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ORIGINAL ARTICLE

Absence of Relationship between Serum Vitamin D Levels, Degree of Hepatic Fibrosis, and Virologic Response to Pegylated Interferon and Ribavirin Therapy in Patients with Chronic Hepatitis C

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ABSTRACT

AIM: In patients with chronic hepatitis C virus (HCV) infection, 25-hydroxyvitamin D3 [25(OH)D] deficiency has been associated with disease progression and decreased odds of sustained virologic response (SVR) to pegylated interferon and ribavirin combination therapy (PegIFN + RBV).

METHODS: In this retrospective study, the pretreatment 25(OH)D levels of patients with genotype 1 HCV infection treated with PegIFN + RBV for 48 weeks were measured and correlated with disease stage and periportal necroinflammatory activity (PPA) on liver biopsy, as well as to virologic response at the end of the treatment period. Serum 25(OH)D levels were quantitated by chemiluminescence.

RESULTS: Of the 201 patients included, 53% were male and 70% were white, with a mean (SD) age of 46 (± 11) years. Subtype 1b was identified in 48% of cases, and 57% of patients had a viral load over 800 000 IU/mL. Overall, 29% of patients had stage 3/4 fibrosis and 40% had grade 3/4 PPA. SVR was achieved in 47% of patients. The mean 25(OH)D concentration was 26.3 (± 11.5) ng/mL. Low 25(OH)D levels (< 30 ng/dL) were detected in 69% of patients, and were not associated with staging (rS = -0.003; P = 0.895) or grading (rS = 0.045; P = 0.105). There were no significant differences in SVR rate between patients with *vs* those without 25(OH)D deficiency (49% *vs* 40%, P = 0.359). The only variables associated with 25(OH)D insufficiency or deficiency were serum albumin (rS = 0.199; P = 0.006), female sex (rS = 0.249; P = 0.002), and age (rS = -0.227; P = 0.001).

CONCLUSION: In this sample of patients with chronic HCV infection, vitamin D deficiency was not associated with increased histologic severity, nor with increased likelihood of SVR.

Key Words: 25-hydroxyvitamin D; Hepatitis C virus; Hepatic fibrosis; Sustained virologic response; Adults

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INTRODUCTION

Vitamin D plays essential roles in the body, including regulation of the transcription of genes involved in cell proliferation, differentiation, immune modulation, inflammation, and fibrogenesis[1,2].

Vitamin D deficiency or insufficiency can be the result of several mechanisms, ranging from dietary deficiencies and inadequate exposure to light to changes in hepatic and renal hydroxylation processes[3]. The association between 25(OH)D deficiency and liver disease has been described since the mid-1950s[4], when authors first reported osteoporosis and osteomalacia in a high percentage of patients with liver cirrhosis and chronic diarrhea, probably due to reduced absorption of vitamin D in the gut. Since then, several studies have demonstrated the association between vitamin D deficiency and chronic liver disease[5-7].

Hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease worldwide. It is estimated that approximately 160 million individuals worldwide are living with chronic HCV infection[8-11]. The long-term impact of chronic HCV infection is highly variable, ranging from mild abnormalities to chronic hepatitis with extensive fibrosis (or cirrhosis) and progression to hepatocellular carcinoma (HCC)[8,9].

Many studies have sought to associate chronic hepatitis C and changes in serum vitamin D levels, from reports of increased prevalence of low bone mineral density in these patients[12] to increased expression of vitamin D receptors in inflammatory liver cells, leading to increased histologic severity, a higher degree of inflammatory activity, and rapid disease progression[13-16]. Some authors have also found lower rates of sustained virologic response (SVR) to pegylated interferon (PegIFN) and ribavirin (RBV) therapy in patients with genotype-1 chronic hepatitic C and vitamin D deficiency[14,17,18]. However, more recent studies have found no association between vitamin D deficiency and degree of fibrosis, inflammatory activity, or SVR[19,20]; accordingly, this remains a controversial topic, and further research is required.

