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Title: Vitamin D deficiency is associated with severity of liver disease in HIV/HCV coinfected patients

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Abstract

Objective: To study the association of plasma 25-hydroxy vitamin D (25(OH)D) levels in HIV/HCV coinfected patients with severity of liver disease and virological response to hepatitis C virus (HCV) therapy with pegylated-interferon-alpha plus ribavirin (pegIFNα/RBV).

Methods: A cross-sectional study in 174 HIV/HCV coinfected patients that underwent a liver biopsy previously to start HCV therapy and a retrospective study of 125 of them. Plasma 25(OH)D levels were quantified by enzyme immunoassay. Liver biopsies were evaluated by METAVIR score. A sustained virological response (SVR) was defined as an undetectable serum HCV viral load (<10 IU/mL) up through 24 weeks after the end of HCV treatment.

Results: The median of plasma 25(OH)D level was 48 nmol/L (p25th: 32.5; p75th: 56.1) and 27 (15.5%) had 25(OH)D deficiency (<25 nmol/L). The percentage of 25(OH)D deficiency was higher in patients with significant fibrosis (F≥2) (92.6% vs. 57.1%; p=0.010) and moderate necroinflammatory activity grade (A≥2) (85.2% vs. 60%; p=0.043). However, adjusted logistic regression analyses showed that 25(OH)D deficiency was only associated with severity of liver disease [F≥2 (OR=8.47 (95% of confidence interval (CI)=1.88; 38.3); p=0.005) and A≥2 (OR=3.25 (95%CI=1.06; 10.1); p=0.040)]. Moreover, any significant relationship was found between 25(OH)D deficiency and SVR after HCV therapy.

Conclusion: Plasma 25(OH)D deficiency was associated with liver disease severity in HIV/HCV coinfected patients, but it was not associated with HCV treatment failure.

Key Words: vitamin D; AIDS; chronic hepatitis C; liver fibrosis; hepatic biopsy; antiviral therapy

INTRODUCTION

Chronic hepatitis C (CHC) is a major cause of morbimortality in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected patients (1-3). The standard of care for HCV infection in HIV/HCV coinfected patients consists of pegylated-interferon alpha (pegIFNα) plus weight-based ribavirin (RBV) (4). The rate of response is lower than in HCV monoinfected patients and sustained virological response (SVR) is achieved only in 27%-40% of HIV/HCV coinfected patients, depending on different factors related to host and virus (4).

Vitamin D is an essential nutrient produced naturally after exposure to ultraviolet B radiation, is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D), and later in the kidneys to its active form, 1,25-dihydroxyvitamin D (1,25(OH)2D). The main circulating vitamin D metabolite is 25(OH)D and its concentration in plasma is the most reliable indicator of vitamin D status (5, 6). There is consensus that plasma 25(OH)D levels below 25 nmol/L (10 ng/mL) are qualified as deficient and below 75 nmol/L (30 ng/mL) are qualified as insufficient or suboptimal (7).

Vitamin D receptor (VDR) is widely expressed in the liver and inflammatory cells of CHC patients and its expression is negatively associated with the severity of liver histology in CHC patients (8). Besides, it has been described a critical role of vitamin D/VDR system in the control of T-cells activation (9). Vitamin D has also antiproliferative and antifibrotic effects on liver, and may be considered as having potential therapeutic value (10); suggesting the interest of vitamin D supplementation as a preventive and/or early treatment strategy for CHC (11).

Vitamin D deficiency is common in HIV infected subjects and recent data have linked vitamin D levels to immunodeficiency, combined antiretroviral therapy (cART), and progression to AIDS and death in HIV infected patients (5). However, there are no data consistent about the associations described above (12-18). Moreover, in CHC patients, low vitamin D levels has also been related to liver disease severity (12, 19-24) and lower chance of response to pegIFNα/RBV therapy (20-22, 25, 26). However, there is also inconsistency in published data regarding to the association of low vitamin D levels with severity of liver disease (16, 27, 28), and failure to pegIFNα/RBV therapy in patients with CHC (12, 23, 27).

The aim was to study the association of plasma vitamin D levels in HIV/HCV-coinfected patients with severity of liver disease and virological response to HCV therapy.

PATIENTS AND METHODS

Patients

A cross-sectional study was performed in 174 HIV/HCV coinfected patients who underwent a liver biopsy between May 2000 and July 2008 at Hospital General Universitario Gregorio Marañón in Madrid (Spain). In addition, we carried out a retrospective study in 144 of them who started HCV antiviral treatment. The study was approved by the Institutional Review Board and the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII) and it was conducted in accordance with the Declaration of Helsinki and patients gave their written consent for the study.
The subjects included in our study were HCV treatment-naive patients who were potential candidates for HCV therapy and underwent a liver biopsy. The inclusion criteria were: availability of a plasma sample collected and frozen, detectable HCV RNA by polymerase chain reaction, negative for hepatitis B surface antigen, no clinical evidence of hepatic decompensation, and stable cART or no need for cART. The exclusion criteria were active opportunistic infections, active drug and/or alcohol addiction, and other concomitant diseases or conditions such as diabetes, nephropathies, autoimmune diseases, hemochromatosis, primary biliary cirrhosis, Wilson's disease, α1-antitrypsin deficiency, and neoplasia.

