The Hepatic Expression of Vitamin D Receptor is Inversely Associated with the Severity of Liver Damage in Genotype 1 Chronic Hepatitis C Patients

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Background/aims: Low 25-Hydroxyvitamin D serum levels have been associated with the severity of liver fibrosis in genotype 1 chronic hepatitis C patients (G1CHC), and experimental evidence suggested a hepatoprotective role of vitamin D via interaction with hepatic vitamin D receptor (VDR). We assessed the hepatic expression of VDR protein and its association with liver disease severity.

Methods: Ninety-one consecutive patients with biopsy-proven G1CHC and available frozen liver tissue were evaluated. Ten subjects without chronic liver diseases and nine patients with autoimmune hepatitis served as controls. The hepatic expression of VDR protein was assessed by western blot for quantification, and by immunohistochemistry for morphological distribution.

Results: Liver VDR protein was mainly localized in hepatocytes and cholangiocytes, and its expression by western blot was similar in CHC and controls (1.83±0.97 vs. 2.18±0.62, p=0.14), but lower in autoimmune hepatitis (0.84±0.14, p<0.001). The expression was lower in CHC with severe necroinflammatory activity (1.44±0.87) vs. both controls and CHC with grade 1–2 inflammation (1.94±0.97)(p=0.01 and p=0.03, respectively), but higher compared to autoimmune hepatitis (p=0.007). A similar difference was observed in CHC patients with F3-F4 fibrosis, whose VDR expression (1.51±1.07) was also lower compared with controls and CHC with F0-F2 fibrosis (1.98±0.89)(p=0.02 and p=0.04, respectively), but higher vs. autoimmune hepatitis (p=0.003). At multivariate logistic regression analysis, low VDR protein expression remained associated with severe necroinflammatory activity and severe fibrosis (OR 0.543,95%CI, 0.288–0.989, p=0.04; and OR 0.484,95%CI 0.268–0.877, p=0.01, respectively) in CHC after correction for clinical, biochemical and histological features.

Conclusion: In a cohort of G1CHC patients, the hepatic expression of VDR protein is associated with the severity of both liver fibrosis and inflammation.

The natural history of patients with chronic hepatitis C (CHC) is largely driven by the development of liver fibrosis with the ultimate progression to cirrhosis and its complications (1). Different lines of research showed a relevant role of metabolic alterations, namely hepatic steatosis (2), insulin resistance (IR) (3), hyperuricaemia (4), and menopause (in females) (5), on liver disease severity and progression to advanced liver damage.

In this complex interplay between the liver and metabolic factors, there is a growing evidence for a role of

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Abbreviations: ABBREVIATIONS: HCV: hepatitis C virus; G1: genotype 1; CHC: chronic hepatitis C; SVR : sustained virological response; VDR: vitamin D receptor.

vitamin D status on liver disease severity. We first reported that fully compensated genotype 1 (G1) CHC patients are characterized by a high prevalence of 25-hydroxyvitamin D (25[OH]D) deficiency compared to a control population, and an independent inverse relationship was described between 25[OH]D serum levels and liver fibrosis severity (6). Along this line, we also reported an association between the severity of liver fibrosis and homozygosity for the gene variant of DHCR7 gene, a gene able to regulate vitamin D serum levels in both the general population and CHC patients (7). Other studies confirmed the association between vitamin D status and the severity of liver fibrosis in CHC (8–9), but negative data were also reported (10). This conflicting evidence is likely to stem from heterogeneity of the studied cohorts, of sample collection for vitamin D dosage, and in the distribution of SNPs affecting vitamin D metabolism (8–9, 11–13)

The biological effects of vitamin D are mediated by the interaction with vitamin D receptor (VDR), an NR1I family receptor with ligand-activated transcription factor activities (14), involved in immune response, fibrogenesis and inflammation, and associated with a liver protective effect in experimental models (15–16).

Considering the limits in the assessment of vitamin D serum levels, and that VDR, not vitamin D, is the ultimate actor in the interplay between vitamin D status and liver damage, aim of the present study was to assess the quantitative and morphological expression of hepatic VDR in a population of patients with G1 CHC. Quantitative data may throw light on the ultimate role of vitamin D in liver disease progression in CHC.

