et al. Safety and efficacy of locally manufactured pegylated interferon in hepatitis C patients. *Arch Iran Med*. 2010;**13**(4):306-12. [PubMed: [20597564](#)].
3. Khodabandehloo M, Roshani D. Prevalence of hepatitis C virus genotypes in Iranian patients: a systematic review and meta-analysis. *Hepat Mon*. 2014;**14**(12):ee22915. doi: [10.5812/hepatmon.22915](#). [PubMed: [25685164](#)].
4. Merat S, Rezvan H, Nouraie M, Jafari E, Abolghasemi H, Radmard AR, et al. Seroprevalence of hepatitis C virus: the first population-based study from Iran. *Int J Infect Dis*. 2010;**14** Suppl 3:e113-6. doi: [10.1016/j.ijid.2009.11.032](#). [PubMed: [20362479](#)].
5. Kermani FR, Sharifi Z, Ferdowsian F, Paz Z, Zamanian M. Distribution of hepatitis c virus genotypes among chronic infected injecting drug users in Tehran, Iran. *Jundishapur J Microbiol*. 2013;**6**(3):265-8.
6. Kheirandish P, SeyedAlinaghi S, Jahani M, Shirzad H, Seyed Ahmadian M, Majidi A, et al. Prevalence and correlates of hepatitis C infection among male injection drug users in detention, Tehran, Iran. *J Urban Health*. 2009;**86**(6):902-8. doi: [10.1007/s11524-009-9393-0](#). [PubMed: [19844670](#)].
7. Manns M, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, et al. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*. 2014;**384**(9941):414-26. doi: [10.1016/S0140-6736\(14\)60538-9](#). [PubMed: [24907224](#)].
8. Namazee N, Sali S, Asadi S, Shafiei M, Behnava B, Alavian SM. Real response to therapy in chronic hepatitis C virus patients: a study from Iran. *Hepat Mon*. 2012;**12**(9):ee6151. doi: [10.5812/hepatmon.6151](#). [PubMed: [23087759](#)].
9. Yu JW, Wang GQ, Sun LJ, Li XG, Li SC. Predictive value of rapid virological response and early virological response on sustained virological response in HCV patients treated with pegylated interferon alpha-2a and ribavirin. *J Gastroenterol Hepatol*. 2007;**22**(6):832-6. doi: [10.1111/j.1440-1746.2007.04904.x](#). [PubMed: [17565637](#)].
10. Chopp S, Vanderwall R, Hult A, Klepser M. Simeprevir and sofosbuvir for treatment of hepatitis C infection. *Am J Health Syst Pharm*. 2015;**72**(17):1445-55. doi: [10.2146/ajhp140290](#). [PubMed: [26294237](#)].
11. Nimer A, Mouch A. Vitamin D improves viral response in hepatitis C genotype 2-3 naïve patients. *World J Gastroenterol*. 2012;**18**(8):800-5. doi: [10.3748/wjg.v18.i8.800](#). [PubMed: [22371640](#)].
12. Watkins RR, Lemonovich TL, Salata RA. An update on the association of vitamin D deficiency with common infectious diseases. *Can J Physiol Pharmacol*. 2015;**93**(5):363-8. doi: [10.1139/cjpp-2014-0352](#). [PubMed: [25741906](#)].
13. Iruzubieta P, Teran A, Crespo J, Fabrega E. Vitamin D deficiency in chronic liver disease. *World J Hepatol*. 2014;**6**(12):901-15. doi: [10.4254/wjh.v6.i12.901](#). [PubMed: [25544877](#)].
14. Van Belle TL, Gysemans C, Mathieu C. Vitamin D in autoimmune, infectious and allergic diseases: a vital player? *Best Pract Res Clin Endocrinol Metab*. 2011;**25**(4):617-32. doi: [10.1016/j.beem.2011.04.009](#). [PubMed: [21872803](#)].
15. Yano M, Ikeda M, Abe K, Dansako H, Ohkoshi S, Aoyagi Y, et al. Comprehensive analysis of the effects of ordinary nutrients on hepatitis C virus RNA replication in cell culture. *Antimicrob Agents Chemother*. 2007;**51**(6):2016-27. doi: [10.1128/AAC.01426-06](#). [PubMed: [17420205](#)].
16. Matsumura T, Kato T, Sugiyama N, Tasaka-Fujita M, Murayama A, Masaki T, et al. 25-Hydroxyvitamin D3 suppresses hepatitis C virus production. *Hepatology*. 2012;**56**(4):1231-9. doi: [10.1002/hep.25763](#). [PubMed: [22487892](#)].