Clinical and Laboratory data

The following information was obtained from medical records at day of sample collection: age, gender, risk category, weight, height, alcohol intake (consumption of more than 50 g of alcohol per day for at least 12 months was considered as high intake), Centers for Disease Control (CDC) clinical category, nadir $CD4^+$ T cell count, cART, HCV genotype, CD4+ T-cells, plasma HIV viral load (HIV-RNA), plasma HCV viral load (HCV-RNA), necroinflammatory activity grade and fibrosis stage of liver biopsies, and biochemistry panel tests. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

HCV-RNA viral load was measured by quantitative polymerase chain reaction (qPCR) (Cobas Amplicor HCV Monitor Test, Branchburg, NJ, USA; and COBAS AmpliPrep/COBAS TaqMan HCV test) and results were reported in International Units per milliliter (IU/mL), with a lower limit of detection of 10 IU/mL.

Biochemistry panel was measured when patients were fasting. The degree of insulin resistance was estimated for each patient using the homeostatic model assessment (HOMA) method described by Matthews et al (29): fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5. In HIV/HCV coinfected patients, HOMA has been associated with liver disease (30, 31) and low rate of SVR in pegIFNα/Ribavirin therapy (32).

Liver biopsy and outcome variables

Liver biopsies were performed following the recommendations of the Patient Care Committee of the American Gastroenterological Association (33). Liver fibrosis was estimated following the criteria established by the METAVIR Cooperative Study Group (34). Fibrosis was scored as follows: F0, no fibrosis; F1, portal fibrosis; F2, periportal fibrosis or rare portal-portal septa; F3, fibrous septa with architectural distortion; no obvious cirrhosis (bridging fibrosis); and F4, definite cirrhosis. Necroinflammatory activity grade was scored as follows: A0, no activity; A1, mild activity; A2, moderate activity; A3, severe activity. Liver steatosis was evaluated according to the existence of hepatocytes containing visible macrovesicular fat droplets. We consider that hepatic steatosis was clinically significant when fatty hepatocytes exceed 10% of hepatic parenchyma.

Hepatitis C therapy

HCV treatment regimens included were pegIFNα 2a or 2b at standard doses (180 μg/week or 1.5 μg/kg/week, respectively) plus weight-adjusted RBV dosing (1000 mg/day for patients weighing <75

kg and 1200 mg/day for patients weighing ≥75 kg). In accordance with well-known guidelines (35), patients with HCV genotypes 1 or 4 received either 48 or 72 weeks of treatment, and patients with HCV genotype 2 or 3 were treated for 24, 48 or 72 weeks, according to virological response at week 4. Early stopping rules were applied for subjects with suboptimal virological response at weeks 12 and 24.

A SVR was defined as an undetectable serum HCV-RNA level (<10 IU/mL) up through 24 weeks after the end of HCV treatment. Patients not fulfilling SVR criteria were considered as non-responders or non-SVR

25-hydroxy vitamin D measurement

Plasma samples were obtained and stored at -80ºC at the same time of the liver biopsy, and 0-12 weeks before starting HCV therapy. Plasma 25-hydroxyvitamin D (25(OH)D) was quantified by enzyme immunoassay according to the manufacturer´s instructions for the test (25-Hydroxy Vitamin D EIA kit, Immunodiagnostic Systems Ltd (IDS Ltd), Boldon, UK). Vitamin D deficiency was defined as a 25(OH)D plasma level <25 nmol/L (<10 ng/ml), vitamin D insufficiency as 25(OH)D plasma level from 25 nmol/L to 74 nmol/L (10-30 ng/ml), and vitamin D sufficiency as a 25(OH)D plasma level above 75 nmol/L (>30 ng/ml) (7, 24).

Clinical outcomes

Two main outcomes: i) Severity of liver disease: a) Significant fibrosis (F≥2); b) Moderate activity grade (A≥2); c) steatosis. ii) Virological response to HCV treatment: SVR.

A secondary outcome: immunodeficiency (prior-ADCs, CD4+ nadir <200 cells/mm^3, and CD4+ count <500 cells/mm^3).

Statistics

The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 19.0 (SPSS INC, Chicago, IL, USA). Values were expressed as absolute number (percentage) and median (percentile 25 (P25th); percentile 25 (P75th)). Statistical significance was defined as $p<0.05$. All p-values were two-tailed.

Categorical data and proportions were analyzed using the chi-squared test or Fisher´s exact test. Mann-Whitney U test or Kruskal-Wallis test were used to compare data among independent groups. We also performed logistic regression analyses to analyze the association between the deficiency of vitamin D (25(OH)D <25 nmol/L) and liver disease severity and HIV disease. These logistic regression analyses were adjusted by the most important clinical and epidemiological characteristics (covariates). We included each outcome parameter (Enter algorithm) along with the most relevant epidemiological and clinical characteristics (Stepwise algorithm, at each step, the factors were considered for removal or entry: a p-value for entry and exit of 0.10 and 0.20, respectively). The covariates used were age, sex, seasonality (winter/spring vs. summer/autumn), intravenous drug users (IVDU), alcohol intake, BMI, HOMA, nadir CD4+, cART, efavirenz, undetectable HIV-RNA (<50 copies/mL), CD4+ cell count, HCV genotype 3 and HCV-RNA ≥500.000 IU/mL. Thus, each logistic regression was always adjusted for significant covariates associated with the outcome variable.