In the present study, a retrospective analysis of serum 25(OH)D levels in Brazilian patients with chronic genotype-1 HCV infection before PegIFN + RBV combination therapy was conducted to evaluate potential associations of these levels with histologic parameters and subsequent virologic response to therapy.

PATIENTS AND METHODS

Patients aged 18-70 years, with chronic hepatitis C confirmed by positive HCV RNA in serum, who were undergoing follow-up at the outpatient clinic of the Hepatology Section, Division of Gastroenterology, Federal University of São Paulo, Brazil, were eligible for the study. Only those patients who were treatment-naive, had undergone baseline liver biopsy before the start of treatment, and provided written consent were included. Between January 2001 and December 2012, these patients received combination treatment with pegylated interferon (peginterferon alfa-2a, 180 mcg/week, or peginterferon alfa-2b, 1.5 mcg/kg/week, via subcutaneous injection) and ribavirin (weight-adjusted dosing) for 48 weeks.

The study was conducted by means of a review of medical records. Patients with HCC, other concomitant liver disease (except non-alcoholic fatty liver disease), hepatitis B virus or HIV coinfection, alcohol intake > 20 g/day, organ transplant recipients, those with comorbidities such as chronic kidney disease or hyperparathyroidism, and those on any medications known to interfere with vitamin D metabolism were excluded.

HCV-RNA detection and quantitation in serum was performed via a polymerase chain reaction (PCR) assay, with a 50 UI/mL limit of detection (Cobas Amplicor Test, Roche Diagnostics, USA) HCV genotyping was performed by sequencing of the five-prime untranslated (5´UTR) region. Response to antiviral therapy was assessed by qualitative HCV-RNA testing on week 4 of treatment (rapid virologic response, RVR), quantitative HCV-RNA testing on week 12 of treatment (early virologic response, EVR), qualitative HCV-RNA testing at the end of treatment (EOT), and qualitative HCV-RNA testing 6 months after EOT (SVR).

Biochemistry panels (AST, ALT, GGT, ALP, bilirubin,albumin, platelet count, prothrombin activity, FRT, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose) were performed in an automated analyzer.

Liver biopsies were obtained up to 6 months before the start of treatment. Histologic staging and grading of necroinflammatory activity (as assessed by periportal activity) were performed in accordance with the criteria recommended by the Brazilian Society of Hepatology (SBH) and Brazilian Society of Pathology (SBP) Classification of Chronic Hepatitis[21], on scales of 0 to 4.

Serum 25(OH)D was measured in samples stored at -20 ℃, with the commercially available LIAISON® 25 OH Vitamin D TOTAL Assay kit (Saluggia, Italy), which uses chemiluminescence immunoassay (CLIA) technology to quantitate 25(OH)D and other hydroxylated metabolites of vitamin D in serum, EDTA plasma, or lithium-heparin plasma. The reference ranges adopted were: <10 ng/mL, deficiency; 10-30 ng/mL, insufficiency; and ⩾ 30 ng/mL, sufficiency. For purposes of analysis, 25(OH)D levels ⩽ 30 ng/ml were categorized as low, and those ⩾ 30 ng/mL as normal or sufficient.

Statistical analysis

The Student *t*-test and Mann-Whitney *U* test were used for between-group comparisons of continuous and non-continuous variables respectively. For comparison of categorical variables, the chi-square or Fisher's exact test were used as appropriate. The significance level was set at $P < 0.05$. All statistical analyses were performed in the IBM SPSS Statistics for Windows, Version 20.0 software environment (Armonk, NY: IBM Corp).

The study protocol was drafted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Federal University of São Paulo Research Ethics Committee with judgment number 1986/11.

RESULTS

Of 299 records reviewed, 8 (2.7%) were excluded due to the presence of factors that could interfere with serum vitamin D levels, 27 (9.0%) because data were insufficient for analysis, and 63 (21.1%) due to issues during collection and/or storage of serum samples for 25(OH) D measurement. Therefore, 201 patients (67.2%) were ultimately included in the study (Figure 1).