Materials and Methods

Patients

The study was carried out in 91 consecutive patients recruited at the Gastrointestinal & Liver Unit at the University Hospital in Palermo, with a histological diagnosis of G1 CHC and with available frozen liver tissue. G1 CHC patients were characterized by the presence of anti-HCV and HCV RNA, with persistently abnormal alanine aminotransferase (ALT), and by alcohol consumption of < 20 g/d in the last year or more, evaluated by a specific questionnaire. Exclusion criteria were: 1) advanced cirrhosis (Child-Pugh B and C); 2) hepatocellular carcinoma; 3) other causes or liver disease of mixed etiology (excessive alcohol consumption, hepatitis B, autoimmune liver disease, Wilson's disease, hemochromatosis, α1-antitrypsin deficiency); 4) HIV infection; 5) previous treatment with antiviral therapy, immunosuppressive drug and/or regular use of steatosis-inducing drugs (corticosteroids, valproic acid, tamoxifen, amiodarone); 6) therapy with medications known to affect vitamin D3 metabolism, including vitamin/mineral supplements; and 7) active i.v. drug addiction.

Nine patients with first diagnosis of autoimmune hepatitis (17), and ten control subjects undergoing elective cholecystectomy, without liver disease and with negligible alcohol consumption, were also enrolled. Control subjects had normal ALT and no evidence of viral infection (anti-HCV, antihuman immunodeficiency virus, and hepatitis B surface antigen (HBsAg) negative). Informed consent for liver biopsy was previously obtained from control subjects.

The study was performed in accordance with the principles of the Helsinki Declaration and its appendices, and with local and national laws. Approval was obtained from the hospital's Institutional Review Board and Ethics Committee, and written informed consent was obtained from all patients and controls.

Clinical and laboratory assessment

Clinical and anthropometric data were collected at the time of liver biopsy. The diagnosis of arterial hypertension was based on the following criteria: systolic blood pressure (BP) $\geq$ 135 mm Hg and/or diastolic BP $\geq$ 85 mm Hg (measured three times within 30 minutes, in the sitting position and using a brachial sphygmomanometer), or use of blood-pressure-lowering agents. The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association, using a value of fasting blood glucose $\geq$ 126 mg/dL on at least two occasions (18). In patients with a previous diagnosis of type 2 diabetes, current therapy with insulin or oral hypoglycemic agents was documented.

A 12-hour overnight fasting blood sample was drawn at the time of biopsy to determine the serum levels of ALT, total cholesterol, triglycerides, glucose, insulin, and platelet count. Insulin resistance (IR) was determined by the homeostasis model assessment (HOMA), using the following equation (19): Insulin resistance (HOMA-IR) = Fasting insulin (μU/mL) x Fasting glucose (mmol/L)/22.5. HOMA-IR has been validated in comparison with the euglycemic/hyperinsulinemic clamp technique in both diabetic and nondiabetic patients (20).

The analysis of serum 25(OH) D was performed using a Chromosystem reagent kit and a chromatographic system equipped with a Waters 1525 binary high-pressure liquid chromatography pump connected to a photo diode array detector (detection wavelength, 265 nm).

All anti-HCV positive patients were tested at the time of biopsy for HCV-RNA (RT-PCR homemade; detection limit: 12 IU/ml). Genotyping was performed by INNO-LiPA, HCV II, Bayer.

Histology

Slides were coded and read by one pathologist (D.C.), who was unaware of patients' identity and history. A minimum length of 15 mm of biopsy specimen or the presence of at least 10 complete portal tracts was required (21). In CHC, biopsies were classified according to the Scheuer numerical scoring system (22). The percentage of hepatocytes containing macrovescicular fat was determined for each 10x field. An average percentage of steatosis was then determined for the entire specimen. Steatosis was assessed as the percentage of hepatocytes containing fat droplets (minimum 5%), and evaluated as a continuous variable. Steatosis was classified as absent-mild when $< 20\%$, or moderate-severe when $\geq 20\%$. In autoimmune hepatitis histology was evaluated according to the simplified score for autoimmune hepatitis diagnosis (17).