RESULTS

Patients

This study included 174 patients coinfected with HIV and HCV, whose characteristics at the time of liver biopsy are shown in **Table 1**.

The median of plasma 25(OH)D level was 48 nmol/L (p25th: 32.5; p75th: 56.1). Besides, 27 (15.5%) patients had vitamin D deficiency (25(OH)D <25 nmol/L), 131 (75.3%) patients had vitamin D insufficiency (25(OH)D between 25 and 74 nmol/L) and 16 (9.2%) patients had normal values of vitamin D (≥75 nmol/L). When patients were stratified according to vitamin D status (**Table 1**), we found important differences in prior AIDS defining conditions (prior-ADCs) (p= 0.022), significant fibrosis (F≥2; p= 0.010), and moderate necroinflammatory activity grade (A≥2; p= 0.043). In addition, the percentage of CD4+ count <500 cells/mm^3 was close to statistical significance (p= 0.070).

Table 1. Characteristics of 174 HIV/HCV coinfected patients according to plasma levels of 25-hydroxyvitamin D.

Patients characteristics	All patients	Vit. D deficiency (<25 nmol/L)	Vit D insufficiency (25-74 nmol/L)	Vit D sufficiency (≥75 nmol/L)	p-value
No. (%)	174	27 (15.5%)	131 (75.3%)	16 (9.2%)	
Epidemiological data					
Sex (male)	130 (74.7)	23 (85.2)	94 (71.8)	13 (81.3)	0.281
Age (years)	40.8 (37.3; 44.6)	41.1 (39; 45.9)	40 (36.9; 44)	42.7 (39.4; 45.6)	0.128
Body mass index	22.5 (20.4; 24.3)	22.2 (20.9; 23.9)	22.6 (20.3; 24.4)	22.2 (20; 24.8)	0.852
High alcohol intake	92 (54.4)	16 (59.3)	66 (52.4)	10 (62.5)	0.642
HCV infection features					
HCV infection parameters					
HCV-genotype-1/4	119 (69.2)	17 (63)	93 (72.1)	9 (56.3)	0.323
HCV-genotype-3	48 (27.9)	9 (33.3)	32 (24.8)	7 (43.8)	0.222
Plasma HCV-RNA ≥500,000 IU/mL	127 (73)	20 (74.1)	97 (74)	10 (62.5)	0.612
Metavir fibrosis stage					
No/Mild fibrosis, F0/F1	54 (31)	2 (7.4)	48 (36.6)	4 (25)	**0.010**
Significant fibrosis, F≥2	120 (69)	25 (92.6)	83 (63.4)	12 (75)	**0.010**
Advanced fibrosis, F3/F4	51 (29.3)	11 (40.7)	33 (25.2)	7 (43.8)	0.112
Metavir activity grade (n=173)					
No/Mild activity, A0/A1	62 (35.8)	4 (14.8)	51 (39.2)	7 (43.8)	0.171
Moderate activity, A≥2	111 (64.2)	23 (85.2)	79 (60.8)	9 (56.3)	**0.043**
Severe activity, A≥3	28 (16.3)	6 (23.1)	19 (14.6)	3 (18.8)	0.544
HIV infection features					
Prior AIDS	83 (47.7)	19 (70.4)	59 (45)	5 (31.3)	**0.022**
HIV acquired by IVDU	152 (87.9)	27 (100)	111 (85.4)	14 (87.5)	0.106
Combined antiretroviral therapy (cART)					
On cART	149 (85.6)	20 (74.1)	115 (87.8)	14 (87.5)	0.176
NRTI based regimen	147 (84.5)	20 (74.1)	113 (86.3)	14 (87.5)	0.265
Non-NRTI based regimen	90 (51.7)	12 (44.4)	68 (51.9)	10 (62.5)	0.517
Protease inhibitor based regimen	46 (26.4)	8 (29.6)	35 (26.7)	3 (18.8)	0.729
Ritonavir	27 (15.5)	4 (14.8)	21 (16)	2 (12.5)	0.929
Efavirenz	51 (29.3)	7 (25.9)	38 (29)	6 (37.5)	0.714

Tenofovir	49 (28.2)	9 (33.3)	35 (26.7)	5 (31.3)	0.753
Abacavir	27 (15.5)	7 (25.9)	17 (13)	3 (18.8)	0.223
HIV infection parameters					
Nadir CD4+ cells/mm^3	198 (92; 323)	166 (60; 270)	200 (100; 320)	241 (100; 391)	0.407
Nadir CD4+ ≤200 cells/mm^3	91 (52.3)	17 (63)	67 (51.1)	7 (43.8)	0.413
CD4+ cells/mm^3	448 (336; 648)	396 (273; 482)	450 (333; 658)	531 (411; 675)	0.171
CD4+ <500 cells/mm^3	68 (39.1)	21 (77.8)	78 (59.5)	7 (43.7)	0.070
Plasma HIV-RNA<50cp/mL	123 (71.1)	18 (66.6)	94 (71.7)	11 (68.7)	0.821
Biochemical parameters					
HOMA	2.3 (1.2; 4.6)	2.4 (1.1; 5.4)	2.2 (1.2; 4.7)	2.5 (1.3; 3.7)	0.978
ALP (UI/dL)	106 (80; 173)	124 (95; 177)	105 (80; 176)	98 (76; 170)	0.366
AST (UI/dL)	65 (43; 91.7)	67 (39; 112)	65 (44; 91)	56.5 (40.5; 128)	0.969
GGT (UI/dL)	104.5 (57; 199.2)	130 (58; 222)	99 (54; 191)	124.5 (64; 196.7)	0.832
ALT (UI/dL)	82.5 (54; 124)	99 (48; 159)	78 (54; 117)	91.5 (55; 140.5)	0.556

