Vitamin D levels in Egyptian HCV patients (Genotype 4) treated with pegylated interferon

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Abstract

Background/Aim : **Vitamin D has been shown to play an important immunomodulatory role. Deficiency of vitamin D has been recently associated to the lack of response to interferon therapy in Hepatitis C virus genotype 1 infected patients.**

This study aims to evaluate serum level of vitamin D and verify whether circulating vitamin D has any independent role in predicting the rates of HCV virologic response after the administration of pegylated interferon to Egyptian patients infected with genotype 4 HCV.

Methods : **Fifty patients infected with HCV genotype 4 and not co-infected with neither Hepatitis B virus nor Human Immunodeffiency Virus were recruited for the study. They were treated with ribavirin-pegylated interferon alpha 2a. Viral titer was determined at baseline, at 12 weeks and at end of treatment (48 weeks). Vitamin D levels and a biochemical profile were obtained for the patients at baseline and at end of treatment. Vitamin D control group consisting of 20 healthy patients of similar age and weight to the study group were recruited to obtain vitamin D levels.**

Results : **Vitamin D levels in HCV infected patients were significantly lower than in healthy subjects. Responders to ribavirin plus pegylated interferon alpha 2a therapy had significantly higher vitamin D levels than non-responders.**

Conclusion : **Vitamin D deficiency predicts an unfavorable response to interferon-based treatment of HCV.** (Acta gastroenterol. belg., **2013**, 76, **38-44**).

Key words : Vitamin D, HCV, Genotype 4, Egyptian patient.

Introduction

Vitamin D is a fat-soluble vitamin that plays a role in many important body functions. It is known for its traditional role in bone mineralization and calcium homeostasis (1). Vitamin D, beyond its known role in calcium and bone metabolism, possesses important immune functions, favoring innate immunity response and cell differentiation. This immune function of vitamin D has been associated with various pathological conditions, autoimmunity disorders, various cancers, cardiovascular disorders, diabetes, allograft survival and several infectious diseases (2-6). Some studies have found low serum 25 (OH)D levels in patients with chronic hepatitis and cirrhosis of different origins (7-9).

Vitamin D exists in several forms including 25-hydroxy vitamin D [25 (OH)D], the primary circulating form, and 1,25-dihydroxy vitamin D [1,25 (OH)D], the active form (1).

Serum 25 (OH)D correlates with overall vitamin D stores and is the most commonly used biomarker for assessing vitamin D deficiency. Deficiency is often defined by circulating 25 (OH)D levels below 30 ng/ml (50 nmol/l) (10).

Hepatitis C virus (HCV) is a major cause of chronic hepatitis and the leading cause of end-stage liver disease including liver cirrhosis and hepatic cell carcinoma (HCC) (11), and the most common indication for liver transplantation (12).

HCC represents an important public health problem in Egypt where up to 90% of HCC cases are attributable to HCV infection (13). HCV is the single most important cause of liver disease in Egypt (14-18), where this prevalence is 10-20 folds higher than that in the United States (19). In Egypt, where hepatitis C is highly endemic (up to 15% of the population), 91% of the patients are infected with HCV genotype 4 (20).

Recently, two novel predictors of response to antiviral treatment for HCV have emerged : the interleukin-28B (IL-28B) rs12979860 C/T polymorphism and vitamin D serum concentration. The serum vitamin D concentration is of a great interest because it is easily modifiable by dietary supplementation (21), and is easy and of low cost to assess in every day practice.

This study aims to evaluate serum level of vitamin D and verify whether circulating vitamin D has any independent role in predicting the rates of HCV virologic response after the administration of pegylated interferon (PEG-IFN) to patients infected with genotype 4 HCV. Finding an inexpensive predictor of the response to an expensive treatment, might improve the management of HCV patients in Egypt where medical care is cost prohibitive.

Methods

Study Subjects

Inclusion Criteria

Adult subjects aged between 18-60 years, who were diagnosed with chronic HCV infection, genotype 4,

presenting a body mass index (BMI) < 40 kg/m2 were enrolled in the study.

Exclusion Criteria

Subjects were excluded if co-infected with Human immuno-deficiency virus (HIV) infection or hepatitis B virus (HBV), had evidence for other liver diseases, had any underlying renal, cardiovascular, gastrointestinal, autoimmunity, or malignancy diseases, or if currently taking any medications or vitamin supplements or any medications known to affect vitamin D metabolism, including vitamin/mineral supplements. Subjects were also excluded if previously were treated with PEG-IFN or if they had active intravenous drug addiction.

Control Group

Healthy subjects of matched age, gender and body mass index to the test group were included in the study to compare vitamin D levels.

