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Hypovitaminosis D and its relation to demographic and laboratory data among hepatitis C patients

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ABSTRACT

Background. The relationship between 25-hydroxyvitamin D [25(OH)D] serum levels and response to antiviral therapy and laboratory data in HCV infection remains unclear. The aim of this study was to determine pre-treatment 25(OH)D serum level among HCV infected individuals and to evaluate the association between vitamin D status, virological response, and laboratory data. **Material and methods.** Baseline serum 25(OH)D levels were measured in 237 chronic HCV infected patients (139 female, age 53.7 ± 11.2 years) using chemiluminescence immunoassay. Correlations between serum 25(OH)D levels, virological and laboratory data regarding HCV infection as well as sustained virological response (SVR) to antiviral therapy were evaluated. **Results.** Mean serum values of 25(OH)D was 26.2 ± 12 ng/mL and prevalence of vitamin D deficiency (< 30 ng/mL) was 66.2%. Advanced age (> 55 years), high mean values of LDL, total cholesterol, HDL and low mean values of alkaline phosphatase and hemoglobin were statistically associated to vitamin D deficiency. Antiviral treatment was underwent by 133 HCV patients and 44.3% of them achieved SVR. Most of individuals that presented SVR also presented 25(OH)D level higher than 30ng/mL (55.9%). SVR was associated to low mean values of LDL, total cholesterol and platelets; high mean values of ALT, AST and low fibrosis grade. Conclusions: In conclusion, low vitamin D levels were observed among HCV infected patients and was associated to laboratory findings, however baseline 25(OH)D level is not independently associated with SVR.

Key words. Vitamin D. Hepatitis C virus. Virological response. Treatment.

INTRODUCTION

Vitamin D, whose active form is 1,25-dihydroxy vitamin D3, is essential for calcium and bone homeostasis, and its deficiency has been associated with a number of diseases, such as cancer, cardiovascular and autoimmune diseases, insulin resistance (IR), and infectious disease.[1-5] Among patients with chronic liver disease, vitamin D deficiency is often observed and that finding has been related to severe fibrosis, low responsiveness to interferon-based antiviral therapy in genotype 1 chronic hepatitis C (CHC) and the presence of mixed cryoglobulinemia and systemic vasculitis among CHC patients.[6-9]

Hepatitis C virus (HCV) infection affects more than 130 million of individuals[10] and its treatment is usually based in pegylated interferon (PEG-IFN) and ribavirin (RBV) for 24 weeks for patients infected with HCV genotypes 2 or 3, or 48 weeks for those infected with HCV genotype 1, with rates of sustained virological response (SVR) ranging from 60-70% among CHC patients with genotypes 2 and 3, but lower than 50% in patients with genotype 1 using conventional IFN therapy.[11] In order to increase SVR rates, the influence of genetic and metabolic factors have been studied, and, in the context, interleukin-28B (IL28B) polymorphism and IR are found to be associated to SVR.[12-14] In addition, Nimer, *et al.*[15] and Abou-Mouch, *et al.*[16] have shown that vitamin D supplementation improves viral response in CHC patients infected with genotypes 1, 2 or 3.

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The aim of our study was to determine serum levels of 25(OH)D in a cohort of CHC patients from Brazil, and to investigate the potential relationships between 25(OH)D levels and laboratory and virological parameters.

MATERIAL AND METHODS

Patients

The study population included CHC patients, resident in Rio de Janeiro and recruited at Viral Hepatitis Ambulatory (Viral Hepatitis Laboratory, Oswaldo Cruz Institute, FIOCRUZ), Hepatology Unit (Clementino Fraga Filho University Hospital, UFRJ), and General Medicine Department (Gaffrée Guinle University Hospital, UNIRIO). Patients were included if they had a virological diagnosis of CHC [anti-HCV and HCV RNA reactive serum sample, with persistently abnormal alanine aminotransferase (ALT), for at least 6 months]. The infecting HCV genotypes were the following: 1, 2, 3, and 5. Exclusion criteria were advanced cirrhosis (Child-Pugh B and C), presence of hepatocellular carcinoma, human immunodeficiency virus (HIV) and/or hepatitis B co-infection, autoimmune liver disease, genetic liver disease (Wilson's disease, hemochromatosis), previous HCV antiviral treatment, excessive alcohol consumption, concomitant use of drugs known to affect serum vitamin D concentration and intravenous drug use.

This study was conducted following the principles of the Declaration of Helsinki and their appendices. Approval was obtained from FIOCRUZ Ethics Committee, and written informed consent was obtained from all subjects.