The mean age was 46 ± 11 years. Of the included patients, 53% (n = 107) were male, 69% were white (n = 138), 27% black or brown, and 4% of Asian descent. One hundred and two patients (53%) were overweight or obese (BMI ⩾ 25 kg/m²).

Characterization of hepatitis C virus

From the 201 HCV positive selected patients for vitamin D determination, 201 had a confirmed genotype and 151 a recent (less than 6 months) viral load.

Genotypes 1a and 1b were found in 38% and 48% of patients respectively. In 57% of patients, the viral load was ⩾ 800 000 IU/mL.

Regarding histologic findings, 71% of patients (n = 143) had stage 0-2 fibrosis and 29% had advanced fibrosis (stage 3-4; n = 58). Overall, 16% were cirrhotic (stage 4). Regarding periportal necroinflammatory activity (PPA), 60% of patients had grade 0-2 PPA (n = 118) and the remaining 40% had grade 3 or 4 PPA (n = 78). Half of the included patients had no evidence of steatosis on liver biopsy specimens; in the other half, steatosis was graded as mild, moderate, or intense.

HCV and virologic response

Of the included patients, 69% were treated with PegIFN alfa-2b

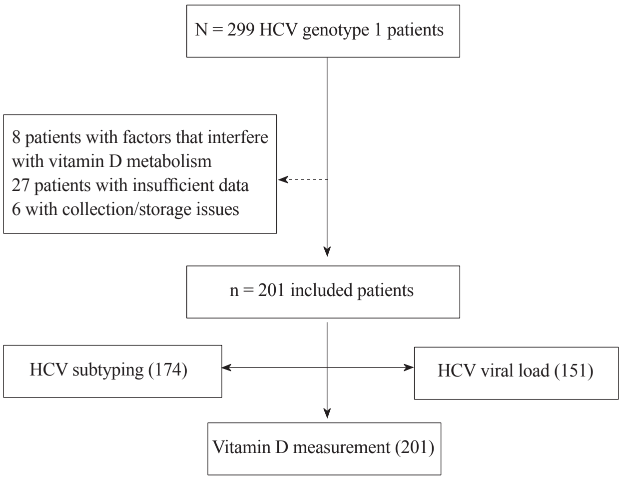

Figure 1 Flow chart of included patients (n = 201).

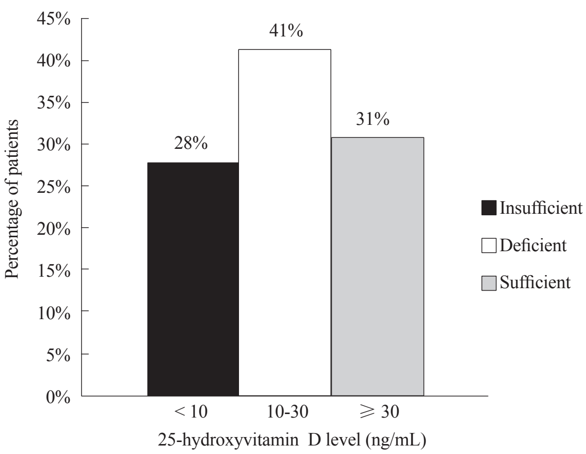

Figure 2 Characterization of 25(OH)D levels in the sample (n = 201).

and 31% with PegIFN alfa-2a. Concerning RVR, of the 88 patients analyzed, 32 (36%) had undetectable HCV-RNA levels by week 4 of treatment. Of these 32 patients, 94% (n = 30) achieved SVR, versus only 21% (n = 12) of patients who did not exhibit RVR (n = 56). Approximately 60% of patients analyzed had EVR and 47% achieved SVR (n = 94).

Comparison of histologic parameters in patients who achieved SVR versus nonresponders found significantly lower treatment response rates in patients with steatosis (χ^2 = 4.632, P = 0.031) and in those with more severe PPA (χ^2 = 5.090, P = 0.024). At the predefined cutoff point of 800 000 IU/mL, HCV viral load was not associated with SVR in the studied patients (P = 0.138).