Study Setting and Design

This is a prospective, parallel, observational study. The study was conducted between September 2010 and August 2011. All the subjects were recruited from the department of internal medicine, Ain Shams University Hospitals.

All recruited HCV genotype 4 patients were treated with subcutaneous (Sc) administration of PEG-IFN-alpha2a (Pegasys, Roche, Basel, Switzerland) at dose of 1.5 mcg/kg/week, plus oral ribavirin at a dose of 1000-1200 mg/day according to body weight (1000 mg/day for a body weight of < 75 kg, 1200 mg/day for a body weight of > 75 kg) for 48 weeks (22).

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices (23). Approval was obtained from the local Institutional Review Board and Ethics Committee, and written informed consent was obtained from all cases.

All the recruited subjects were clinically screened prior to drug administration and again at the end of treatment (48 week). Screening included also age, sex, body weight, height, and BMI as well.

Liver biopsy at inclusion was available for all patients. Hepatic inflammation and fibrosis were graded according to Modified Knodell's (Ishak) fibrotic Score (24,25). Clinical and anthropometric data were collected at the time of liver biopsy.

Assessment of Efficacy of PEG-IFN

The efficacy of the PEG-IFN was assessed based on the end-of-treatment response (EOT) adopting viral titer (Real Time PCR System-*Applied Biosystem*) using a detection limit of 50 IU (100 copies) × 103/L. The viral titer was obtained at the time of biopsy from all the screened patients at baseline just before injection of PEG-IFN, at 12 weeks (early viral response, EVR) and at end-of-treatment. For this assessment, 5 mL blood samples were withdrawn for the entire screened subjects and frozen at -80ºC for further investigations. Genotyping was performed by INNO-LiPA, HCV.

Baseline Characteristics

A 12-hour overnight fasting blood sample was drawn at the time of biopsy to determine serum levels of alanine aminotransferase (ALT) (IU/mL), aspartate aminotransferase (AST) (IU/mL), blood glucose concentration (mg/dL), total bilirubin (mg/dL), direct bilirubin (mg/dL), albumin (g/dL), alpha-fetoprotein (AFP) (ng/dL), pro-thrombin time (PT) %, thyroid stimulating hormone (TSH) (mIU/L), creatinine (mg/dL), and hemoglobin (Hb) (g/L).

Vitamin D Analysis

The analysis of serum 25 (OH) D was performed using 25-OH-Vitamin D direct ELISA- quantitative sandwich enzyme-linked immunosorbant assay (IBL International, Hamburg, Germany). Patients were classified according to the serum vitamin D level into patients with insufficient (< 30 ng/ml) and those with sufficient levels (> 30 ng/ml).

Statistical Analysis

Pairwise comparisons were performed using Mann-Whitney, Wilcoxon-Signed-Rank, or Pearson's Chi square tests as needed at a level of significance of 0.05. Correlations were tested using Spearman's correlation. Univariate linear regression was performed to test the association of EVR with different patient characteristics.

Results

Patient Characteristics

A total of 50 HCV patients were recruited in the study. Baseline characteristics, treatment regimen, and HCV virologic response to PEG-IFN treatment of the 50 patients included in this study are summarized in-Table 1. Almost half of the patients (46%) had an initial moderate viral load. EOT was achieved by 32 (64%) patients.

Twenty control subjects were matched for age, gender and BMI with those diagnosed with HCV. More than 50% of the patients were in the normal body weight range (BMI < 25 Kg/m2).

Vitamin D Deficiency

Mean serum vitamin D level in the control group was 30.95 ± 2.7 ng/ml, where the majority of the patients showed sufficient 25 (OH)D levels (70%).

The mean serum 25 (OH)D level in the patient group was 25.74 ± 6.285 ng/ml before initiating the PEG-IFN therapy, with only 38% of the patients having sufficient 25 (OH)D levels (> 30 ng/ml). After 48 weeks of PEG-IFN therapy, the mean serum 25 (OH)D level declined to 22.24 ± 5.909 ng/ml, with 20% of the patients having levels above 30 ng/mL. Both values were signifi-

Table 1. — **Baseline characteristics of 50 patients infected with HCV Genotype 4. Data represented as mean and (Standard Deviation)**