Clinical and laboratory assessment

Clinical and anthropometric data were collected simultaneously from all patients. Body mass index (BMI) was calculated on the basis of weight in kilograms and height (in meters). Waist circumference (cm) was measured at the midpoint between the lower border of the rib cage and the iliac crest. A 12-h overnight fasting blood sample was drawn to determine serum levels of ALT, gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein cholesterol (LDL), triglycerides, ferritin, plasma glucose concentration, and platelet count. Serum insulin was determined by a chemiluminescence immunoassay (LIASON Insulin assay, Diasorin, Italy). IR was determined with the homeostasis model assessment method.[17]

The analysis of serum 25(OH) D was performed using a chemoluminescent immunoassay on a Liaison automatic analyzer (Liason 25 OH Vitamin D, DiaSorin). Data were expressed as nanograms per milliliter. In accordance with the manufacturer's instructions, serum 25(OH)D concentration of 30 ng/mL was considered the threshold value for identifying low levels of vitamin D. Individuals were classified as vitamin D deficient when serum 25(OH)D concentration was below 30ng/mL.

All patients were tested for HCV RNA by qualitative polymerase chain reaction (Cobas Amplicor HCV Test version 2.0, Roche, Austria; limit of detection: 50 IU/mL). HCV RNA positive samples were quantified by COBAS TaqMan HCV Test (Roche) and expressed as IU/mL. HCV genotyping was performed with INNO-LIPA HCV II kit (Innogenetics, Zwijnaarden, Belgium), which were used according to the manufacturer's instructions or using genotype-specific primers for entire core region and a part of 5' noncoding region (5'NCR).[18]

Hepatic fibrosis was assessed using serum biochemical markers.[19] Fib-4 index [age (years) x AST (IU/L)]/[platelet count (10^9/L) x ALT (IU/L)1/2] and FORNS index [7.811-3.131 X ln platelet count + 0.781 x ln GGT (IU/L) + 3.467 x ln age (years) - 0.014 x cholesterol (mg/dL) were used. If FIB-4 was less than 1.45, individual was considered as low fibrosis and if FIB-4 was $\geq$ 1.45, Forns was considered. Forns lower than 4.2 was classified as low fibrosis and Forns $\geq$ 4.2 was classified as high fibrosis.

Statistical analysis

Continuous variables were summarized as mean ($\pm$SD) and categorical variables as frequency and percentage. Nonparametric tests such as Mann-Whitney and Kruskal-Wallis tests were used to compare continuous variables that were not normally distributed (Kolmogorov-Smirnov test). Continuous variables with normal distribution were compared by using unpaired Student t-test. χ^2 test was used for comparison of categorical variables.

Univariate regression analysis was performed using 25(OH)D serum levels as a categorical dependent variable, and as candidate independent factors related to low serum levels of 25(OH)D, we selected age, gender, BMI, baseline ALT, AST, platelet count, GGT, ferritin, total cholesterol, HDL, LDL, triglycerides, alkaline phosphatase, hemoglobin,

blood glucose, insulin, homeostasis model assessment score, fibrosis, SVR, HCV genotype, HCV RNA levels. Univariate regression analysis was also done to identify predictors of SVR as a category dependent variable among HCV individuals who underwent antiviral therapy. Variables associated with the dependent variable in the univariate regression analyses (probability threshold, $P < 0.05$) were included in the multivariate logistic regression models 20. We used SPSS software, version 20.0 (IBM, USA) for all statistical analyses.

RESULTS

Patient characteristics

A total of 237 CHC patients were included in the study and the baseline features are shown in table 1. Mean age of HCV patients was 53.7 ± 11.2 years and most of them were female (58.6%). Mean BMI was 28.1 ± 3.8 kg/m^2 and 79.6% of them were in the overweight to obesity range.

HCV patients presented mean values of biochemical parameters (ALT, AST, GGT, alkaline phosphatase, ferritin) above normal values, while mean lipid values (HDL, LDL, triglycerides) were classified as normal values. Mean blood glucose values were considered elevated (104.9 ± 45.5 ng/mL) as well as mean HOMA score value (3.6 ±3.7). Using HOMA score, IR was found among 59.9% of CHC patients. Mean hemoglobin values was 14.0 ± 1.3 ng/mL and 17 (7.1%) were classified as anemic (hemoglobin values < 12 ng/mL for women and < 13 ng/mL for men). HCV genotype 1 was the most prevalent (87%), and mean HCV RNA was considered high (9.4 ± 85.0 x 10^6 UI/mL). Using non invasive methods, most of individuals presented high fibrosis grade (70.04%).