The mean (± SD) serum level of vitamin D in the analyzed population was 26.3 ± 11.5 ng/mL (range, 4-75 ng/mL). Of the included patients, 69% had 25(OH)D deficiency or insufficiency (n = 139) (Figure 2). Low 25(OH)D levels were found predominantly in female and older patients (Table 1). Low 25(OH)D levels were not associated with BMI (54% of these patients were overweight or obese, defined as BMI ⩾ 25 kg/m²) or blood glucose levels (mean fasting glucose 95.5 mg/dL) (no significant difference). There were no between-group differences in platelet counts, ferritin levels, cholesterol (total or fractions), triglycerides, AST, ALT, ALP, or GGT (Table 2). Among the other liver function tests, only albumin was significantly different between groups (P = 0.006).

Regarding histologic features, there were no significant between-group differences in staging, not even after patients were stratified by

Table 1 Pretreatment histologic profile of the sample, stratified by serum 25(OH)D levels.

Variable	Normal vitamin D n (%)	Deficient or insufficient vitamin D n (%)	P
Staging	(n = 62)	(n = 139)	
⩾ 2	34 (55)	83 (60)	0.518
⩾ 3	17 (27)	41 (30)	0.764
4	11 (33)	22 (67)	0.895
Periportal activity	(n = 60)	(n = 136)	
PPA3/PPA4	29 (48)	49 (36)	0.105
Steatosis	(n = 56)	(n = 125)	
Present	25 (45)	66 (53)	0.310

Table 2 Pretreatment clinical and biochemical profile of the sample, stratified by serum 25(OH)D levels (n = 201).

Variable	Normal vitamin D (n = 62)	Deficient or insufficient vitamin D (n = 139)	P
Age, years#	43 ± 11	48 ± 11	0.001*
Sex (female), n (%)	19 (31%)	75 (54%)	0.002*
Skin color (white), n (%)	45 (75%)	90 (67%)	0.291
Overweight/obesity, n(%)	31 (51%)	71 (54%)	0.701
AST (U/L)	64 ± 39	62 ± 40	0.871
ALT (U/L)	98 ± 82	84 ± 59	0.336
ALP (U/L)	134 ± 67	140 ± 78	0.511
GGT (U/L)	74 ± 50	86 ± 88	0.899
BIL (mg/dL)	0.4 ± 0.4	0.4 ± 0.2	0.697
ALB (g/dL)	4.5 ± 0.5	4.3 ± 0.5	0.006*
PTp (%)	92 ± 13	94 ± 11	0.258
Platelets (× 10^9/L)	202 ± 68	185 ± 62	0.118
Glucose (mg/dL)	94.1 ± 20.1	95.5 ± 24	0.520
HDL-c (mg/dL)	46 ± 13	53 ± 21	0.595
LDL-c (mg/dL)	98 ± 30	95 ± 32	0.740
TG (mg/dL)	101 ± 36	111 ± 63	0.530

*P < 0.05. #μ ± SD = mean ± standard deviation; *P < 0.05; chi-square test or Student t-test.

significant fibrosis (stage ⩾ 2 [n = 117] *vs*. stage < 2 [n = 84], P = 0.518), advanced fibrosis (stage ⩾ 3 [n = 58] *vs*. stage < 3 [n = 143], P = 0.764), or presence versus absence of cirrhosis (stage 4 [n = 33] *vs*. stage ⩽ 3 [n = 168], P = 0.895) (Table 1). Concerning the grade of PPA, patients were divided into two groups: PPA 0-2 and PPA 3-4. Among those patients with 25(OH)D deficiency or insufficiency, 36% had PPA grade 3 or 4, which did not represent a significant difference versus patients with normal 25(OH)D levels (P = 0.105). Assessment of liver biopsy specimens for steatosis revealed that 53% of patients with 25(OH)D deficiency or insufficiency had evidence of steatosis, versus 45% of those with normal 25(OH)D levels (P = 0.310).

