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RESEARCH PAPER

Efficacy of vitamin D supplementation in combination with conventional antiviral therapy in patients with chronic hepatitis C infection: a meta-analysis of randomised controlled trials

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Keywords
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Abstract

Background: Although a contributory role of vitamin D levels for the development of chronic hepatitis C has been suggested, the efficacy of vitamin D supplementation in combination with conventional antiviral therapy consisting of pegylated interferon-α (Peg-IFN-α) injection and oral ribavirin (RBV) remains unclear. We investigated its efficacy in the treatment of chronic hepatitis C via a meta-analysis of randomised controlled trials.
Methods: We searched PubMed, EMBASE, the Cochrane Library, ClinicalTrials.gov and the bibliographies of relevant articles to locate additional publications in September 2016. Three evaluators independently reviewed and selected eligible studies based on predetermined selection criteria.
Results: Of 522 articles meeting our initial criteria, a total of seven open-label, randomised controlled trials involving 548 participants, were included in the final analysis. Vitamin D supplementation in combination with Peg-IFN-α injection and oral RBV significantly increased the rate of viral response for hepatitis C at 24 weeks after treatment in a random-effects meta-analysis (relative risk = 1.30; 95% confidence interval = 1.04–1.62; I^2 = 75.9%). Also, its significant efficacy was observed in patients with hepatitis C virus genotype 1, which is known to be refractory to antiviral therapy.
Conclusions: In summary, we observed that additional use of vitamin D has a positive effect on sustained viral response rates of patients with chronic hepatitis C infection. However, we cannot establish the efficacy because of substantial heterogeneity, a small sample size and a low methodological quality.

Introduction

More than 170 million individuals have hepatitis C virus (HCV) infection worldwide, and approximately four million people are newly infected with HCV every year[(1)]. Up to 80% of patients with HCV infection proceed to chronic hepatitis C (CHC), which can ultimately lead to the development of liver cirrhosis and hepatocellular

carcinoma [2]. Previously, the standard treatment of CHC was a combination therapy with weekly pegylated interferon-α (Peg-IFN-α) injection and daily oral ribavirin (RBV) [2]. For example, a large randomised trial reported that the combination therapy of Peg-IFN-α and RBV increased sustained viral response (SVR) up to 52% [3], which is defined as the absence of detectable HCV RNA 24 weeks after completion of antiviral therapy for CHC. Before first-generation direct-acting antivirals (DAAs) were licensed for use in HCV genotype 1 infection in 2011, their combination for 24 or 48 weeks was the approved treatment for CHC [4].

In the meantime, observational epidemiological studies have reported that there is an association between low levels of vitamin D and the risk of CHC. A retrospective cohort study with 468 study participants showed that the mean level of vitamin D in noncirrhosis CHC patients was 17 ng mL^{-1} and 25% of them had a level of vitamin D <10 ng mL^{-1}, whereas only 12% of controls had that level [5]. Also, in a nested case–control study, CHC patients with genotype 1 had lower serum levels of 25-hydroxyvitamin [6].

However, the correlation between serum vitamin D levels and SVR in CHC infection remains controversial. CHC patients whose baseline level of vitamin D was more than 30 ng mL^{-1} showed high SVR rates [odds ratio (OR) = 1.57; 95% confidence interval (CI) = 1.12–2.2] and vitamin D supplementation was also positively associated with a high rate of SVR (OR = 4.59; 95% CI = 1.67–12.63), irrespective of HCV genotype in a meta-analysis [7]. Also, vitamin D deficiency was associated with a low rate of SVR in patients with HCV genotypes 2 and 3 [5]. However, a meta-analysis including 11 studies reported that initial vitamin D status was not associated with the rate of SVR to the combination therapy of Peg-IFN-α and RBV, regardless of HCV genotypes [8]. Furthermore, no meta-analysis on the efficacy of vitamin D supplementation for the treatment of CHC has been published so far.

In the present study, we investigated the associations between vitamin D supplementation and the success rate of Peg-IFN-α and RBV in CHC via a meta-analysis of randomised clinical trials.

Materials and methods

Literature search

We searched PubMed, EMBASE, the Cochrane Library and ClinicalTrials.gov using common keywords related to vitamin D supplementation in addition to the combination treatment of peg-IFN-α and RBV and the SVR of CHC in September 2016. The keywords were: 'vitamin D', '25(OH)D', '1,25(OH)D', 'cholecalciferol', 'alfacalcidol' and 'ergocalciferol' for exposure factors; 'hepatitis C', 'HCV', 'liver disease', 'viral response' and 'hepacivirus' for outcome factors. Also, we reviewed the bibliographies of relevant articles to locate additional studies. The language of publication was not restricted.