17. Petta S, Ferraro D, Camma C, Cabibi D, Di Cristina A, Di Marco V, et al. Vitamin D levels and IL28B polymorphisms are related to rapid virological response to standard of care in genotype 1 chronic hepatitis C. *Antivir Ther*. 2012;**17**(5):823-31. doi: [10.3851/IMP2100](#). [PubMed: [22505587](#)].
18. 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. *J Zhejiang Univ Sci B*. 2014;**15**(10):900-6. doi: [10.1631/jzus.B1400073](#). [PubMed: [25294379](#)].
19. Abu-Mouch S, Fireman Z, Jarchofsky J, Zeina AR, Assy N. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. *World J Gastroenterol*. 2011;**17**(47):5184-90. doi: [10.3748/wjg.v17.i47.5184](#). [PubMed: [22215943](#)].
20. Yokoyama S, Takahashi S, Kawakami Y, Hayes CN, Kohno H, Kohno H, et al. Effect of vitamin D supplementation on pegylated interferon/ribavirin therapy for chronic hepatitis C genotype 1b: a randomized controlled trial. *J Viral Hepat*. 2014;**21**(5):348-56. doi: [10.1111/jvh.12146](#). [PubMed: [24716637](#)].
21. Esmat G, El Raziky M, Elsharkawy A, Sabry D, Hassany M, Ahmed A, et al. 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**(1):49-54. doi: [10.1089/jir.2014.0060](#). [PubMed: [25061714](#)].
22. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. *Dig Dis Sci*. 2010;**55**(9):2624-8. doi: [10.1007/s10620-009-1069-9](#). [PubMed: [19960254](#)].
23. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc*. 2010;**85**(8):752-7. doi: [10.4065/mcp.2010.0138](#). [PubMed: [20675513](#)] quiz 757-8.

24. Terrier B, Lapidus N, Pol S, Serfaty L, Ratzu V, Asselah T, et al. Vitamin D in addition to peg-interferon-alpha/ribavirin in chronic hepatitis C virus infection: ANRS-HC25-VITAVIC study. *World J Gastroenterol*. 2015;**21**(18):5647-53. doi: [10.3748/wjg.v21.i18.5647](#). [PubMed: [25987791](#)].
25. Kitson MT, Sarrazin C, Toniutto P, Eslick GD, Roberts SK. Vitamin D level and sustained virologic response to interferon-based antiviral therapy in chronic hepatitis C: a systematic review and meta-analysis. *J Hepatol*. 2014;**61**(6):1247-52. doi: [10.1016/j.jhep.2014.08.004](#). [PubMed: [25135863](#)].
26. Berak H, Laskus T, Kolakowska-Rzadzka A, Wasilewski M, Stanczak JJ, Bardadin K, et al. Peginterferon alfa-2a and peginterferon alfa-2b combined with ribavirin in patients with genotype 1 chronic hepatitis C: results of a prospective single-centre study. *Adv Med Sci*. 2014;**59**(2):261-5. doi: [10.1016/j.advms.2014.01.005](#). [PubMed: [25117425](#)].
27. Alavian SM, Ahmadzad M, Keshvari M. Efficacy and safety of interferon-alpha (Peginterferon B) and ribavirin combination therapy in patients with chronic hepatitis C in Iran. *Hepat Mon*. 2006;**6**:11-8.
28. Chen EQ, Shi Y, Tang H. New insight of vitamin D in chronic liver diseases. *Hepatobiliary Pancreat Dis Int*. 2014;**13**(6):580-5. [PubMed: [25475859](#)].
29. Jimenez-Sousa MA, Rallon N, Berenguer J, Pineda-Tenor D, Lopez JC, Soriano V, et al. TLR3 polymorphisms are associated with virologic response to hepatitis C virus (HCV) treatment in HIV/HCV coinfected patients. *J Clin Virol*. 2015;**65**:62-7. doi: [10.1016/j.jcv.2015.02.004](#). [PubMed: [25766991](#)].
30. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr*. 2004;**80**(6 Suppl):1689S-96S. [PubMed: [15585789](#)].
31. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol*. 2005;**289**(1):F8-28. doi: [10.1152/ajprenal.00336.2004](#). [PubMed: [15951480](#)].