Values expressed as absolute number (percentage) and median (percentile 25th; percentile 75th).
Abbreviations: ALP: alkaline phosphatase. ALT: alanine transaminase. AST: aspartate transaminase. GGT: gamma glutamyl transpeptidase. NRTI, Nucleoside Reverse Transcriptase Inhibitor. HCV: Hepatitis C virus. HCV-RNA: HCV plasma viral load. HIV-1: Human immunodeficiency virus type 1. HIV-RNA: HIV plasma viral load. HOMA: homeostasis model assessment. IVDU: intravenous drug users.
(†), We considered the consumption of greater than 50 grams of alcohol per day for ≥12 months as a high alcohol intake.

Plasma vitamin D levels and seasonal variation

A significant seasonal variation of plasma 25(OH)D levels was observed (**Figure 1A**). Patients evaluated in the first semester (winter/spring) had lower plasma 25(OH)D levels than patients evaluated in the second semester (summer/autumn) (p <0.001; **Figure 1A**). Besides, a higher percentage of patients with vitamin D deficiency (25(OH)D <25 nmol/L) was found in the first semester (winter/spring) (p <0.001; **Figure 1B**).

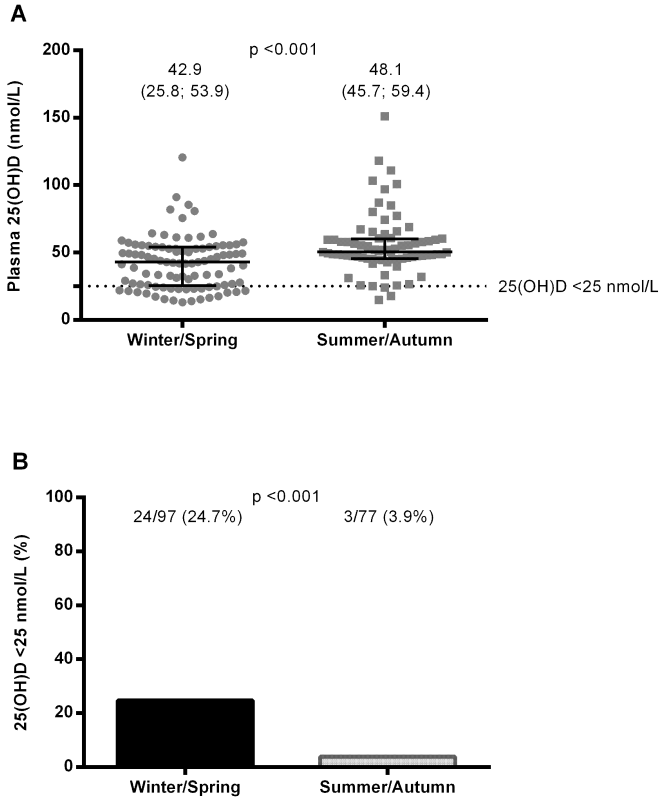

Figure 1. Plasma vitamin D (25(OH)D) levels and seasonality in HIV/HCV coinfected patients. (A) Seasonal variation of plasma 25(OH)D levels. (B) Distribution of the patients according to plasma 25(OH) vitamin D deficiency (25(OH)D <25 nmol/L).

Association between vitamin D deficiency and severity of liver disease

Supplementary table (ST) 1 shows the characteristics of HIV/HCV coinfected patients according to severity of liver disease. Patients with significant fibrosis (F≥2) had higher percentage of male, high alcohol intake, and HCV GT3; and higher values of HOMA, AST, GGT, and ALT than patients with mild fibrosis (F0/F1) (p<0.05; **ST 1**). In addition, patients with moderate necroinflammatory activity grade (A≥2) had only higher values of AST than patients with low necroinflammatory activity grade (A0/A1) (p= 0.001; **ST 1**).

Moreover, patients with F≥2 had lower plasma 25(OH)D levels than patients with F0/F1 (p= 0.037; **Figure 2A).** Conversely, F≥2 was found in 92.6% (25/27) of patients with 25(OH)D deficiency, whereas only in 57.1% (84/147) of patients without 25(OH)D deficiency (p <0.001)**.** In addition, plasma 25(OH)D levels were significantly lower in patients with A≥2 than in patients with A0/A1 (p= 0.021; **Figure 2B).** A value of A≥2 was found in 85.2% (23/27) of patients with 25(OH)D deficiency, and only in 60% (87/145) of patients without 25(OH)D deficiency (p= 0.012).