Serum 25(OH)D levels

Mean serum values of 25(OH)D in CHC patients were 26.2 ± 12.0 ng/mL Table 1. One hundred and fifty seven (66.2%) patients had vitamin D deficiency (< 30 ng/mL). Table 2 shows the results of comparative analysis of demographic and clinical variables and serum vitamin D concentration, categorized according to previously defined cutoff values.

Age (P = 0.003), high total cholesterol (P = 0.001), high LDL cholesterol (P = 0.023), high HDL cholesterol (P = 0.049), low alkaline phosphatase (P = 0.009) and low hemoglobin levels (P = 0.018), were associated with lower 25(OH)D levels in CHC at univariate regression analysis. At multivariate analysis, age (P = 0.019) and haemoglobin levels (P = 0.017) were found independent factors in multiple linear regression analysis (Table 2).

Mean values of blood glucose, GGT, AST and HCV RNA viral load were elevated among vitamin D deficiency individuals, though these variables were not statistically significant. Although vitamin D deficiency was common among anemic individuals (13/17), no statistical significance was observed (p = 0.428). Interestingly, most of individuals

Table 1. Baseline demographic, laboratory, metabolic and histological features of 237 chronic hepatitis C (CHC) patients.

Variable*	CHC patients* (n = 237)
Age, years	53.7 ± 11.2
Sex	
Male/Female (n)	98/139
Body mass index, kg/m^2	28.1 ± 3.8
Platelet count x 10^3/mm	185.3 ± 79.6
HCV RNA, x 10^6 IU/mL	9.4 ±85.0
ALT, IU/L	72.0 ± 56.3
AST, IU/L	76.4 ± 53.4
Phosphatase alkalin, IU/L	140.1 ± 102.4
γ-GT, IU/L	105.6 ± 116.0
Cholesterol, mg/dL	198.7 ± 125.8
HDL cholesterol, mg/dL	51.8 ± 17.9
LDL cholesterol, mg/dL	125.5 ± 125.2
Triglycerides, mg/dL	105.1 ± 54.4
Ferritin, ng/mL	210.5 ± 118.6
Blood glucose, ng/mL	104.9 ± 45.5
Insulin, µU/mL	13.1 ± 11.8
Haemoglobin, ng/mL	14.0 ± 1.3
HOMA score	3.6 ±3.7
HOMA index, n (%)	
< 2	95 (40.1%)**
≥ 2	142 (59.9%)**
Serum 25-Hydroxyvitamin D, n (%)	26.2 ± 12.0
< 30 ng/mL	157 (66.2%)**
≥ 30ng/mL	80 (33.8%)**
HCV genotypes, n (%)	
1	206 (86.9%)**
Non 1	31 (13.1%)**
Hepatic fibrosis, n (%)	
Low	71 (29.9%)**
High	166 (70.1%)**

* Continuous variables are expressed as mean value ± standard deviation. ** Percentages in parenthesis referred to the total of patients (n = 237). ALT: alanine aminotransferase. AST: aspartate aminotransferase. γ-GT, gamma glutamyltransferase. HDL: high-density lipoprotein. LDL: low-density lipoprotein. HOMA: homeostatic model assessments.

presenting vitamin D deficiency also presented high fibrosis grade (71.4%) and IR (61.1%).

Factors associated with SVR

One hundred and thirty three HCV patients underwent and completed the antiviral treatment program. SVR was achieved in 59 individuals (44.2%) and all of them belonged to genotype 1. Low LDL (P = 0.019), low total cholesterol (P = 0.004), high AST (P = 0.000), high ALT (P = 0.000), low platelets (P = 0.036) and low fibrosis (P = 0.011) were associated with SVR (Table 3) in the univariate analysis, but none of them were associated to SVR at multivariate analysis. SVR was more prevalent in those patients without IR (52.5%) presenting high 25(OH)D levels (55.9%). However, these variables were not statistically significant.

DISCUSSION

In the present study, serum 25(OH)D levels were evaluated in HCV infected patients according to antiviral therapy response and clinical-biochemical

Table 2. Univariate and multivariate regression analysis of factors associated with serum 25(OH)D levels in 237 chronic hepatitis C patients.