Selection criteria

We included randomised clinical trials that met all of the criteria: (i) had investigated the efficacy of vitamin D supplements for the SVR of CHC with a combination therapy of Peg-IFN-α and RBV; (ii) had enrolled at least 30 study participants; (iii) had followed the study participants for at least 24 weeks; and (iv) had reported measurements of SVR in both the vitamin D supplementation and control groups. If data were duplicated or shared in more than one study, the first published study was included in the analysis. We only included articles that were published in peer-reviewed journals and excluded abstracts, letters and posters presented in academic conferences.

Selection of relevant studies

Three investigators independently assessed the eligibility of all studies based on the predetermined selection criteria. Disagreements about the selection of studies between investigators were resolved by consensus.

Assessment of methodological quality

The methodological quality of included studies was assessed based on the Jadad scale [9], which is the most widely applied measurement tool. Its score ranges from zero (very poor) to five-point (rigorous). The five-point quality scale consists of points for randomisation (described as randomised, one point; table of random numbers or computer-generated randomisation, additional one point), double-blind (described as double-blind, one point; use masking such as identical placebo, additional one point) and follow-up (state the numbers and reasons for withdrawal in each group; one point) in the report of each trial. Those with a score of 3 or higher were considered as a high-quality study, and we conducted subgroup meta-analysis by quality.

Main and subgroup analysis

We investigated the association between vitamin D supplementation in combination with Peg-IFN-α and RBV and SVR in patients with CHC infection. Also, subgroup meta-analyses were carried out by various factors: vitamin D dosage (<2000 IU daily versus ≥2000 IU daily),

methodological quality (high versus low), duration of supplementation (less than 48 weeks versus 48 weeks or longer), number of participants (<70 versus 70>), HCV genotype, geographical region, mean age (<46 years versus >46 years), funding source (pharmaceutical industry versus independent organisation), pretreatment with vitamin D before antiviral therapy, time of viral response, initial HCV-RNA titre, body mass index (BMI), initial vitamin D status, vitamin D assay and initial alanine aminotransaminase/aspartate aminotransaminase (ALT/AST) levels. A main outcome measure was SVR at 24 weeks after antiviral therapy for CHC. Also, we investigated its efficacy on RVR (i.e. rapid viral response at 4 weeks), EVR (i.e. early viral response at 12 weeks) and SVR at 48 weeks.

Statistical analysis

We computed relative risks (RRs) with 95% CIs by using crude 2 × 2 tables based on an intention-to-treat analysis, whenever possible. For the assessment of heterogeneity across studies, we used Higgins I^2, which measures the percentage of total variation across publications [10]. I^2 was calculated as:

$$I^2 = 100\% \times (Q - \mathrm{df})/Q,$$

where Q is Cochran's heterogeneity statistic and d.f. indicates the degrees of freedom. Negative values of I^2 were set at zero; the I^2 results are between 0% (no observed heterogeneity) and 100% (maximal heterogeneity). An I^2 value >50% was considered to indicate substantial heterogeneity [10]. We used a random-effects model meta-analysis based on the DerSimonian and Laird method because individual trials were conducted in the different populations [11].

We evaluated publication bias using Begg's funnel plot and Egger's test. When publication bias exists, Begg's funnel plot presents asymmetry or $P < 0.05$ by Egger's test. We used Stata SE, version 13.0 (StataCorp, College Station, TX, USA) for the statistical analysis.

Results

Identification of relevant studies

Figure 1 represents a flow diagram of the selection of relevant studies. A total of 522 articles were found by searching four databases (PubMed, EMBASE, the Cochrane Library and ClinicalTrials.gov) and hand-searching relevant bibliographies. After excluding 189 duplicated articles, three of the investigators independently examined and excluded additional 307 articles that did not satisfy the predetermined selection criteria on the basis of each article's title and abstract. Among these, 19 articles were excluded after review of the full texts because of: use of vitamin D status as an exposure variable ($n = 6$); use of acute biomarkers as exposure ($n = 3$); no use of a randomisation method ($n = 4$); inclusion of HIV/HCV co-infected participants ($n = 2$); use of only one arm ($n = 2$); insufficient data ($n = 1$); and use of alfacalcidol supplementation in the nonsupplementation control group ($n = 1$). Studies not using a randomisation method include two retrospective case–control studies, one prospective case–control study and one pilot study. The remaining seven open-label, randomised controlled trials [12–18] were included in the final analysis.