32. Muller K, Bendtzen K. 1,25-Dihydroxyvitamin D3 as a natural regulator of human immune functions. *J Invest Dermatol Symp Proc*. 1996;**1**(1):68-71. [PubMed: [9627696](#)].
33. Hewison M. Vitamin D and the intracrinology of innate immunity. *Mol Cell Endocrinol*. 2010;**321**(2):103-11. doi: [10.1016/j.mce.2010.02.013](#). [PubMed: [20156523](#)].
34. Trochoutsou AI, Kloukina V, Samitas K, Xanthou G. Vitamin-D in the Immune System: Genomic and Non-Genomic Actions. *Mini Rev Med Chem*. 2015;**15**(11):953-63. [PubMed: [25985946](#)].
35. Sabry D, Al-Ghusein MA, Hamdy G, Abul-Fotouh A, Motawi T, El Kazaz AY, et al. Effect of vitamin D therapy on interleukin-6, visfatin, and hyaluronic acid levels in chronic hepatitis C Egyptian patients. *Ther Clin Risk Manag*. 2015;**11**:279-88. doi: [10.2147/TCRM.S66763](#). [PubMed: [25737638](#)].
36. Bitetto D, Fattovich G, Fabris C, Ceriani E, Falletti E, Fornasiere E, et al. Complementary role of vitamin D deficiency and the interleukin-28B rs12979860 C/T polymorphism in predicting antiviral response in chronic hepatitis C. *Hepatology*. 2011;**53**(4):1118-26. doi: [10.1002/hep.24201](#). [PubMed: [21480318](#)].
37. Petta S, Camma C, Scazzone C, Tripodo C, Di Marco V, Bono A, et al. 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**(4):1158-67. doi: [10.1002/hep.23489](#). [PubMed: [20162613](#)].
38. Lange CM, Gouttenoire J, Duong FH, Morikawa K, Heim MH, Moradpour D. Vitamin D receptor and Jak-STAT signaling crosstalk results in calcitriol-mediated increase of hepatocellular response to IFN-alpha. *J Immunol*. 2014;**192**(12):6037-44. doi: [10.4049/jimmunol.1302296](#). [PubMed: [24821973](#)].
39. Duan X, Guan Y, Li Y, Chen S, Li S, Chen L. Vitamin D Potentiates the Inhibitory Effect of MicroRNA-130a in Hepatitis C Virus Replication Independent of Type I Interferon Signaling Pathway. *Mediators Inflamm*. 2015;**2015**:508989. doi: [10.1155/2015/508989](#). [PubMed: [26060358](#)].
40. Alipour S, Saberi A, Seifollahi A, Shirzad N, Hosseini L. Risk factors and prevalence of vitamin d deficiency among Iranian women attending two university hospitals. *Iran Red Crescent Med J*. 2014;**16**(10):eee15461. doi: [10.5812/ircmj.15461](#). [PubMed: [25763193](#)].
41. Heshmat R, Mohammad K, Majdzadeh SR, Forouzanfar MH, Bahrami A, Omrani GHR. Vitamin D deficiency in Iran: A multi-center study among different urban areas. *Iran J Public Health*. 2008;**37**(suppl).
42. Pinzone MR, Di Rosa M, Malaguarnera M, Madeddu G, Foca E, Caccarelli G, et al. Vitamin D deficiency in HIV infection: an underestimated and undertreated epidemic. *Eur Rev Med Pharmacol Sci*. 2013;**17**(9):1218-32. [PubMed: [23690192](#)].
43. Clark PJ, Thompson AJ, Vock DM, Kratz LE, Tolun AA, Muir AJ, et al. Hepatitis C virus selectively perturbs the distal cholesterol synthesis pathway in a genotype-specific manner. *Hepatology*. 2012;**56**(1):49-56. doi: [10.1002/hep.25631](#). [PubMed: [22318926](#)].
44. Lambert AA, Drummond MB, Mehta SH, Brown TT, Lucas GM, Kirk GD, et al. Risk factors for vitamin D deficiency among HIV-infected and uninfected injection drug users. *PLoS One*. 2014;**9**(4):eee95802. doi: [10.1371/journal.pone.0095802](#). [PubMed: [24756000](#)].
45. Grammatikos G, Lange C, Susser S, Schwendy S, Dikopoulos N, Buggisch P, et al. Vitamin D levels vary during antiviral treatment but are unable to predict treatment outcome in HCV genotype 1 infected patients. *PLoS One*. 2014;**9**(2):ee87974. doi: [10.1371/journal.pone.0087974](#). [PubMed: [24516573](#)].