Antiviral Treatment Schedule and Outcomes

Patients were treated with Pegylated-interferon (PEG-IFN) α-2a (Pegasys, Roche, Basel, Switzerland) 180 mcg/wk plus Ribavirin (RBV) according to body weight (1,000 mg/d for a body weight of $<$ 75 kg, 1200 mg/d for a body weight of $\geq$ 75 kg) for 48 weeks. Patients were withdrawn from treatment if they did not achieve a virological response, defined as undetectable serum HCV RNA by PCR within 24 weeks after start of treatment.

Hepatic Vitamin D receptor evaluation

Immunohistochemical evaluation

For immunohistochemical analysis sections were deparaffinized with xylene and rehydrated to water through a graded alcohol series. Antigen retrieval was performed using 10 mMol citrate buffer (pH 6.0) at high temperature for 20 minutes. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide. Sections were incubated with antihuman VDR (Clone D-6: sc-13 133, Santa Cruz) at room temperature for 1 hour. Staining was performed with the Novolink Max Polymer Detection System (Leica Microsystems) using DAB (3,3′-Diaminobenzidine) substrate chromogen (Leica Microsystems). Finally, sections were counterstained with hematoxilyn. Slides were evaluated using a Leica DMD108 microscope.

Western blot evaluation

Liver samples were stored at −80°C and disrupted/homogenized using the TissueRuptor apparatus (Qiagen) immediately before nucleic acid extraction. DNA, RNA and proteins from biopsy were extracted using the AllPrep DNA/RNA Micro Kit (Qiagen) according to manufacturer's instructions.

Protein lysates were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and blotted over night onto a PVDF transfer membrane (Amersham) using an SDS electroblotting system (BioRad) at a constant voltage of 50 V. Blots were probed with anti-VDR monoclonal antibody (Clone D-6: sc-13 133, Santa Cruz) and anti-GAPDH monoclonal antibody (Sigma-Aldrich) over night. Filters were incubated for one hour with a secondary antibody (Goat antimouse immunglobulin horseradish peroxidase-conjugate; Bio-Rad) and specific proteins were visualized with the ECL detection system (Amersham) (Appendix Figure 1). Results were expressed as signal intensity of VDR lines with respect to GAPDH as loading control.

Statistics

Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequency and percentage. The Student t test and analysis of variance (ANOVA) were used as appropriate. For statistical purposes, the values of VDR were multiplied by 10 and log-transformed in the regression.

Multiple logistic regression models were used to assess independent predictors of both liver necroinflammatory activity and fibrosis in CHC patients. In the first model, the dependent variable was severe liver necroinflammatory activity, coded as 1 (grading 3) vs 0 (grading 1–2). In the second model, the dependent variable was severe liver fibrosis, coded as 1 (F3-F4) vs 0 (F0-F2). As candidate risk factors (independent variables) we selected age, sex, body mass index (BMI), baseline ALT, platelet count, total cholesterol, triglycerides, blood glucose, insulin, HOMA score, diabetes, arterial hypertension, VDR liver protein expression, Log HCV-RNA levels, steatosis, activity score, and fibrosis.

Variables associated with the dependent variable at univariate analyses (probability threshold, $P < .10$) were included in the multivariate regression models. Regression analyses were performed by SAS (23).

Results

Patient Features and Histology

The baseline features of the 91 patients with CHC are shown in Table 1. Thirty percent of patients had fibrosis F3-F4 by Scheuer score, with a prevalence of 22.2% of severe necroinflammation (grade 3). Steatosis was graded as moderate/severe in 27.5% of patients. Mean serum values of 25(OH)D were 24.6 ± 8.4 μg/L.

Control healthy subjects (5 men and 5 women; median age 53 years; BMI, 27.1 ± 3.2 kg/m^2) did not differ from the HCV population for demographic and anthropometric measures. None had histological evidence of either hepatic steatosis, or significant inflammatory and fibrotic changes.

Patients with autoimmune hepatitis were mostly women (7/9), had a median age of 51 years, and a mean BMI of 24.5 ± 2.3 kg/m^2. All patients had histological evidence of ≥F2 fibrosis and severe necroinflammatory activity with interface hepatitis and predominatly lymphoplasmacytic infiltrate, whereas 5 (55.5%) had also evidence of rosetting of liver cells.

Liver VDR protein expression

In CHC, VDR expression was strongly present on cholangiocytes, both in the cytosol and in the nucleus. Diffuse and moderate expression was found in hepatocytes, while a weak positivity was demonstrated in inflammatory cells (Figure 1A). A similar morphological distribution was observed in controls (Figure 1B) and in autoimmune hepatitis (Figure 1C).