Characteristics of studies included in the final analysis

A total of seven studies which were published between 2011 and 2016 included 548 participants (270 in the vitamin D supplementation group and 278 in the control group). The mean (range) age of the study participants was 46.3 (7–82) years. Table 1 shows the general characteristics of the studies included in the final analysis. They were conducted in: Japan ($n = 2$), Egypt ($n = 2$), Israel ($n = 1$), US ($n = 1$) and Iran ($n = 1$). The dosage regimens used in the trials were 1000/1600/2000 IU daily and 15 000 IU weekly of vitamin D supplements. In three studies, study participants received vitamin D supplements before the administration of antiviral therapy for CHC. Among these studies, participants took vitamin D supplements for 4 weeks before the treatment of Peg-IFN-α and RBV in two studies [12,17] and for 12 weeks before the antiviral treatment in one study [13]. Two trials reported funding sources: one [17] was funded by pharmaceutical companies, whereas the other [18] was funded by an independent scientific foundation. Three trials [14,17,22] enrolled CHC patients whose genotype was 1 (two trials [14,17] with genotype 1b); two trials [13,16] enrolled those who had genotype other than 1; and only one study [18] included those with all genotypes. All participants received a standard HCV regimen (Peg-IFN-α and RBV) as antiviral therapy for CHC except for the study by Atsukawa *et al.* [17] in which participants received simeprevir as a DAA in addition to a standard regimen.

Methodological quality of studies

We assessed the methodological quality of studies included in the final analysis based on the Jadad Scale. Scores ranged between 2 and 3, and the mean score was 2.1 (see Supporting information, Appendix S1). Only one study [17] was considered as s a high-quality study with a score of 3.

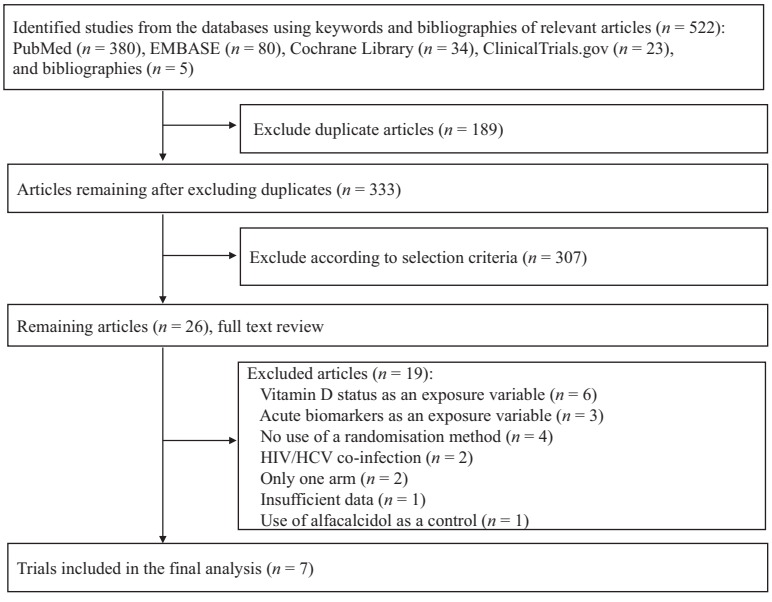

Figure 1 Flow diagram for the identification of relevant studies.

Main analysis

As shown in Fig. 2, in the random-effects meta-analysis of all seven studies, vitamin D supplementation with Peg-IFN-α and RBV therapy for CHC significantly increased SVR at 24 weeks after treatment (RR = 1.30; 95% CI = 1.04–1.62; I^2 = 75.9%).

Subgroup meta-analyses

Table 2 shows the findings from the subgroup meta-analyses by various factors. In the subgroup meta-analysis excluding a trial for children, a significant effect was found. Also, vitamin D supplementation was consistently associated with the increased rate of SVR in the subgroup meta-analyses by funding sources. Subgroup meta-analyses by HCV genotype revealed a significant positive association in three studies including HCV genotype 1 (RR = 1.71; 95% CI = 1.38–2.13; I^2 = 9.9%), whereas no significant association was observed in two studies with HCV genotypes 2, 3 or 4 (see Supporting information, Appendix S2). Also, vitamin D supplementation showed an efficacy when administered at low dosage (<2000 IU day^{-1}) but not at high dosage (≥2000 IU day^{-1}). Pretreatment of vitamin D showed a significantly increased SVR (RR = 1.64; 95% CI = 1.20–2.46; I^2 = 77.8%), whereas vitamin D supplementation without pretreatment failed to show a significantly increased SVR (RR = 1.11; 95% CI = 0.82–1.50; I^2 = 77.7%).

Regarding BMI status, a significantly increased SVR (RR = 1.39; 95% CI = 1.10–1.75; I^2 = 61.3%) was observed in the participants with low BMI (<25 kg m^{-2}) but not in those with high BMI status (≥25 kg m^{-2}). There was a significant positive association in participants with high initial vitamin D levels (>21 ng mL^{-1}; RR = 1.39; 95% CI = 1.10–1.75; I^2 = 61.3%) but not in those with low initial vitamin D levels. For initial laboratory measurements, a positive association was observed in low HCV-RNA titre and low initial ALT/AST levels but not in high levels.