Parameter	Before treatment	After treatment (48 weeks)	P*
Gender Distribution (male %)	68% (N = 34)		
Age (years) (range, SD)	40 (range : 20-54, 8)		
Viral Load			
Number (%) of Patients with Low Load (< 100000)	(N = 14) 28%		
Number (%) of Patients with Moderate (100000-1000000)	(N = 23) 46%		
Number (%) of Patients with High (> 1000000)	(N = 13) 26%		
Aspartate Aminotransferase (IU/mL)	61 (19)	46 (23)	0.00
Alanine Aminotransferase (IU/mL)	57 (15)	36 (10)	0.10
Total Bilirubin (mg/dL)	1.08 (0.41)	0.97 (0.5)	0.68
Direct Bilirubin (mg/dL)	0.26 (0.17)	0.22 (0.2)	0.71
Albumin (g/dL)	3.83 (0.33)	3.70 (0.4)	0.22
Blood Glucose Level (mg/dL)	99 (17)	104 (22)	0.06
Alpha-fetoprotein (ng/dL)	9 (5)	9.54 (3.6)	0.11
Prothrombin Time %	93 (7)	90 (6)	0.04
Thyroid Stimulating Hormone (mIU/L)	3.67 (0.76)	4.29 (1.5)	0.12
Creatinine (mg/dL)	1.13 (1.15)	1.08 (0.3)	0.82
Vitamin D Level	26 (6)	22 (6)	0.00
Hemoglobin (g/L)	12 (3)	10 (1)	0.00
BMI (kg/m²)	27 (9)	24 (8)	0.00

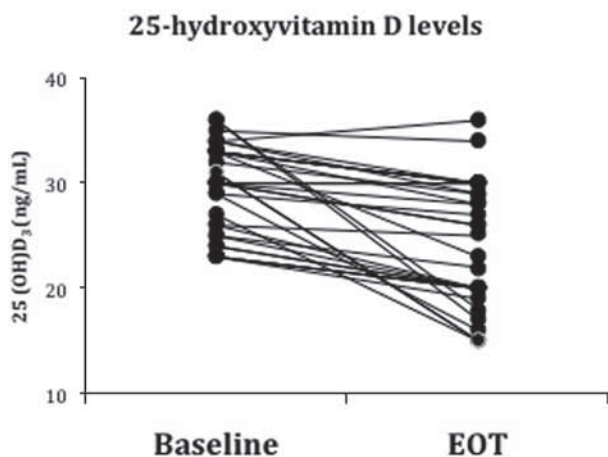*Wilcoxon-Signed-Rank test at a level of significance 0.05.

Fig. 1. — 25-Hydroxyvitamin D (25 (OH)D) Serum Concentrations of 32 HCV Genotype 4 Patients, who Responded to Interferon/Ribavirin Therapy, at Baseline and at End-of-Treatment.

cantly (p = 0.000) lower than those recorded in the control group. The decline in vitamin D levels in the study group was associated with significant decrease in both Hb levels (at 48 weeks : 10.3 ± 1.3, p = 0.003) and BMI (at 48 weeks : 24.4 ± 7.7, p = 0.000).

Association of EOT Response with Vitamin D Serum Concentrations

All patients who had initial 25 (OH)D levels ≥ 30 ng/mL responded to PEG-IFN therapy, while 41% of the responders had initial 25 (OH)D levels < 30 ng/mL. (Fig. 1) None of the patients who had initial 25 (OH)D levels < 20 ng/mL (20% of all patients) responded to PEG-IFN therapy. Average initial 25 (OH)D levels for responders was 24.5 ± 5.7 ng/mL and was significantly different from levels for non-responders 18.3 ± 3.7 (p = 0.000).

Fibrosis, Liver Histology and Liver Functions

Hepatic inflammation and fibrosis were graded according to Modified Knodell's (Ishak) fibrotic Score (24,25). Eighty percent of the patients had a fibrotic score of either 3 or 4 out of 6. The Modified Histological Activity Index (HAI), indicating the necro-inflammatory process, was 5 or 6 out of 18 in 62% of the patients. Vitamin D levels were not significantly different among patient groups with different fibrosis score or HAI (Fig. 2).

Assessing liver function, AST levels significantly changed at 48 weeks (46 ± 23, p = 0.000) as well as the PT%, which significantly decreased to 90 ± 6 (p = 0.036). All other baseline characteristics did not significantly change post-treatment.