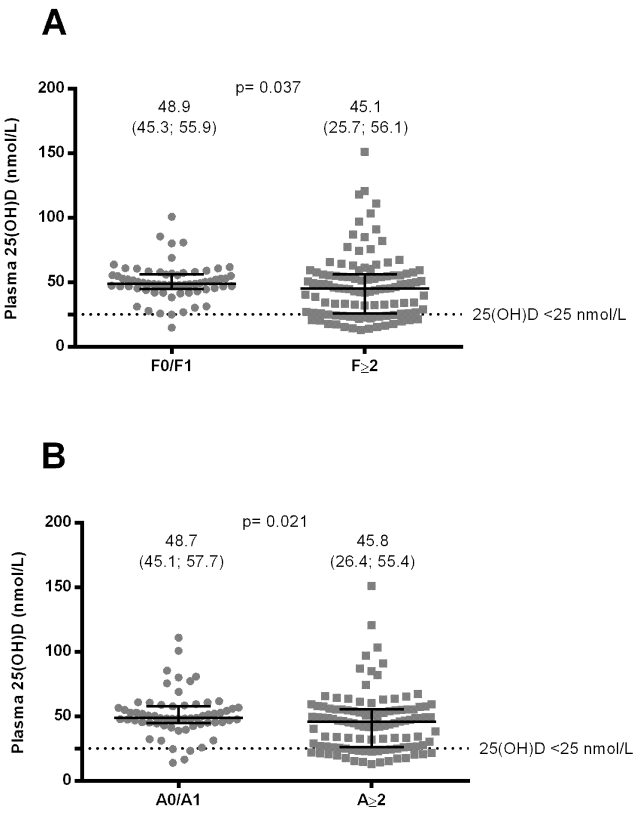

Figure 2. Plasma vitamin D (25(OH)D) levels and characteristics of HCV infection in HIV/HCV coinfected patients. (A) Distribution of plasma 25(OH)D levels according to significant fibrosis (F≥2). (B) Distribution of plasma 25(OH)D levels according to moderate necroinflammatory activity grade (A≥2).

Furthermore, the adjusted logistic regression analyses showed that vitamin D deficiency (25(OH)D <25 nmol/L) was associated with F≥2 (OR= 8.47 (95% confidence interval (CI)= 1.88; 38.3); p= 0.005) and A≥2 (OR= 3.25 (95%CI= 1.06; 10.1); p= 0.040).

Association between plasma 25-hydroxyvitamin D levels and virologic response to HCV therapy

On the one hand, 142 out of 174 patients were treated with pegIFNα/RBV, who were used by intent-to-treat analysis (**Supplementary figure (SF) 1**). The SVR rate was 43% (61/142). We did not find any significant differences between non-SVR and SVR patients when plasma vitamin D (25(OH)D) levels were analysed for all patients (**SF 1A**), HCV GT2/3 patients (**SF 1B**), and HCV GT1/4 patients (**SF 1B**). Additionally, when SVR rate was stratified by vitamin D status (7, 24), we did not find any significant relationship between plasma vitamin D deficiency (25(OH)D <25 nmol/L) and SVR for all patients (**SF 1C**), HCV GT2/3 patients (**SF 1D**), and HCV GT1/4 patients (**SF 1D**).

On the other hand, the statistical analysis was performed by an on-treatment approach (**SF 2**). Thus, 17 out of 142 patients were prematurely interrupted HCV therapy (7 adverse events and 10 abandonments). Thereby, 125 patients were only included in this analysis. The SVR was found in 48.8% (61/125) of HIV/HCV coinfected patients on HCV antiviral therapy. As in the previous analysis, we did not find any significant relationship between SVR and plasma vitamin D (25(OH)D) levels (**SF 2**).

Association between vitamin D levels and HIV infection characteristics

Patients with prior-ADCs had lower plasma 25(OH)D levels than patients without prior-ADCs (p= 0.002; **Figure 3A).** Conversely, prior-ADCs was found in 70.4% (19/27) patients with 25(OH)D deficiency versus only in 43.5% (64/147) patients without 25(OH)D deficiency (p= 0.010). Additionally, plasma 25(OH)D levels were significantly lower in patients with CD4+ count <500 cells/mm^3 than patients with CD4+ count ≥500 cell/mm^3 (p= 0.016; **Figure 3B).** A CD4+ count <500 cells/mm^3 was found in 77.8% (21/27) patients with 25(OH)D deficiency versus only in 57.8% (85/147) patients without 25(OH)D deficiency (p= 0.051)**.**

However, the adjusted logistic regression analyses showed that vitamin D deficiency (25(OH)D <25 nmol/L) was not significantly associated with prior-ADCs (OR= 2.05 (95%CI= 0.51; 8.25); p= 0.312) nor with CD4+ counts <500 cells/mm^3 (OR= 1.42 (95%CI= 0.44; 4.52); p= 0.551).