The subgroup meta-analysis by time of viral response showed no significant association between vitamin D supplementation and SVR. Regarding geographical region, the efficacy of vitamin D supplementation was observed only in East Asia but not in Western countries and Middle Asia. Also, its efficacy was not observed in the younger age group (mean age <46 years) and in high-quality studies.

No publication bias was found. The Begg's funnel plot was symmetrical and P for bias was 0.58 in the Egger's test (see Supporting information, Appendix S3).

Discussion

In the current meta-analysis of randomised controlled trials, we found that vitamin D supplementation in combination with a standard therapy was associated with an increased rate of SVR of CHC. Additionally, this association was observed in favour of HCV genotype 1, pretreatment with vitamin D, a high initial vitamin D level, patients with low BMI, a low initial HCV-RNA titre and a low initial ALT/AST status. However, we found that the included trials had substantial heterogeneity across studies, a small sample size and a low methodological quality.

Table 1 Characteristics of trials* included in the final meta-analysis (n = 7)

Number	Study	Country	Participants (average age, years; women, %)	Duration of supplementation, weeks (follow-up period)	Intervention versus control	Main outcome measures used	Number of participants achieving SVR/number of participants	
							Vitamin D supplementation group	Control group
1	Abu-Mouch *et al.* (2011)	Israel	72 patients with chronic hepatitis C virus genotype 1 (48; 44)	52 (48)	Vitamin D_3 2000 IU day^{-1} versus no supplementation	SVR at 24 weeks	31/36	15/36
2	Nimer *et al.* (2012)	USA	50 patients with chronic hepatitis C virus genotypes 2 or 3 (46; 38)	24 (24)	Vitamin D_3 2000 IU day^{-1} versus no supplementation	SVR at 24 weeks	19/20	23/30
3	Yokoyama *et al.* (2014)	Japan	84 patients with chronic hepatitis C virus genotype 1b (59; 49)	72 (72)	Vitamin D_3 1000 IU day^{-1} versus no supplementation	SVR at 24 weeks	33/42	23/42
4	Eltayeb *et al.* (2015)	Egypt	66 children with hepatitis C virus (11; 35)	48 (48)	Vitamin D_3 2000 IU day^{-1} versus no supplementation	SVR at 24 weeks	24/31	17/29
5	Esmat *et al.* (2015)	Egypt	101 patients with chronic hepatitis C virus genotype 4 (40; 25)	48 (72)	Vitamin D_3 15 000 IU $week^{-1}$ versus no supplementation	SVR at 72 weeks	22/50	35/51
6	Atsukawa*et al.* (2015)	Japan	115 patients with chronic hepatitis C virus genotype 1b (64; 50)	48 (48)	Vitamin D_3 2000 IU day^{-1} versus no supplementation	SVR at 24 weeks	40/57	22/58
7	Vosoghinia *et al.*. (2016)	Iran	66 patients with chronic hepatitis C virus (39; 32)	24 (24)	Vitamin D_3 1600 IU day^{-1} versus nonsupplementation	SVR at 24 weeks	34/34	27/32

IU, international unit; SVR, sustained viral response.

*All the included trials are open-label, randomised, controlled trials.