Table 2. — **Correlation of vitamin D serum levels to the biochemical profile of 50 patients before interferon/ribavrin treatment**

Parameter	Correlation Coefficient (Spearman)	P*
Age (Years)	-0.16	0.26
Aspartate Aminotransferase (IU/mL)	-.507	0.00
Alanine Aminotransferase (IU/mL)	0.03	0.86
Total Bilirubin (mg/dL)	-0.14	0.32
Direct Bilirubin (mg/dL)	-0.25	0.08
Albumin (g/dL)	0.14	0.32
Blood Glucose Level (mg/dL)	0.07	0.64
Alpha-fetoprotein (ng/dL)	-0.10	0.47
Prothrombin Time %	-0.08	0.58
Thyroid Stimulating Hormone (mIU/L)	0.24	0.09
Creatinine (mg/dL)	-0.05	0.71
Hemoglobin (g/L)	-0.25	0.08
BMI (kg/m^2)	-.571	0.00
Viral Load at 12 Weeks (EVR)	-.653	0.00
Viral Load at Baseline	-0.25	0.09

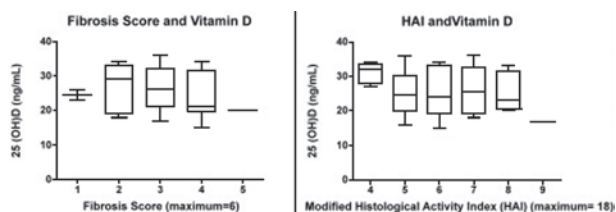*Wilcoxon-Signed-Rank test at a level of significance 0.05.

Fig. 2. — Correlation of 25 (OH)D Serum Concentrations with Fibrosis (A) and Liver Immunohistology (B) According to Modified Knodell's (Ishak) Fibrotic Score.

Correlation of variables with EOT and vitamin D serum levels

Using Spearman correlation, AST, BMI and EVR (early virological response at week 12) showed significant negative correlation to serum 25 (OH)D baseline levels (p < 0.05). Gender was neither significantly associated with EOT nor with 25 (OH)D deficiency. Using univariate linear regression, EVR was significantly associated with vitamin D levels, BMI and with viral load at baseline (p < 0.05).

To assess the association of different factors with EOT, variables before treatment were compared for responders versus non-responders using Mann-Whitney for continuous variables and Pearson's Chi-square for categorical variables (Table 3). AST, BMI, vitamin D levels and viral load at 12 weeks were significantly associated with response to therapy.

Discussion

Vitamin D has been well known for its critical role in calcium homeostasis and bone re-mineralization. However, its immunomodulatory role has been recently introduced. Experimental findings spotted the critical role of vitamin D and its receptor, in the control of T-cell antigen receptor signaling and activation of human T cells, providing a strong rationale for the use of vitamin D in chronic persistent infections (26).

It has been recognized that, vitamin D deficiency is common among patients with chronic liver disease, and this includes those suffering from chronic hepatitis C infection (27). HCV sustained virological response after PEG-IFN therapy has been found to be associated with 25 (OH)D serum levels. Low vitamin D levels seemed to predispose the patients failed to respond to the therapy (27-28). However, in HCV-HIV co-infected patients no correlation was found between vitamin D levels and response to IFN-based therapy (29). Based on the relationship between viral response rates and baseline vitamin D serum level, Bitetto *et al*., concluded that, vitamin D plays an important role in early HCV decline after antiviral treatment (21).

In the present study, serum 25 (OH)D levels in HCV genotype 4 infected patients were analyzed and correlations between 25 (OH)D levels, chronic hepatitis C features, and HCV virologic response to IFN therapy were constructed.

It was shown that, the biochemical profile of genotype 4 HCV infected Egyptian patients is characterized by lower-than-normal serum 25 (OH)D levels. This may be

Table 3. — **clinical and demographic characteristics of the studied population (n = 50). patients' characteristics before treatment are divided according to response at EOT. categorical variables are presented as number of patients (percentage) and continuous variables are presented as medians (range)**

Parameter	Responders (N = 32)	Non Responders (N = 18)	P*
Male gender	20 (63%)	14 (78%)	NS**
Age (years)	40 (20-52)	40 (28-54)	NS***
Aspartate Aminotransferase (IU/mL)	50 (22-95)	69 (42-105)	P < 0.05***
Alanine Aminotransferase (IU/mL)	57 (34-87)	57(35-87)	NS***
Total Bilirubin (mg/dL)	0.90 (0.50-2.50)	1.0 (0.6-2.0)	NS***
Direct Bilirubin (mg/dL)	0.20 (0.10-0.90)	0.20 (0.10-0.80)	NS***
Albumin (g/dL)	3.85 (3.40-5.00)	3.60 (3.00-4.20)	NS***
Blood Glucose Level (mg/dL)	100 (70-153)	90 (80-150)	NS***
Alpha-fetoprotein (ng/dL)	8.50 (3.00-25)	9.00 (3.50-20)	NS***
Prothrombin Time %	93 % (80-105%)	90% (80-100%)	NS***
Thyroid Stimulating Hormone (mIU/L)	3.75 (2.00-6.00)	3.40 (2.00-4.60)	NS***
Creatinine (mg/dL)	0.95 (0.60-9.00)	1.00 (0.70-1.20)	NS***
Vitamin D (ng/mL)	30 (23-36)	19 (15-21)	P < 0.05***
Hemoglobin (g/L)	12 (9.00-15.00)	13 (10-15)	NS***
BMI (kg/m²)	19 (18-41)	36 (19-41)	P < 0.05***
Viral Load at 12 Weeks (EVR) × 10³	0.985 (0-65)	270 (3.34-7300)	P < 0.05***
Viral Load at Baseline × 10³	134 (0.30-7500)	405 (5.60-8400)	NS***
Fibrosis + (1-2)	7 (22%)	2 (11%)s	NS**
Fibrosis Score (3-6)	25 (78%)	16 (89%)	NS**
HAI* (4-7)	29 (91%)	15 (83%)	NS**
HAI (8-9)	3 (9%)	3 (17%)	NS**