Regarding HCV genotype, of the 174 patients analyzed and categorized into subtype 1a or 1b, 118 (68%) had 25(OH)D deficiency or insufficiency. Of these, 40% had genotype 1a and 58% had genotype 1b; again, the difference was not significant (P = 0.469, χ^2 test).

Concerning pretreatment viral load, in the 151 patients in whom this parameter was analyzed and dichotomously stratified as ⩾ 800 000 IU/mL or < 800 000 IU/mL, there were no between-group differences (P = 0.204, χ^2 test).

There was no association between serum vitamin D levels and the virologic characteristics of interest (genotype 1 subtype and viral load). Furthermore, there was no association between 25(OH)D levels and virologic response during, at the end of, or after antiviral therapy (Table 3).

DISCUSSION

Studies have shown that treatment-naive patients with HCV

Table 3 Characteristics related to virologic response to therapy, stratified by serum 25(OH)D levels.

Variable	Normal vitamin D *n*/total (%)	Deficient or insufficient vitamin D *n*/total (%)	*P*
RVR	11/33 (33)	21/55 (38)	0.647
EVR	31/59 (53)	81 / 129 (63)	0.228
SVR	26 / 62 (40)	68/139 (49)	0.359

RVR: Rapid virologic response; EVR: Early virologic response; SVR: Sustained virologic response. RVR and EVR were not inclusion criteria, so RVR were available only for 88 patients and EVR for 187 patients. SVR was available for all 201 patients.

genotype 1 infection experience SVR rates in the range of 40-46% when treated with PegIFN alfa-2a or alfa-2b plus RBV as combination therapy[22-24]. These results are consistent with the findings of the present study. As in previous investigations, among the histologic factors analyzed, steatosis and periportal activity were associated with virologic response[14,25-27], but not liver fibrosis. In cirrhotic patients, the SVR rate was 33% versus nearly 50% in patients without cirrhosis, but the difference did not reach statistical significance, perhaps due to the small number of patients with cirrhosis in the sample.

Regarding the association between liver disease and 25(OH)D deficiency, studies published to date have reported a high prevalence of vitamin D deficiency among patients with a variety of chronic liver diseases, and especially in those with chronic hepatitis C. Although this topic remains controversial, alterations in hepatic 25-hydroxylase enzymes appear to play a role in reduced serum levels of 25(OH)D, at least in patients with advanced liver disease[28].

The technique employed for 25(OH)D measurement in the present study (chemiluminescence) has been considered the most sensitive method for this purpose[29].

The mean 25(OH)D level in the studied population (26.3 ± 11.5 ng/mL) was similar to those reported in previously published studies[14,30]; however, even lower values have been reported in other populations of patients with HCV genotype 1[31,32].

The cutoff point for 25(OH)D sufficiency used in this study was the standard value used in many prior investigations[14,19,30,31,33,34]. We found levels consistent with 25(OH)D deficiency or insufficiency in 69% of our patients, which corroborates findings from countries as diverse as Australia/New Zealand[21], with a 64% rate of 25(OH)D deficiency or insufficiency, and Germany[20], with a 64.1% rate of 25(OH)D deficiency or insufficiency. Even higher rates of deficiency were found in countries such as Italy[33], with 25(OH)D deficiency or insufficiency in 75.4% of patients, and Israel[31], in 80% of tested patients. All of these studies were conducted in populations with HCV genotype 1 infection.

Past studies have reported a higher prevalence of 25(OH)D deficiency and insufficiency in nonwhite individuals[19,34,35]. No such finding was observed in the present study, probably due to widespread miscegenation of the Brazilian population[36]. On the other hand, we found an association between advanced age and low 25(OH)D levels, mostly in female patients, which is consistent with previous studies conducted in patients with hepatitis C[14,30], and in the general Brazilian population[37,38].