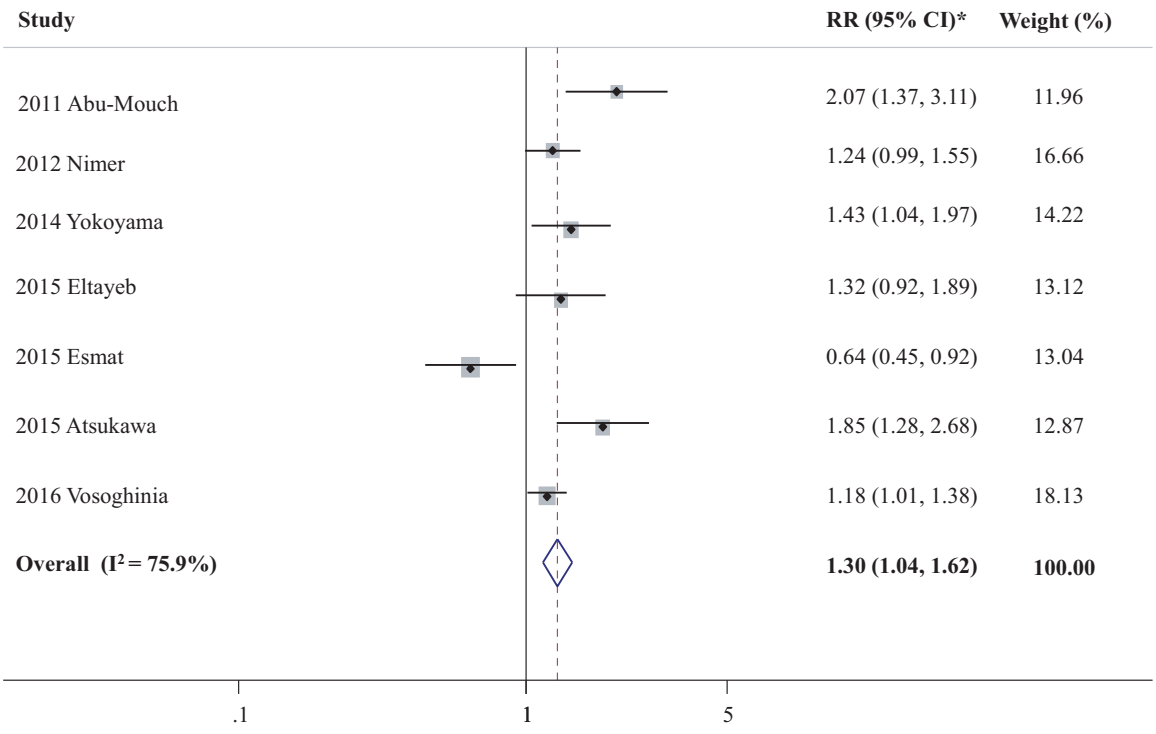

Figure 2 Efficacy of vitamin D supplementation in combination with conventional antiviral therapy in treatment of chronic hepatitis C in a meta-analysis of open-label, randomised controlled trials (n = 7). *Random-effects model. RR, relative risk; CI, confidence interval.

It remains unclear how vitamin D supplementation improves SVR in CHC patients taking standard antiviral therapy. However, inflammation is suggested as a possible biological explanation for its efficacy. The stability between stimulatory and inhibitory immune cytokines is changed by continual HCV infection, which can raise the degree of inflammation, and this phenomenon can result in chronic liver disease and liver cirrhosis [19]. Vitamin D supplementation can suppress virus productions by generating the innate immune system in an *in vitro* study [20]. Almost all types of cells related to immunity express a vitamin D receptor (VDR) [21] and vitamin D appears to control the immune system mainly by handling T-cell antigen-presenting cell functions [22].

CHC infection is also related to elevated circulating levels of multiple inflammatory cytokines such as tumour necrosis factor (TNF), which is involved in systemic inflammation and makes up the acute phase reaction. Circulating levels of TNF-α and soluble TNF receptor (TNFR) in CHC patients were increased compared to those of controls, and the severity of liver inflammation is associated with serum TNFR status [23,24]. A randomised, double-blinded, placebo-controlled trial of 50 CHC patients conducted by Zein *et al.* [25] showed an additional evidence of the significance of TNF-α in HCV infection by therapy with etanercept, acting as TNF-α antagonist, which enhanced SVR rates of Peg-IFN-α and RBR treatment. Vitamin D can play an important role in modulation of inflammatory reactions and fibrosis in CHC patients by inhibiting TNF-α, which affects fibrosis progression through suppression of TGF-β [26].

Another biological mechanism by which vitamin D contributed to increased rates of SVR in CHC might be through insulin resistance. *De novo* progression of insulin resistance can be caused by CHC infection itself [27] and is one of the conventional factors in the characters related to difficult-to-treat patients [28]. The direct efficacy of vitamin D may be interceded through binding of its circulating active form to the pancreatic B cell VDR [29].

In addition, vitamin D can have a pivotal role for a novel anti-HCV agent through reinforcement of IFN-β expression and induction of IFN-stimulated genes, which exhibit effector molecules with antiviral activities [16].

One of the most important factors that can anticipate the successive SVR rate of Peg-IFN-α and RBV is the HCV genotype. SVR rates in patients with genotype 1 HCV infection measure 40–60%, whereas those in patients with genotypes 2 or 3 HCV infection reach approximately 70%–80% [4,30] when treated by standard antiviral therapy. In the present study, vitamin D supplementation showed an antiviral effect on genotype 1 HCV infection, which is refractory to treatment compared with easy-to-treat genotype. Also, HCV

Table 2 Efficacy of vitamin D supplementation in combination with conventional antiviral therapy in treatment of chronic hepatitis C in subgroup meta-analyses by various factors