Variable*	25 (OH) Vitamin D levels		Bivariate analysis P Value	Multivariate analysis OR (95% CI)	P value
	< 30 ng/mL (n = 157)	≥ 30 ng/mL (n = 80)			
Age (years)	55.2 ±10.6	50.6 ±11.8	0.003	0.958 (0.929-0.987)	0.005
Sex			0.07		
Female	99	40			
Male	58	40			
Body mass index (kg/m^2)	28.1 ±3.8	28.3 ±4.1	0.896	NA	NA
Glucose (mg/dL)	108.2 ±50.7	99.1 ±33.7	0.494	NA	NA
LDL (mg/dL)	139.5 ±152.4	100.7 ±37.9	**0.023**	0.999 (0.980-1.020)	0.956
Triglycerides (mg/dL)	104.7 ±59.6	105.8 ±43.6	0.431	NA	NA
Total cholesterol (mg/dL)	214.5 ±151.3	169.5 ±40.9	**0.001**	0.992 (0.973 - 1.011)	0.41
HDL (mg/dL)	53.6 ±19.2	48.6 ±14.9	**0.049**	0.995 (0.969 - 1.022)	0.716
Insulin (mU/mL)	13.7 ±12.4	12.2 ±10.9	0.577	NA	NA
GGT (IU/L)	110.2 ±125.0	96.6 ±96.3	0.62	NA	NA
AST (IU/L)	80.2±56.7	69.1 ±45.8	0.062	NA	NA
ALT (IU/L)	69.3 ±55.9	77.3 ±56.9	0.206	NA	NA
Alkaline phosphatase (IU/L)	134.2 ±109.1	151.8 ±87.7	**0.009**	1.001 (0.998-1.004)	0.386
Hemoglobin (ng/mL)	13.9 ±1.3	14.3 ±1.3	**0.018**	**1.328 (1.053-1.675)**	**0.017**
Platelets (10^3/mm)	186.7 ±76.1	182.8 ±86.1	0.739	NA	NA
Ferritin (ng/dL)	210.0 ±115.9	214.0 ±144.9	0.926	NA	NA
HCV RNA (IU/mL)	1.2 x 10^7 ± 1.0 x 10^8	1.6 x 10^6 ± 2.7 x 10^6	0.289	NA	NA
Sustained virological response,§ n			0.6	NA	NA
Yes	26	33			
No	37	37			
Fibrosis, n				NA	NA
Low	45	26	0.638		
High	112	54			
HOMA, n			0.35	NA	NA
< 2	61	34			
≥ 2	96	46			
HCV genotype, n	141	65	0.134	NA	NA
1	16	15			
Non 1					

* Continuous variables data are expressed as mean value ± standard deviation. NA: not available. Values in bold indicate significant values (p < 0.05). ALT: alanine aminotransferase. AST: aspartate aminotransferase. γ-GT, gamma glutamyltransferase. HDL: high-density lipoprotein. LDL: low-density lipoprotein. HOMA: homeostatic model assessments.

Table 3. Univariate and multivariate regression analysis of risk factors associated with sustained virological response (SVR) in 133 chronic hepatitis C patients.

Variable*	No sustained viral response (n = 74)	Sustained viral response (n = 59)	Bivariate analysis P Value	Multivariate analysis OR (95% CI)	P value
Age (years)	53.0 ± 12.8	52.0 ± 10.0	0.638	NA	NA
Glucose (mg/dL)	98.4 ± 36.2	108.1 ± 46.7	0.223	NA	NA
LDL (mg/dL)	110.5 ± 37.2	95.4 ± 35.8	**0.019**	0.995 (0.971-1.020)	0.705
Triglycerides (mg/dL)	112.4 ± 50.9	103.3 ± 35.5	0.534	NA	NA
Total cholesterol (mg/dL)	181.7 ± 41.1	163.1 ± 35.5	**0.004**	1.014 (0.991-1.038)	0.226
HDL (mg/dL)	50.2 ± 13.1	48.1 ± 12.6	0.432	NA	NA
Insulin (μU/mL)	13.4 ± 12.6	12.4 ± 14.5	0.268	NA	NA
GGT (IU/L)	103.4 ± 150.9	96.9 ± 94.3	0.932	NA	NA
AST (IU/L)	58.4 ± 43.3	98.8 ± 60.2	**0.000**	0.995 (0.981-1.009)	0.465
ALT (IU/L)	67.6 ± 48.6	102.8 ± 66.2	**0.000**	0.993 (0.981-1.005)	0.249
Alkaline phosphatase(IU/L)	187.5 ± 118.1	168.64 ± 80.6	0.655	NA	NA
Hemoglobin(ng/mL)	14.3 ± 1.5	13.9 ± 1.3	0.129	NA	NA
Platelets (10^3/mm)	178.3 ± 80.2	149.1 ± 74.6	**0.036**	0.997 (0.991-1.004)	0.464
HCV RNA (IU/mL)	$2.9 \times 10^7 \pm 1.6 \times 10^8$	$7.7 \times 10^5 \pm 1.0 \times 10^6$	0.066	NA	NA
Vitamin D, n§			0.600	NA	NA
< 30 ng/mL	37	26			
≥ 30 ng/mL	37	33			
Fibrosis, n§			**0.011**	2.157 (0.6675-6.895)	0.195
Low	29	10			
High	45	49			
HOMA, n§			0.061	NA	NA
< 2	30	31			
≥ 2	44	28			
HCV genotype, n§			0.982	NA	NA
1	71	59			
Non 1	03	00			