By western blot analysis, liver VDR expression was, on average, similar in CHC compared to controls (1.83 ± 0.97 vs. 2.18 ± 0.62, $P = .14$), but much higher compared to values measured in autoimmune hepatitis (0.84 ± 0.14, $P < .001$). When CHC patients were stratified according to the severity of liver damage, CHC with severe necroinflammatory activity had a significantly lower VDR expression compared to both controls and CHC with low (grade 1–2) necro-inflammatory activity (1.44 ± 0.87 vs. 2.18 ± 0.62 and vs. 1.94 ± 0.97, $P = .01$ and $P = .03$ respectively), but again much higher compared to autoimmune hepatitis ($P = .007$) (Figure 2A). A similar difference was observed when CHC patients were stratified according to fibrosis; F3-F4 CHC cases had a significantly

Table 1. Demographic, laboratory, metabolic and histological features of 91 patients with genotype 1 chronic hepatitis C.

Variable	Chronic hepatitis C genotype 1 (n = 91)
Age -- yrs	51.7 ± 11.3
Male Gender - n (%)	42 (46.2)
Mean Body Mass Index -- kg/m^2	26.8 ± 4.3
Arterial Hypertension - n (%)	22 (24.2)
Type 2 Diabetes - n (%)	9 (9.9)
Alanine aminotransferase -- IU/mL	90.0 ± 72.6
Platelet -- n/mmc	199.0 ± 56.0
Cholesterol -- mg/dL	172.9 ± 38.0
Triglycerides -- mg/dL	92.5 ± 35.6
Blood glucose -- mg/dL	97.0 ± 31.7
Insulin -- μU/mL	13.8 ± 8.2
HOMA-score	3.58 ± 3.15
25(OH)D - μg/liter	24.3 ± 8.3
Liver VDR protein expression	1.83 ± 0.97
LOG10 HCV-RNA	5.7 ± 0.6
Histology at biopsy	
Steatosis:	
-continuous variable	11.7 ± 20.5
-categorical variable	
<20%	66 (72.5)
≥20%	25 (27.5)
Stage of fibrosis	
0	7 (7.7)
1	25 (27.5)
2	31 (34.1)
3	20 (22.0)
4	8 (8.8)
Grade of activity	
1	23 (25.3)
2	48 (52.7)
3	20 (22.2)

Abbreviation: yrs, years; IU, international units; HOMA, homeostasis model assessment; VDR, vitamin D receptor; HCV-RNA, hepatitis C virus ribonucleic acid. Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequency and percentage.

lower VDR expression compared to controls and to CHC with F0-F2 fibrosis (1.51 ± 1.07 vs. 2.18 ± 0.62 and vs. 1.98 ± 0.89, $P = .02$ and $P = .04$ respectively), but higher with respect to autoimmune hepatitis ($P = .003$)(Figure 2B).

In CHC patients, no association was found between liver VDR protein expression and serum vitamin D levels, metabolic factors and HCV viral load.

Factors associated with severe necroinflammatory activity

The univariate and multivariate comparison of variables between CHC patients with and without severe necroinflammatory activity (grade 3) are reported in Table 2. High baseline values of ALT and triglycerides, as well as low liver VDR protein expression, were associated with severe necroinflammatory activity ($P < .10$). Multivariate logistic regression analysis identified the following variables as independently linked to severe necroinflammatory activity (grade 3): high ALT (OR, 1.016; 95% CI, 1.005–1.027, $P = .005$) and low liver VDR protein expression (OR, 0.543; 95%CI, 0.288–0.989; $P = .04$).

Factors associated with severe fibrosis

The univariate and multivariate comparison of variables between CHC patients with and without severe fibrosis (F3-F4) are reported in Table 3. Older age, high baseline values of ALT, high triglycerides, low VDR liver protein expression, moderate-severe steatosis, and hepatic necroinflammatory activity were all associated with severe fibrosis ($P < .10$). Multivariate logistic regression analysis showed that the following features were independently linked to severe fibrosis (Scheuer score ≥ 3): older age (OR, 1.072; 95% CI, 1.014–1.134, $P = .001$), low VDR liver protein expression (OR, 0.484; 95%CI, 0.268–0.877, $P = .01$), moderate-severe steatosis (OR, 4.055; 95%CI, 1.331–12.358; $P = .01$), and liver necroinflammatory activity (OR, 4.341; 95%CI, 1.317–14.308; $P = .01$).