Factor	Number of Trials	Summary RR (95% CI)	Heterogeneity, I^2
All	7	1.30 (1.04–1.62)	75.9
All excluding the trial by Etlayeb *et al.* [15] with children	6	1.30 (1.01–1.68)	79.8
Vitamin D dosage			
<2000 IU daily	2	1.26 (1.02–1.56)	40.5
≥2000 IU daily	5	1.31 (0.91–1.88)	82.7
Methodological quality			
Low quality	6	1.23 (0.98–1.55)	74.7
High quality	1	1.85 (1.28–2.68)	NA
Duration of supplementation			
<48 weeks	3	1.34 (1.02–1.77)	75.5
≥48 weeks	4	1.25 (0.79–1.99)	84.7
Number of participants			
$n < 70$	3	1.21 (1.08–1.37)	0.0
$n \geq 70$	4	1.36 (0.82–2.26)	87.1
HCV genotype			
Genotype 1	3	1.71 (1.38–2.13)	9.9
Genotype 2–4	2	0.90 (0.41–1.97)	92.6
All genotypes	1	1.18 (1.01–1.38)	NA
Region			
Western	2	1.57 (0.86–2.87)	85.3
East Asia	2	1.60 (1.24–2.06)	7.8
Middle Asia	3	1.01 (0.66–1.56)	85.0
Mean age (years)			
<46	3	1.01 (0.66–1.56)	85.0
>46	4	1.56 (1.20–2.04)	63.3
Funding sources			
Pharmaceutical industry	1	1.85 (1.28–2.68)	NA
Independent organisation	1	1.18 (1.01–1.38)	NA
Pretreatment with vitamin D			
No pretreatment	4	1.11 (0.82–1.50)	77.7
Pretreatment	3	1.64 (1.20–2.46)	77.8
Pretreatment for 4 weeks	2	1.94 (1.48–2.56)	0
Pretreatment for more than 4 weeks	1	1.24 (0.99–1.55)	NA
Time of viral response			
RVR	3	3.45 (0.71–16.70)	89.5
EVR	3	2.96 (0.84–10.42)	80.2
SVR at 48 weeks	1	1.29 (0.88–1.87)	NA
Initial HCV-RNA titre			
Low levels (<log 6.3 IU mL^{-1})	3	1.71 (1.38–2.13)	9.9
High levels (>log 6.3 IU mL^{-1})	3	1.01 (0.66–1.56)	85.0
BMI			
Low (<25 kg m^{-2})	4	1.39 (1.10–1.75)	61.3
High (≥25 kg m^{-2})	3	1.17 (0.66–2.08)	89.2
Initial vitamin D status			
Low (<21 ng mL^{-1})	3	1.17 (0.66–2.08)	89.2
High (>21 ng mL^{-1})	4	1.39 (1.10–1.75)	61.3
Vitamin D assay			
^{125}I-immunoassay	4	1.43 (1.15–1.78)	46.1
Non-^{125}I-immunoassay	2	1.09 (0.38–3.08)	93.8
Initial ALT levels			
Low (<63)	3	1.45 (1.12–1.87)	55.5
High (≥63)	3	1.01 (0.66–1.56)	85.0
Initial AST levels			
Low (<59)	3	1.43 (1.03–1.98)	76.4
High (≥59)	2	0.92 (0.45–1.89)	87.3

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; EVR, early viral response at 12 weeks; HCV, hepatitis C virus; IU, international unit; NA, not applicable; RNA, ribonucleic acid; RVR, rapid viral response at 4 weeks.
All analyses were performed based on a random-effects meta-analysis.

RNA titres lower than 800 000 IU mL^{-1} with low viral load are associated with approximately 2.4–2.7 times higher SVR rates than those more than 800 000 IU mL^{-1} [31,32]. Elevated baseline ALT levels are significantly associated with the persistence of HCV infection [33]. Being overweight is also reported to be another factor that can lessen the achievement of SVR [34]. Regarding HCV-RNA titres, ALT levels and BMI, we found that additional vitamin D supplementation was not associated with an increased SVR rate of standard antiviral therapy in severe cases of CHC. Furthermore, there were differences in the rates of SVR after vitamin D supplementation by ethnicity. For example, in an open-labelled, nonrandomised trial, Hispanic and African people are less likely to react to standard treatment [35]. This might be attributable to the differences of single nucleotide polymorphisms of the interleukin (IL)-28B gene, vitamin D deficiency or polymorphism of VDR among various ethnicity [6,36].

Recently, with the exception of the EASL (European Association for the Study of the Liver), many other organisations commonly recommend IFN-free regimens as first-line therapy options because new DAAs have shown higher SVR rate than IFN therapy [37]. However, because of the high prices of DAAs, IFN-based treatment is still the preferred choice for standard treatment of HCV hepatitis, especially in developing countries. Besides

cost, IFN also has a curative role in HCV patients with favourable IFN-response characteristics, such as a favourable IL28B genotype, which has a high prevalence in Asia [38]. Thus, the additional use of vitamin D for HCV could be applied in those countries.