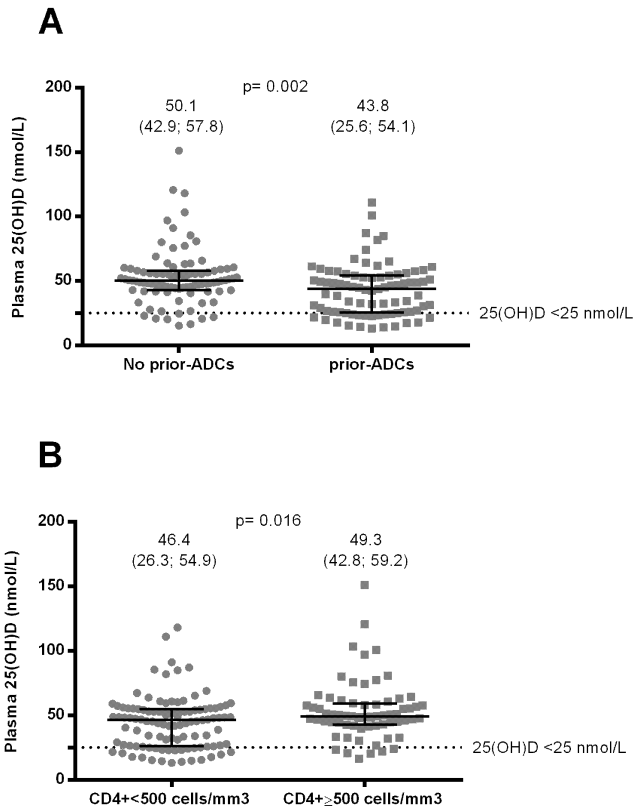

Figure 3. Plasma vitamin D (25(OH)D) levels and characteristics of HIV infection in HIV/HCV coinfected patients. (A) Distribution of plasma 25(OH)D levels according to prior AIDS-defining conditions (ADCs). (B) Distribution of plasma 25(OH)D levels according to CD4+ count below or above 500 cells/mm3.

DISCUSSION

The major results of the present study were: (1) a high prevalence of plasma vitamin D insufficiency (25(OH)D <75 nmol/L) and deficiency (25(OH)D <25 nmol/L) in our study population; (2) a significant association between plasma vitamin D deficiency and severity of liver disease; and (3) a lack of association between plasma vitamin D levels and virological response to IFN-based therapy. Furthermore, no association was found between plasma vitamin D deficiency and HIV infection characteristics.

Vitamin D deficiency is almost universal among patients with chronic liver disease (36). Moreover, vitamin D metabolism is affected by HIV infection and cART (37). Thus, low plasma levels of 25(OH)D have been previously reported in HIV (13, 15) and HCV (20, 21, 36) monoinfected patients, as well as in HIV/HCV coinfected patients (12, 23, 24). Besides, prevalence of vitamin D deficiency in HCV (20) and HIV monoinfected patients (13), and HIV/HCV coinfected patients (23), is higher than in general population. In our study, around 15% of HIV/HCV coinfected patients had vitamin D deficiency and around 75% had insufficient vitamin D levels. These percentages are similar or close to those published by other authors in European HIV/HCV coinfected patients (12, 24); and, as expected, plasma 25(OH)D levels were strongly correlated with seasonal variations.

The role of vitamin D in CHC patients is controversial (6). Many studies have reported that low values of vitamin D are associated with more severity of liver disease (12, 19-24)**.** However, others articles have not found any association of vitamin D levels with severity of liver disease in HCV monoinfected (27, 28) and HIV/HCV coinfected patients (16). In our study, lower plasma 25(OH)D levels were significantly associated with significant fibrosis (F≥2) and moderate necroinflammatory activity grade (A≥2).). In addition, as observed in HCV-monoinfected patients (20) and HIV/HCV coinfected patients (12), no association was found between serum 25(OH)D levels and steatosis (data not shown).

Interestingly, more severe liver disease is usually associated with more vitamin D deficiency (6), while more liver fibrosis usually equals a lower SVR rate in CHC patients on HCV therapy (38). Therefore, it would be expected that levels of vitamin D may be associated with SVR. However, in our study, we did not find any significant association between lower plasma 25(OH)D levels and HCV virologic response to pegIFNα/RBV therapy. Low vitamin D levels have been linked to higher chance of failure to HCV therapy among HCV monoinfected patients (20-22, 25, 26) and HIV/HCV coinfected patients (24). However, there are also several articles that have found no association of vitamin D levels with failure to HCV treatment in HCV monoinfected patients (27), and in all recent reports about HIV/HCV coinfected patients (12, 23, 39). This discordancy, between reports of HIV/HCV coinfected patients and HCV monoinfected patients, could be due to the more complex situation of HIV/HCV coinfected patients, which does not allow detecting any significant difference.

As vitamin D deficiency may contribute to liver fibrosis, vitamin D supplementation may have anti-fibrotic effects in HCV-positive patients and positively contribute to treatment outcome (40). Then, vitamin D levels should be monitoring and deficient patients should be treated with vitamin D supplementation (26, 41, 42).

The role of vitamin D on HIV disease progression is also controversial (5). Overall, cART, and particularly efavirenz, increase risk for low vitamin D; and substance abuse, advanced AIDS, low CD4 counts, higher HIV viral loads, and death have been linked to suboptimal vitamin D in patients with HIV infection (5). However, the results published in recent years have not been very consistent with the associations described above (12-18). In our study, we found a relationship between vitamin D deficiency and prior-ADCs and CD4+<500 cells/mm^3; but these results did not remain significant after adjusting the regression analysis for the main clinical and epidemiological characteristics. Besides, we did not find any association between plasma vitamin D levels and efavirenz in our HIV/HCV coinfected patients (data not shown). The previous report and our data should be interpreted with caution since the studies designs were different, vitamin D metabolites were measured by different methods, and most of the studies were not specifically designed to evaluate the effect of vitamin D.