Few published studies have assessed potential associations between serum albumin and 25(OH)D levels in patients with HCV genotype 1. Kitson *et al* (2013) found no association between these parameters[19]. Although this difference reached statistical significance, albumin levels were within normal limits, and there may be no clinical relevance. However, ionized calcium levels are strictly controlled by parathyroid hormone (PTH) and vitamin D[1]. In healthy individuals, variations in total plasma calcium probably occur as a result of changes in plasma albumin concentration. An Indian study[39] assessed 30 patients (15 men, 15 women) in an attempt to establish a relationship between serum levels of total calcium, ionized calcium, vitamin D, and albumin, and found strong associations both between calcium and 25(OH)D and between calcium and albumin. Therefore, the association between low vitamin D and lower albumin levels found in the present study could be justified by abnormalities in calcium and vitamin D metabolism.

The relationship between 25(OH)D deficiency or insufficiency and severity of liver fibrosis (stage) and necroinflammatory activity (grade) has been observed in several studies, which reported associations between low 25(OH)D levels and more advanced degrees of fibrosis and necroinflammatory activity[14,30-32,34]. In the present study, we found no association between 25(OH)D levels and these histologic variables, as in the Corey *et al* (2012) study, which also found no relationship between 25(OH)D levels and histologic patterns of fibrosis and necroinflammatory activity in 258 cases[33]. Conversely, Kitson *et al* (2013), in a study of 274 treatment-naive patients with genotype 1, found an association only between necroinflammatory activity and 25(OH)D deficiency[19].

The overall SVR rate was 47% in the selected patients, but this response rate was not associated with absolute serum vitamin D levels or with the percentage of patients with deficiency of this fat-soluble vitamin, corroborating the findings of some previous studies[18,19,40] and contradicting the findings of others[14,41,42,45].

We also found no relationship between serum vitamin D levels and virologic response during or at the end of treatment, in contrast to the findings of Kitson *et al*, who studied a population with very similar characteristics, but, on multivariate analysis, identified vitamin D deficiency as an independent predictor or EVR (OR = 14.1; 95%CI = 1.3; $P = 0.03$) in patients who received standard care (PegIFN + RBV)[19].

Limitations of the present study include the fact that we did not consider seasonal variation in 25(OH)D levels, while the Kitson *et al* study did. However, the impact of these factors on the findings of the present study is likely to be less relevant than in other regions of the world, as climates in Brazil are mostly tropical. Another limitation was the absence of lifestyle assessment, as the body surface area exposed to sunlight may also interfere with vitamin D production[43-45]. Again, this evaluation would have been less relevant than in colder climates, where the population is generally less exposed to sunlight.

In light of the conflicting results observed in this study and elsewhere in the international literature, we cannot establish any definitive conclusion as to a potential association between baseline 25(OH)D levels and SVR or histologic findings in patients with chronic HCV genotype 1 infection who receive PegIFN + RBV combination therapy. Within this context, a systematic review could elucidate the importance of vitamin D status to treatment response in chronic hepatitis C.

Abbreviations

25(OH)D: 25-hydroxyvitamin D3; ALB: albumin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BIL: bilirubin; BMI: body mass index; CLIA: chemoluminescence immunoassay; EDTA: ethylene diamine tetraacetic acid; EOT: end of treatment; EVR: early virologic response; FRT: ferritin; GGT: gamma-glutamyl transferase; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HCV-RNA: hepatitis C virus ribonucleic acid; HDL: high-density lipoprotein; HIV: human immunodeficiency virus; LDL: low-density lipoprotein; n: number of patients; NAFLD: nonalcoholic fatty liver disease; PCR: polymerase chain reaction; PegIFN alfa-2a: peginterferon alfa-2a; PegIFN alfa2b: peginterferon alfa-2b; PegIFN: pegylated interferon; PPA: periportal activity (degree of necroinflammatory activity); PTp: prothrombin activity percentage; RBV: ribavirin; RVR: rapid virologic response; SBH; Brazilian Society of Hepatology; SBP: Brazilian Society of Pathology; SD: standard deviation; SVR: sustained virologic response; TC: total cholesterol; TG: triglycerides.

CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

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