There are several important limitations in the present study. First, substantial heterogeneity was observed in the main analysis. This heterogeneity might be attributable to clinical and methodological diversity in individual trials. To explore the reasons for the heterogeneity, we performed subgroup meta-analyses by various factors. However, substantial heterogeneity was still found in most subgroup meta-analyses. Second, the number of the included studies in this analysis was relatively small. Also, except for two trials, the sample sizes were small (less than 100). It is usually known that a phase 1 clinical trial screening for safety covers a 'small' group of study participants less than 100. Generally, to establish the efficacy of the drug, trials with a larger group of participants (i.e. several hundred or a thousand) participants are needed. Further large trials are required. Third, except for one study [17], the overall methodological quality of the included trials in the present study was low. Fourth, the included trials analysed data on the basis of surrogate outcomes such as SVR instead of reporting clinical outcomes such as the complications of cirrhosis, decompensation of cirrhosis, hepatocellular carcinoma, extra-hepatic manifestations and mortality. Furthermore, all of the included trials did not use either a double-blind method or an identical placebo. Also, only one study [17] clearly described randomisation methods. Last, six out of seven included studies did not disclose the source of the funding, which might have influenced the study designs, results and data interpretation.

In conclusion, our meta-analysis of open-label, randomised controlled trials found that the additional use of vitamin D has a positive effect on SVR rates of patients with CHC infection. However, there is insufficient evidence to establish the efficacy of additional vitamin D supplementation in combination with conventional antiviral therapy because of substantial heterogeneity, a small sample size and a low methodological quality, etc. Further large randomised, double-blind, placebo-controlled trials are warranted to confirm its efficacy.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained. The reporting of this work is compliant with CONSORT[1]/STROBE[2]/PRISMA[3] guidelines.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.

No funding declared.

S-KM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. S-KM and H-BK were responsible for the study concept and design. S-KM, H-BK, Y-JL and B-JP were responsible for acquisition, analysis or interpretation of data. H-BK was responsible for drafting the manuscript. S-KM and H-BK were responsible critical revision of the manuscript for important intellectual content. S-KM and H-BK were responsible for the statistical analysis. All authors critically reviewed the manuscript and approved the final version submitted for publication.

Ethical approval

Not applicable.

References

1. Averhoff FM, Glass N & Holtzman D (2012) Global burden of hepatitis C: considerations for healthcare providers in the United States. *Clin Infect Dis* **55**, S10–S15.
2. Ghany MG, Strader DB, Thomas DL *et al.* (2009) Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* **49**, 1335–1374.
3. Fried MW, Shiffman ML, Reddy KR *et al.* (2002) Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* **347**, 975–982.
4. European Association for Study of Liver (2015) EASL recommendations on treatment of hepatitis C. *J Hepatol* **63**, 199–236.
5. Lange CM, Bojunga J, Ramos-Lopez E *et al.* (2011) Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. *J Hepatol* **54**, 887–893.
6. Petta S, Camma C, Scazzone C *et al.* (2010a) Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* **51**, 1158–1167.
7. Villar LM, Del Campo JA, Ranchal I *et al.* (2013) Association between vitamin D and hepatitis C virus infection: a meta-analysis. *World J Gastroenterol* **9**, 5917–5924.
8. Kitson MT, Sarrazin C, Toniutto P *et al.* (2014) Vitamin D level and sustained virologic response to interferon-