Thus, the role of vitamin D status on HIV disease progression remains unclear and further studies are needed for elucidating this potential association.

This study has other limitations that must be taken into account: i) the cross-sectional design with which is impossible to dissect the temporal relation between 25(OH)D and liver disease severity; ii) the low number of patients, especially in various strata analysed, which could make that we did not find any significant p-value; iii) the lack of data regarding other factors, such as dietary intake, prevalence of osteoporosis, polymorphisms of vitamin D hydroxylating enzymes, and on other variables involved in vitamin D metabolism, such as parathyroid hormone; iv) a possible selection bias since the patients selected for our study were patients who met a set of criteria for starting HCV treatment (eg. little alcohol abuse, high CD4 cell counts, controlled HIV replication, and good treatment adherence).

In conclusion, plasma vitamin D deficiency was associated with severity of liver disease in HIV/HCV coinfected patients, but it was not associated with HCV treatment failure.

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Acquisition of data: JB, JC, PM, TA, and JCL.
Evaluation of hepatic biopsy: EA.
Analysis and interpretation of data: MGF, MGA and SR.
Drafting of the manuscript: MGF, MGA, MAJS, DPT, and SR.
Critical revision of the manuscript for important intellectual content: JB.
Statistical analysis: MGF, MGA and SR.
Administrative, technical, or material support: MGF, MGA, MAJS, and DPT.
Study supervision: SR.

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SUPPLEMENTAL MATERIALS

Supplementary table 1. Characteristics of 174 HIV/HCV coinfected patients according to severity of liver disease.

Patients characteristics	F0/F1	F2/F3/F4	p-value	A0/A1	A2/A3	p-value
No. (%)	54 (31)	120 (69)		62 (35.8)	111 (64.2)	
Epidemiological data						
Sex (male)	35 (64.8)	95 (79.2)	**0.044**	43 (69.4)	86 (77.5)	0.239
Age (years)	39.8 (37.3; 43.3)	41.1 (37.2; 44.8)	0.236	40.4 (37.6; 44.1)	41.6 (37.5; 44.9)	0.251
Body mass index	23.2 (21.2; 25.1)	22.2 (20.3; 24.0)	0.144	23 (20.8; 24.9)	22.4 (20.4; 24.1)	0.417
High alcohol intake	22 (42.3)	70 (59.8)	**0.035**	30 (50)	62 (56.9)	0.390
HCV infection features						
HCV infection parameters						
HCV-genotype-1/4	41 (77.4)	78 (65.5)	0.121	43 (71.7)	76 (68.5)	0.664
HCV-genotype-3	8 (15.1)	40 (33.6)	**0.012**	14 (23.3)	33 (29.7)	0.371
Plasma HCV-RNA ≥500,000 IU/mL	40 (74.1)	87 (72.5)	0.829	44 (71)	83 (74.8)	0.587
HIV infection features						
Prior AIDS	20 (37)	63 (52.5)	0.059	24 (38.7)	58 (52.3)	0.087
HIV acquired by IVDU	43 (81.1)	109 (90.8)	0.072	51 (83.6)	100 (90.1)	0.214
Combined antiretroviral therapy (cART)						
On cART	46 (85.2)	103 (85.8)	0.910	56 (90.3)	92 (82.9)	0.182
NRTI based regimen	45 (83.3)	102 (85.0)	0.779	56 (90.3)	90 (81.1)	0.108
Non-NRTI based regimen	26 (48.1)	64 (53.3)	0.527	36 (58.1)	53 (47.7)	0.193
Protease inhibitor based regimen	15 (27.8)	31 (25.8)	0.788	13 (21)	33 (29.7)	0.211
Ritonavir	10 (18.5)	17 (14.2)	0.463	7 (11.3)	20 (18)	0.242
Efavirenz	13 (24.1)	38 (31.7)	0.309	20 (32.3)	30 (27)	0.467
Tenofovir	12 (22.2)	37 (30.8)	0.243	18 (29)	31 (27.9)	0.877
Abacavir	6 (11.1)	21 (17.5)	0.282	9 (14.5)	18 (16.2)	0.768
HIV infection parameters						
Nadir CD4+ cells/mm^3	219 (120; 335.5)	195.5 (81; 326.7)	0.492	195 (100; 318.5)	200 (81; 343)	0.900
Nadir CD4+ ≤200 cells/mm^3	26 (48.1)	65 (54.2)	0.462	33 (53.2)	57 (51.4)	0.813