VDR expression and sustained virological response

Seventy-eight out of 91 patients (male gender: 51%; mean age: 51.9 ± 11.3 years; F3-F4 fibrosis: 32%; liver VDR expression: 1.78 ± 0.95) with similar characteristics to the entire population underwent antiviral therapy with PEG-IFN and RBV. A sustained virological response (SVR) was obtained in 41 cases (52.5%). Compared to no SVR, the patients achieving a SVR were younger ($P = .01$), had lower viral load ($P = .03$), lower amounts of both steatosis ($P = .04$) and fibrosis ($P = .02$), and a signifi-

Table 2. Univariate and multivariate analysis of risk factors associated with severe liver necroinflammatory activity (grading 3) in 91 patients with genotype 1 chronic hepatitis C.

Variable	Mild-moderate grading $n = 71$	Severe grading $n = 20$	Univariate Analysis p value	Multivariate Analysis	
				OR (95% CI)	p value
Age -- yrs	51.0 ± 11.5	54.3 ± 10.6	0.25	-	
Male Gender					
Male *vs.* Female	32/39	10/10	0.69	-	
Body Mass Index -- kg/m^2	26.8 ± 4.6	26.6 ± 2.9	0.88	-	
Alanine aminotransferase -- IU/mL	71.4 ± 40.2	156.1 ± 114.1	<0.001	1.016 (1.005 – 1.027)	0.005
Cholesterol -- mg/dL	172.3 ± 38.8	175.0 ± 35.7	0.78	-	
Triglycerides -- mg/dL	86.3 ± 28.1	114.5 ± 49.4	0.001	1.012 (0.993 – 1.032)	0.21
Blood glucose -- mg/dL	98.0 ± 35.1	93.3 ± 14.5	0.53	-	
Insulin -- μU/mL	13.3 ± 8.7	15.4 ± 6.0	0.35	-	
HOMA-score	3.57 ± 3.49	3.63 ± 1.49	0.94	-	
Arterial Hypertension					
absent *vs.* Present	53/18	16/4	0.62	-	
Type 2 Diabetes					
absent *vs.* Present	64/7	18/2	0.98	-	
25(OH)D - μg/liter	24.4 ± 8.6	24.0 ± 7.2	0.88	-	
Liver VDR protein expression	1.94 ± 0.97	1.44 ± 0.87	0.03	0.543 (0.288 – 0.989)	0.04
LOG10 HCV-RNA	5.6 ± 0.6	5.8 ± 0.5	0.62	-	
Steatosis <20% *vs.* ≥20%	53/18	13/7	0.39	-	

Abbreviation: yrs, years; IU, international units; HOMA, homeostasis model assessment; VDR, vitamin D receptor; HCV-RNA, hepatitis C virus ribonucleic acid. Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequency and percentage.