based antiviral therapy in chronic hepatitis C: a systematic review and meta-analysis. *J Hepatol* **61**, 1247–1252.
9. Jadad AR, Moore RA, Carroll D *et al.* (1996) Assessing the quality of reports of randomised clinical trials: is blinding necessary? *Control Clin Trials* **17**, 1–12.
10. Higgins JP & Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* **21**, 1539–1558.
11. Borenstein M, Hedges LV, Higgins JP *et al.* (2010) A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res Synth Methods* **1**, 97–111.
12. Abu-Mouch S, Fireman Z, Jarchovsky J *et al.* (2011) Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. *World J Gastroenterol* **17**, 5184–5190.
13. Nimer A & Mouch A (2012) Vitamin D improves viral response in hepatitis C genotype 2-3 naïve patients. *World J Gastroenterol* **18**, 800–805.
14. Yokoyama S, Takahashi S, Kawakami Y *et al.* (2014) Effect of vitamin D supplementation on pegylated interferon/ribavirin therapy for chronic hepatitis C genotype 1b: a randomized controlled trial. *J Viral Hepat* **21**, 348–356.
15. Eltayeb AA, Abdou MA, Abdel-aal AM *et al.* (2015) Vitamin D status and viral response to therapy in hepatitis C infected children. *World J Gastroenterol* **21**, 1284–1291.
16. Esmat G, El Raziky M, Elsharkawy A *et al.* (2015) 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* **35**, 49–54.
17. Atsukawa M, Tsubota A, Shimada N *et al.* (2016) Effect of native vitamin D3 supplementation on refractory chronic hepatitis C patients in simeprevir with pegylated interferon/ribavirin. *Hepatol Res* **46**, 450–458.
18. Vosoghinia H, Esmaeilzadeh A, Ganji A *et al.* (2016) Vitamin D in standard HCV regimen (PEG-interferon plus ribavirin), its effect on the early virologic response rate: a clinical trial. *Razavi Int J Med* **4**, e36632.
19. Larrubia JR, Benito-Martínez S, Calvino M *et al.* (2008) Role of chemokines and their receptors in viral persistence and liver damage during chronic hepatitis C virus infection. *World J Gastroenterol* **14**, 7149–7159.
20. Gal-Tanamy M, Bachmetov L, Ravid A *et al.* (2011) Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. *Hepatology* **54**, 1570–1579.
21. Hewison M (2010) Vitamin D and the intracrinology of innate immunity. *Mol Cell Endocrinol* **321**, 103–111.
22. Müller K & Bendtzen K (1996) 1,25-Dihydroxyvitamin D3 as a natural regulator of human immune functions. *J Investig Dermatol Symp Proc* **1**, 68–71.
23. Zylberberg H, Rimaniol AC, Pol S *et al.* (1999) Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. *J Hepatol* **30**, 185–191.
24. Itoh Y, Okanoue T, Ohnishi N *et al.* (1999) Serum levels of soluble tumor necrosis factor receptors and effects of interferon therapy in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* **94**, 1332–1340.
25. Zein NN (2005) Etanercept as an adjuvant to interferon and ribavirin in treatment-naive patients with chronic hepatitis C virus infection: a phase 2 randomized, double-blind, placebo-controlled study. *J Hepatol* **42**, 315–322.
26. Tan X, Li Y & Liu Y (2007) Therapeutic role and potential mechanisms of active Vitamin D in renal interstitial fibrosis. *J Steroid Biochem Mol Biol* **103**, 491–496.
27. Wong RJ & Gish RG (2016) Metabolic manifestations and complications associated with chronic hepatitis C virus infection. *Gastroenterol Hepatol (N Y)* **12**, 293–299.
28. Grasso A, Malfatti F, De Leo P *et al.* (2009) Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin. *J Hepatol* **51**, 984–990.
29. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
30. Park SH, Park CK, Lee JW *et al.* (2012) Efficacy and tolerability of peginterferon alpha plus ribavirin in the routine daily treatment of chronic hepatitis C patients in Korea: a multi-center, retrospective observational study. *Gut Liv* **6**, 98–106.
31. Thompson AJ, Muir AJ, Sulkowski MS *et al.* (2010) Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* **139**, 120–129.
32. von Wagner M, Huber M, Berg T *et al.* (2005) Peginterferonalpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* **129**, 522–527.
33. Esmat G, Hashem M, El-Raziky M *et al.* (2012) Risk factors for hepatitis C virus acquisition and predictors of persistence among Egyptian children. *Liver Int* **32**, 449–456.
34. Rodriguez-Torres M, Sulkowski MS, Chung RT *et al.* (2010) Factors associated with rapid and early virologic response to peginterferon alfa-2a/ribavirin treatment in HCV genotype 1 patients representative of the general chronic hepatitis C population. *J Viral Hepat* **17**, 139–147.
35. Rodriguez-Torres M, Jeffers LJ, Sheikh MY *et al.* (2009) Peginterferon alfa-2a and ribavirin in Latino and non-Latino whites with hepatitis C. *N Engl J Med* **360**, 257–267.
36. Ge D, Fellay J, Thompson AJ *et al.* (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* **461**, 399–401.
37. Lynch SM & Wu GY (2016) Hepatitis C virus: a review of treatment guidelines, cost-effectiveness, and access to therapy. *J Clin Transl Hepatol* **4**, 310–319.
38. Suwanthawornkul T, Anothaisintawee T, Sobhonslidsuk A *et al.* (2015) Efficacy of second generation direct-acting antiviral agents for treatment naïve hepatitis C genotype 1:

a systematic review and network meta-analysis. *PLoS ONE* **10**, e0145953.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Methodological quality of trials based on the Jadad Scale (n = 7).

Appenix S2. Efficacy of vitamin D supplementation in combination with conventional antiviral therapy in treatment of chronic hepatitis C in a meta-analysis of open-label, randomized controlled trials according to genotype of hepatitis C virus (n = 7). * Random-Effects Model. RR, relative risk; CI, confidence interval.

Appendix S3. Begg's funnel plot and Egger's test for identifying publication bias in a meta-analysis of open-label, randomized controlled trials (n = 7). RR, relative risk.