* Continuous variables data are expressed as mean value ± standard deviation. § Total values were not 237 individuals, since only 133 underwent antiviral treatment. NA: not available. Values in bold indicate significant values ($p < 0.05$). ALT: alanine aminotransferase. AST: aspartate aminotransferase. γ-GT: gamma glutamyltransferase. HDL: high-density lipoprotein. LDL: low-density lipoprotein. HOMA: homeostatic model assessments.

features of HCV infection. To our knowledge, this is the first study that describes vitamin D levels among HCV infected individuals from South America giving some new insight about this parameter in tropical regions. The major findings of this study were:

- High prevalence of hypovitaminosis D in HCV infected patients.
- The association between low serum 25OHD levels and some demographic (age) and laboratory data (LDL, cholesterol, HDL, alkaline phosphatase, hemoglobin).
- Lack of association between serum 25OHD levels and SVR to IFN-based therapy.

High prevalence of hypovitaminosis D (66.3%) was observed in Brazilian HCV infected individuals similar to that observed among European and American HCV patients. Prevalence of hypovitaminosis D varying from 46.4 to 73% among monoinfected HCV patients from Italy[1,14,21] and 86% among HIV-HCV infected individuals from France.[9] A recent metanalysis also showed that 71% of HCV infected individuals from Europe and North America had low vitamin D levels.[22] In the present study, low serum 25OHD levels was associated to higher values of mean age, LDL, total cholesterol, HDL, and low mean values of alkaline phosphatase, hemoglobin in the univariate regression analysis, although only age was statistically significant in the multivariate analysis. Petta, *et al.*[1] have also demonstrated that age and HDL cholesterol are independently associated to low vitamin D levels, however the reason for this finding it is not explained.

This study also demonstrated that low vitamin D levels were not associated to fibrosis grade as observed by Kitson, *et al.*[23] and Bitetto et al.[14] among HCV infected individuals from Australia and France, respectively. The differences observed in these studies could be due to different reasons:

- Absence of data regarding the season when blood sampling was performed; during the summer individuals could be more exposed to sun and consequently they could present higher levels of vitamin D[24].
- High frequency of high fibrosis grade (88%) in the present study and
- Different methods for vitamin D determination (HPLC for Petta, *et al.*[7] and Kitson, *et al.*[23] and electroquimioluminescence at Bitetto, *et al.*[14] and in the present study).

Vitamin D levels were not associated to SVR in the present study, as previously observed among CHC patients from Australia[23] and among HIV/HCV coinfected individuals.[14,24] This fact could be due to the large number of individuals included in the present study or some genetic characteristics of Brazilian population, such as, miscegenous population. In the present study, SVR was statistically associated with low mean values of LDL and total cholesterol, high mean values of AST, ALT and platelets and low fibrosis grade. Berg, *et al.*[25] found that LDL levels (≥ 2.6 mmol/L) are associated with SVR for telaprevir-based therapy in HCV genotype 1 patients. Low fibrosis grade have been associated to high SVR rates, as demonstrated among patients infected with HCV genotypes 2 or 3 receiving PEG-IFN and ribavirin (Niederau, *et al.*[26]). In addition, Ferreira, *et al.*[27] showed that high ALT values are associated with SVR among HIV/HCV infected patients in Brazil. The data found in the present study would be useful for defining successful strategies for PEG-IFN plus ribavirin treatment.

The main limitation of this study lies in its cross-sectional nature and its inability to dissect the temporal relation between 25(OH)D and laboratory data. Another limitation of this study is the lack of data on the potential confounders that may influence the levels of vitamin D, such as exposure to sunshine, dietary intake, and the prevalence of osteoporosis. Therefore, all patients involved in this study lived in Rio de Janeiro, where sunshine is abundant throughout the year. The absence of data on polymorphisms of vitamin D hydroxylating enzymes, and on other variables involved in vitamin D metabolism, such as parathyroid hormone, and in vitamin D signaling regulation also could affect the interpretation of our results.

CONCLUSION

Vitamin D deficiency was common among Brazilian HCV infected patients and it was no associated with SVR; however a relationship of vitamin D status and some laboratory data that could potentially influence the response to therapy was observed.

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