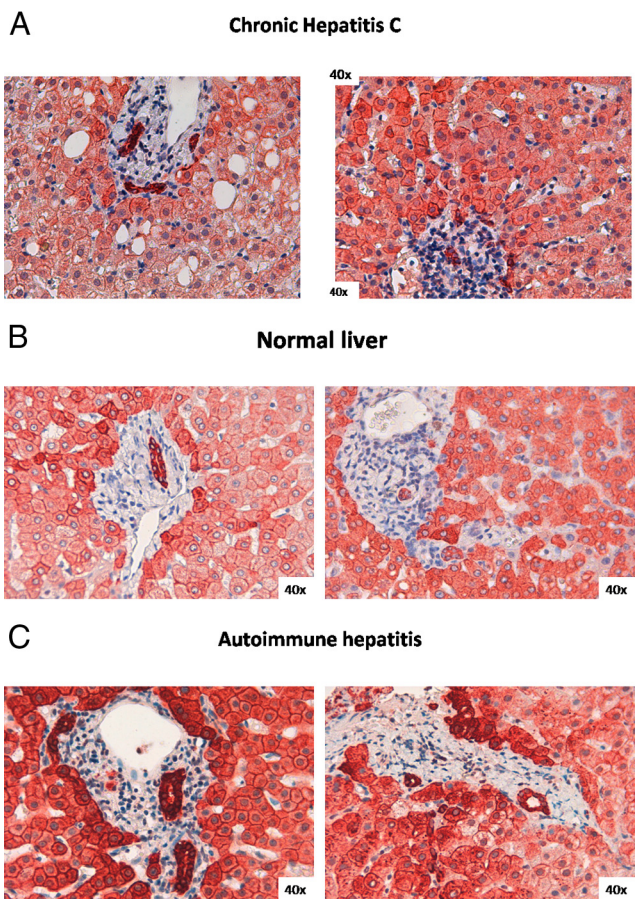

Figure 1. VDR expression was evaluated via immunohistochemistry in hepatocytes, cholangiocytes, and liver inflammatory cells in CHC patients (A), in controls (B) and in autoimmune hepatitis patients (C). VDR was strongly expressed on cholangiocytes, both in the cytosol and in nucleus. Diffuse and moderate expression was found in hepatocytes; weak positivity was also evident in inflammatory cells. Original magnification 400x.

cantly higher expression of hepatic VDR (1.98 ± 0.95 vs 1.48 ± 0.96; $P = .02$).

Discussion

As far as we know, this is the first report on the quantitative hepatic expression of VDR in the liver of G1 CHC. In these biopsies, VDR is localized in inflammatory cells, hepatocytes and cholangiocytes, and its protein expression was similar in CHC patients and controls. However, when patients were stratified according to the severity of liver damage, VDR expression was reduced in G1 CHC patients in relation to the degree of liver necroinflammatory activity or fibrosis, resulting independently linked to these histological alterations.

Our data on necroinflammatory activity confirm a recent report by Barchetta et al (24); they reported an inverse relationship between VDR and liver inflammation in a small group of 36 patients with CHC. However, in their study VDR liver expression was assessed by an immunohistochemical semiquantitative score, less accurate than the quantitative VDR evaluation by western blot analysis performed in our study. In addition, data from the above study (24) do not clarify whether the inverse relationship between liver VDR and liver inflammation is an HCV-specific mechanism. To give an answer to this relevant issue, we also measured liver VDR expression in subjects with autoimmune hepatitis, where necroinflammation is definitely higher compared to CHC patients. We were thus able to demonstrate an inverse relationship between various degrees of necroinflammation, with hepatic VDR expression being progressively lower moving from CHC pa-

