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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 PegINF/RBV therapy.

Patients and Methods: In a randomized-controlled trial 66 patients with HCV (30 with genotype 1or 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 PegINF-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 $\pm$ 15 ng/mL), but increased after 12 weeks of vitamin D therapy to a mean level of 52 $\pm$ 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 populationare 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 sofosvobir and ledasprevir 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 affording 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 asses this hypothesis.

2. Objectives

Given the high cost of new oral drugs including sofosvobir 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 mm^3, a platelet count above 90,000 per mm^3, 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

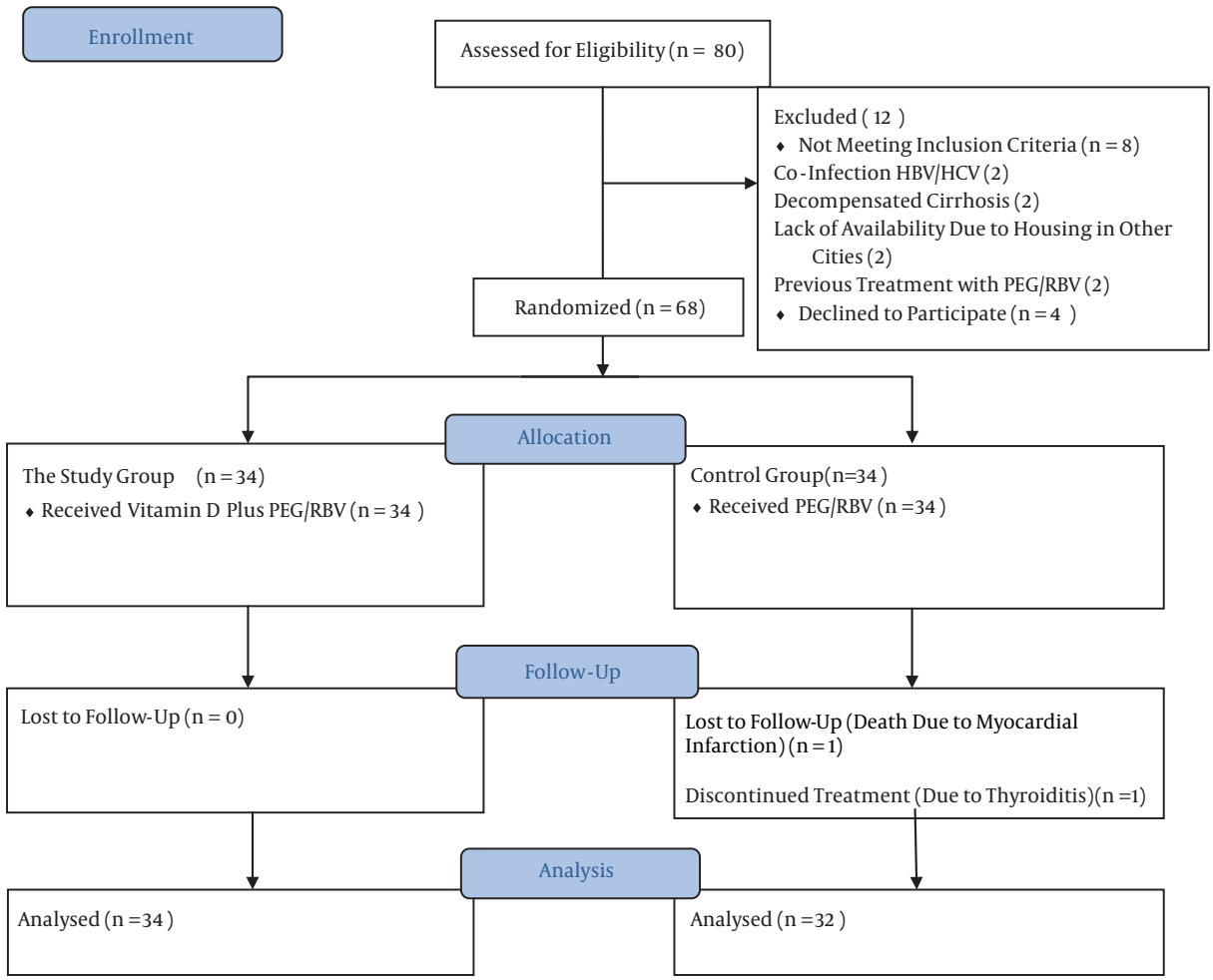


Figure 1. Study Design

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 D_3 (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-α2a at the standard dose of 180 μg 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-INF-α2a 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 ± 9.48	43.21 ± 12.08	0.57
Sex	29 ± 34	29 ± 32	> 0.99
Male			
Percent	87.9	90	
BMI, kg/m^2	23.89 ± 3.2	25.51 ± 6.3	0.37