CD4+ cells/mm^3	460 (323.5; 682)	442 (350; 643.5)	0.759	465 (354; 693)	432 (336; 630)	0.202
CD4+ <500 cells/mm^3	31 (57.4)	75 (62.5)	0.524	33 (53.2)	72 (64.9)	0.133
Plasma HIV-RNA<50cp/mL	35 (66)	88 (73.3)	0.329	47 (77)	75 (67.6)	0.190
Biochemical parameters						
HOMA	1.8 (1.1; 2.9)	2.8 (1.3; 5.3)	**0.004**	2 (1.2; 4.3)	2.4 (1.2; 4.6)	0.517
ALP (UI/dL)	99 (72; 130)	116 (83; 172)	0.054	95 (73; 146)	119 (83; 177)	0.092
AST (UI/dL)	53.5 (35.5; 69.4)	70 (48; 112)	**<0.001**	54 (37.5; 74.5)	69 (48; 112)	**0.001**
GGT (UI/dL)	85 (31.7; 152.2)	113 (63; 218)	**0.012**	91 (52.5; 165.5)	115 (58; 213)	0.176
ALT (UI/dL)	73.9 (47; 95.2)	87 (55; 143)	**0.033**	75 (50; 101.5)	85 (55; 143)	0.073

Values expressed as absolute number (percentage) and median (percentile 25th; percentile 75th).
Abbreviations: ALP: alkaline phosphatase. ALT: alanine transaminase. AST: aspartate transaminase. GGT: gamma glutamyl transpeptidase. NRTI, Nucleoside Reverse Transcriptase Inhibitor. HCV: Hepatitis C virus. HCV-RNA: HCV plasma viral load. HIV-1: Human immunodeficiency virus type 1. HIV-RNA: HIV plasma viral load. HOMA: homeostasis model assessment. IVDU: intravenous drug users.
(†), We considered the consumption of greater than 50 grams of alcohol per day for ≥12 months as a high alcohol intake.

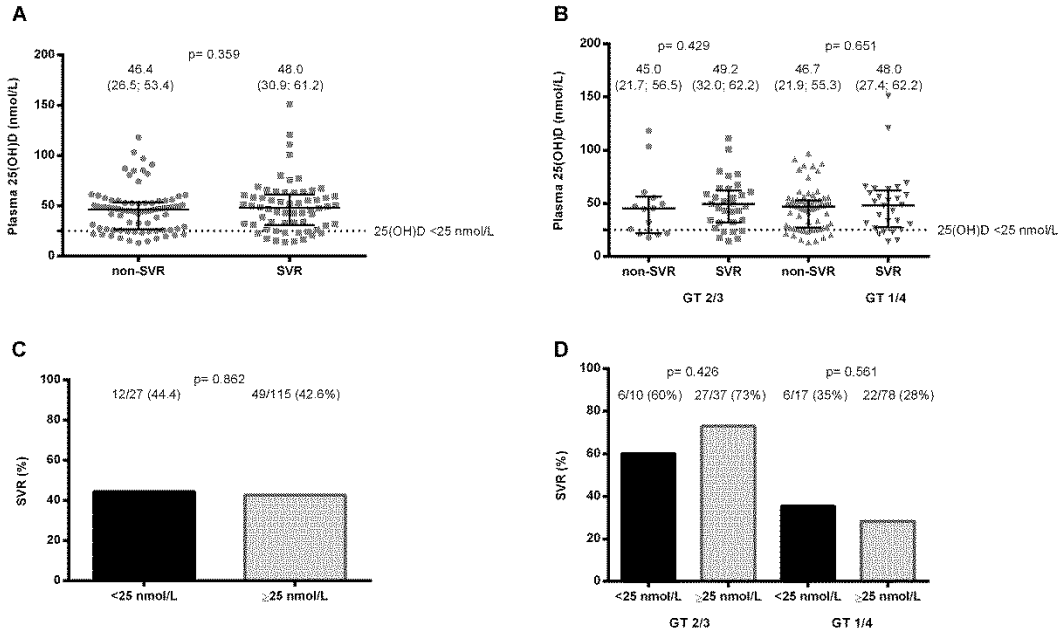
Supplemental figure 1. Plasma vitamin D (25(OH)D) levels and sustained virologic response (SVR) according to HCV genotype in HIV/HCV coinfected patients on HCV therapy by intention-to-treat analysis

(A) Distribution of plasma 25(OH)D levels according to virologic response to HCV therapy.

(B) Distribution of plasma 25(OH)D levels according to virologic response to HCV therapy and HCV genotypes.

(C) Distribution of the patients with sustained virologic response (SVR) according to plasma 25(OH) vitamin D deficiency (25(OH)D <25 nmol/L).

(D) Distribution of the patients with sustained virologic response (SVR) according to plasma 25(OH) vitamin D deficiency (25(OH)D <25 nmol/L) and HCV genotypes.



Supplemental figure 2. Plasma vitamin D (25(OH)D) levels and sustained virologic response (SVR) according to HCV genotype in HIV/HCV coinfected patients on HCV therapy by <u>on-treatment analysis</u>

(A) Distribution of plasma 25(OH)D levels according to virologic response to HCV therapy.

(B) Distribution of plasma 25(OH)D levels according to virologic response to HCV therapy and HCV genotypes.

(C) Distribution of the patients with sustained virologic response (SVR) according to plasma 25(OH) vitamin D deficiency (25(OH)D <25 nmol/L).

(D) Distribution of the patients with sustained virologic response (SVR) according to plasma 25(OH) vitamin D deficiency (25(OH)D <25 nmol/L) and HCV genotypes.