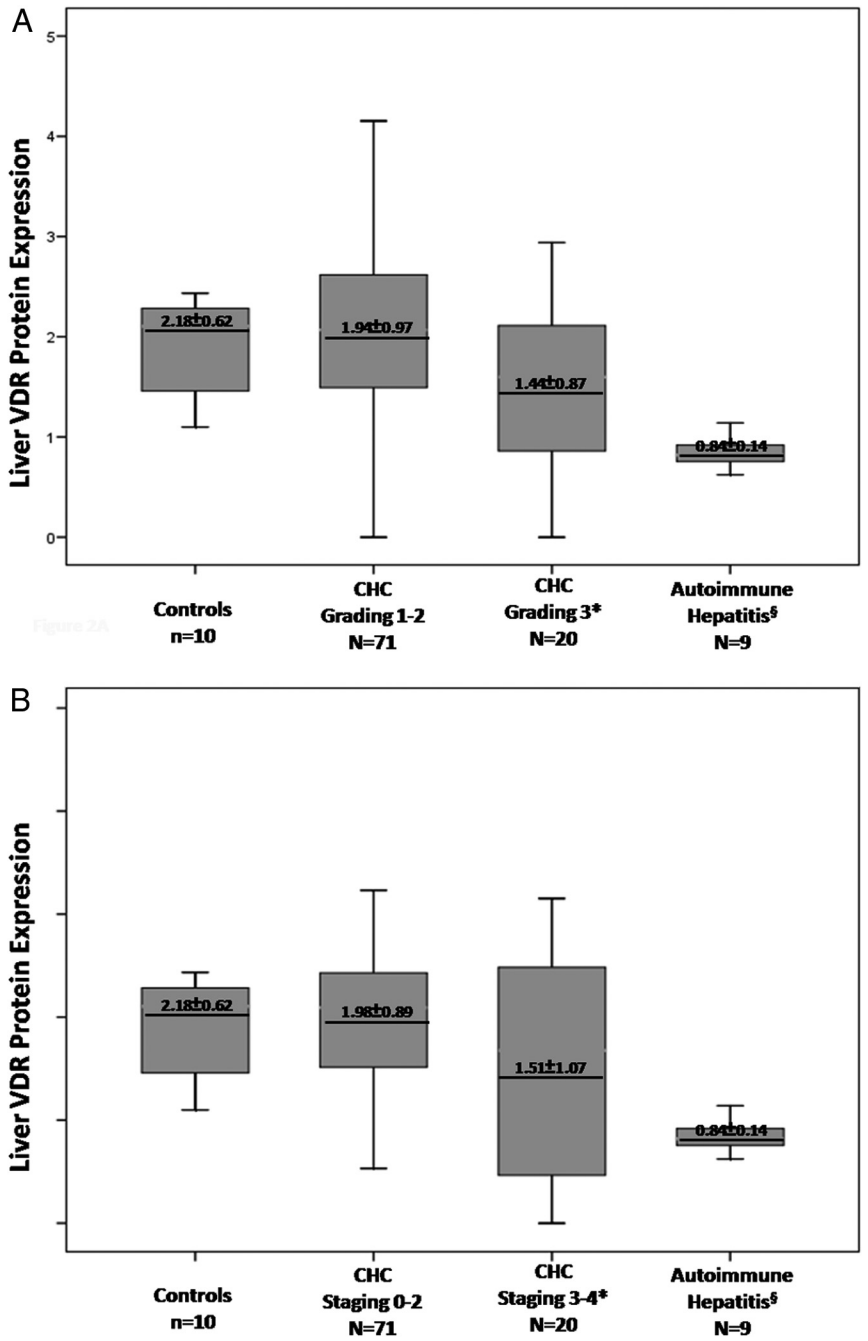

Figure 2. A. Log liver vitamin D receptor protein expression by western blot in controls, and in CHC patients according to the severity of liver necroinflammatory.*$P < .05$;§$P < .01$. B. Log liver vitamin D receptor protein expression by western blot in controls, and in CHC patients according to the severity of liver fibrosis.*$P < .05$;§$P < .005$.

tients with low-to-moderate necroinflammation, CHC cases with high necroinflammation and finally autoimmune hepatitis. Our data suggest that the reduced liver expression of VDR is thus linked to inflammatory mechanisms occurring in the liver, not specifically related to HCV infection, but and maximally present in a highly inflammatory liver disease such as autoimmune hepatitis. Unfortunately, the assessment of VDR by Western Blot represents the combination of VDR in multiple cell types, but we were not able to determine the VDR expression of specific cell types. This aspect could be of particular interest for inflammatory cells, particularly T cells, since vitamin D and VDR expression are able to control T cell antigen receptor signaling and activation (25). Further studies are needed in this setting.

Our study also offers the first evidence of a reduced VDR liver expression in CHC according to the severity of liver fibrosis, after correction for well-known metabolic and histological risk factors for fibrosis. This issue was not observed in the paper by Barchetta et al (24), possibly due to the small study sample and the less accurate method of assessment of VDR liver expression. The independent association of both high hepatic inflammatory activity and low VDR with liver fibrosis could be explained by a cause-effect relationship, where low liver VDR expression, driven by inflammation, could promote and amplify fibrogenic mechanisms.

In agreement with Barchetta et al (24), we were able to confirm the nuclear and cytoplasmic distribution of VDR not only in inflammatory cells promoting collagen deposition, but also in hepatocytes and mainly in cholangiocytes, this morphological picture being observed in CHC as well as in controls and autoimmune hepatitis.

Accordingly, low hepatic VDR expression, not directly related to HCV-induced mechanisms, could be a factor involved in liver disease severity, also mediated by hepatocytes, cholangiocytes and other inflammatory cells.

Finally, in the subgroup of patients treated with PEG-IFN and RBV, we reported a significantly higher VDR liver expression in those obtaining a SVR compared to those who did not achieve it. This finding is coherent with previous evidence showing a link between vitamin D status and the likelihood of achieving a SVR after treatment with PEG-IFN e RBV in patients with G1 CHC (6).