Iv drug abuser	23 ± 34	18 ± 32	0.9
Percent	69.7	56	
Genotype			0.8
1 and 4	18 ± 52	15 ± 46	
2 and 3	16 ± 47	17 ± 53	
Stage of fibrosis > F2 In genotype 1 and 4	6 ± 15	5 ± 15	0.23
Percent	40	73	
AST, IU/L	56.07 ± 29.63	89.96 ± 59.42	0.01
ALT, IU/L	86 ± 59.84	107.04 ± 110.7	0.38
ESR	6.46 ± 9.6	3.5 ± 1.7	0.58
HCV RNA, IU/m	2.7 ×10^6 ± 8.2 × 10^6	3.03 × 10^6 ± 2.6 × 10^6	0.85
Serum Vitamin D baseline, ng/mL	22 ± 15	23 ± 13	0.65

[a]Values are expressed as mean ± SD.

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

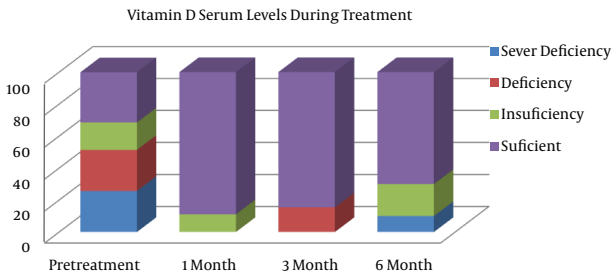
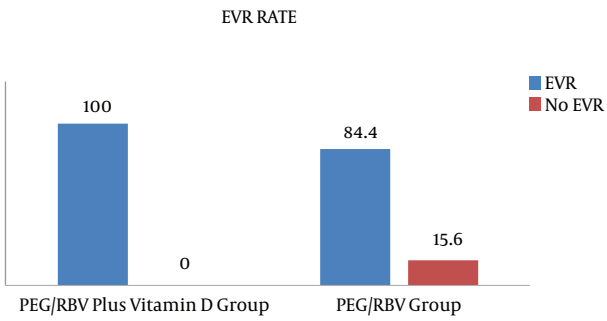


Figure 2. Classification of Serum [25(OH) D] Levels in Vitamin D Group Patients Throughout the Study Period

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 and2 versus 3) the complete EVR rate showed no

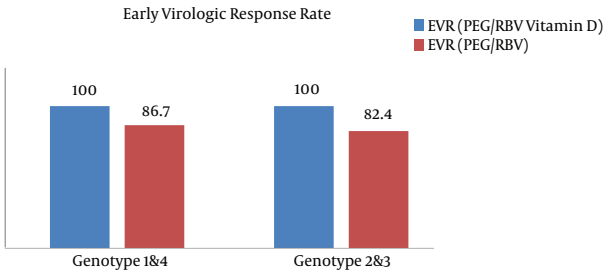
meaningful difference between the two groups (100% versus 86.7% (P = 0.19) and 100% versus 82.4% (P = 0.22), respectively) (Figure 4).

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



EVR, early virological 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)2D3 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-naive 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 Vosoghinia, 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 Sahebari 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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