Table 3. Univariate and multivariate analysis of risk factors associated with severe fibrosis (F3-F4) in 91 patients with genotype 1 chronic hepatitis C.

Variable	No severe Fibrosis (Scheuer score 0–2) $n = 63$	Severe Fibrosis (Scheuer score 3–4) $n = 28$	Univariate Analysis p value	Multivariate Analysis OR (95% CI)	Multivariate Analysis p value
Age -- yrs	49.4 ± 11.6	57.1 ± 8.7	0.002	1.072 (1.014 – 1.134)	0.001
Male Gender					
Male *vs.* Female	30/33	12/16	0.67	-	
Body Mass Index -- kg/m^2	26.8 ± 4.8	26.7 ± 3.1	0.87	-	
Alanine aminotransferase-- IU/mL	69.5 ± 47.5	136.1 ± 95.9	<0.001	-	
Cholesterol -- mg/dL	176.7 ± 40.4	164.4 ± 30.9	0.16	-	
Triglycerides -- mg/dL	85.7 ± 34.1	107.8 ± 34.6	0.006	-	
Blood glucose -- mg/dL	94.3 ± 29.8	103.0 ± 35.6	0.24	-	
Insulin -- μU/mL	13.0 ± 7.9	15.5 ± 8.6	0.20	-	
HOMA-score	3.26 ± 2.11	4.32 ± 4.70	0.13	-	
Arterial Hypertension					
absent *vs.* Present	50/13	18/10	0.19	-	
Type 2 Diabetes					
absent *vs.* Present	59/4	23/5	0.10	-	
25(OH)D - μg/liter	24.3 ± 8.8	24.3 ± 7.5	0.98	-	
Liver VDR protein expression	1.98 ± 0.89	1.51 ± 1.07	0.04	0.484 (0.268 – 0.877)	0.01
LOG10 HCV-RNA	5.7 ± 0.6	5.5 ± 0.7	0.33	-	
Histology at biopsy					
Steatosis <20% *vs.* ≥20%	51/12	15/13	0.007	4.055 (1.331 – 12.358)	0.01
Liver necroinflammatory activity	22/33/8	1/15/12	0.002	4.341 (1.317 – 14.308)	0.01

Abbreviation: yrs, years; IU, international units; HOMA, homeostasis model assessment; VDR, vitamin D receptor; HCV-RNA, hepatitis C virus ribonucleic acid. Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequency and percentage.

The cross-sectional design of the study cannot provide any mechanistic explanation, but our data fit with those reported in experimental studies. Specifically, in primary hepatic stellate cells (HSCs) isolated from rats, and in a rat model of liver fibrosis induced by thioacetamide, VDR was highly expressed in quiescent HSCs, its expression decreased up to 40% during activation, and its silencing demonstrated that suppression of cyclin D1 and collagen Iα1 protein expression was VDR dependent (15). Along this line, another experimental study also showed that vitamin D deficiency up-regulated hepatic inflammatory and oxidative stress genes in a rat model of nonalcoholic fatty liver disease (16). Finally, cholangiocytes, cells highly expressing VDR according to our and other published data (24), can express several profibrogenic and chemotactic proteins activating inflammatory and fibrogenic cells (26–28).

The main limitation of this study lies in its cross-sectional nature and its inability to clearly dissect the temporal relation between hepatic VDR protein expression and both liver necroinflammation and fibrosis. Another relevant limit is the lack of experimental data providing a mechanistic explanation for our association. However, a growing body of literature demonstrates the anti-inflammatory and antifibrotic effect of VDR (15–16). Furthermore, we have not evaluated the relationship between VDR expression and tissue vitamin D levels. Finally, a methodological drawback is the potentially limited external validity of the results for different populations and settings, however tempered by data of controls and AIH cases.

In conclusion, in a homogeneous cohort of compensated biopsy-proven G1 CHC patients, the expression of hepatic VDR protein is inversely and independently associated with the severity of both liver fibrosis and inflammation. This finding translates experimental results on human liver, also suggesting a strong link between liver inflammation and liver VDR expression not directly related to virus-induced mechanisms.

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Appendix Figure 1. VDR and GAPDH liver expression in different CHC patients by western blot analysis.