*Significant level at P < 0.05.
**Pearson Chi-square test for categorical variables and
***Manns-Whitney test for continuous variables (Level of Significance = 0.05).
+Fibrosis score and HIA (The Modified Histological Activity Index) are graded according to Modified Knodell's (Ishak) fibrotic Score (24,25).
NS: None significant.

attributed to inadequate sun exposure and/or to a low supply of vitamin D from the diet. It is worth mentioning that calcium level was normal in all recruited subjects. Vitamin D level was found to influence the achievement of viral clearance after antiviral therapy in patients with chronic HCV infection. Patients with sufficient vitamin D levels (> 30 ng/mL) achieved viral clearance at the end of treatment (48 weeks) while those with vitamin D levels below 20 ng/mL were associated with failure to respond to therapy. This has been previously established for HCV genotype 1 (27).

By comparing the whole biochemical profile i.e., vitamin D, Hb levels, as well as BMI, for all the subjects at baseline and at EOT, it was found that these parameters significantly decreased. When it comes to the different liver function tests, it is known that, patients with chronic liver disease are known to have elevated levels of liver functions (AST and ALT), and eradicating HCV is expected to decrease their levels. Even though ALT level is more specific for liver function than AST level, and

although both AST and ALT decreased by the end of treatment, only AST level significantly declined by the end of treatment.

The present study showed a significant decline in circulating vitamin D post-treatment. This decline could be attributed to the extensive consumption of the internal vitamin D in inhibiting the viral production where, vitamin D is known to have a direct inhibitory effect on viral production. This inhibition may be attributed to empowering the innate immune response, where vitamin D up-regulates the expression of IFN therapy, the immediate cellular response to viral infection (30).

The anti-viral effects of vitamin D could be explained by cathelicidin (in the form of LL-37), human beta defensin, and through the release of reactive oxygen. (31) Cathelicidins are a family of proteins with a C-terminal cationic anti-microbial domain activated by cleavage from the N-terminal cathelin domain (32). LL-37's anti-bacterial effect is linked to its ability to disrupt bacterial membranes through electrostatic interactions. It was

found that, hepatitis C replicon replication reduction in human hepatoma cells may be mediated by vitamin D induced oxidative stress (33).

Liver fibrosis was previously found to correlate to vitamin D levels in HCV infected patients (27). However, in our current study, vitamin D levels were not significantly different among patients with different fibrosis scores and liver fibrosis scores did not correlate with EOT. Nimer *et al.*, showed that, fibrosis alone is not the key to understanding the impact of chronic hepatitis C on vitamin D level (34).

In addition to the significance of vitamin D levels in relation to end of treatment, AST, BMI and EVR were significant predictors of EOT. High BMI was previously found to be a predictor of noresponse (35). However, it was later shown that, adjusting ribavarin levels to body weight made BMI a non-significant predictor of response (36).

HCV represents a huge burden in Egypt on both the health and economical sectors. According to the 2008 Egypt Demographic and Health Survey (2008 EDHS) (37), about 15% of the population is infected with the virus while 10% have active infection. According to amedia statement of the Egyptian Ministry of Health in 2010, the cost of HCV treatment per person in Egypt is about $3000 and the total annual expenditure for HCV treatment in Egypt is about 900 million Egyptian pounds (about $158 millions). (Public Statement) If an intervention as simple as vitamin D supplementation would help improve the outcomes of interferontherapy, this would lift a huge burden. Further studies need to confirm the suitability of supplementing patients with vitamin D along with the standard therapy to enhance response.

It is worth mentioning, that predictors for treatment responses to HCV i.e., IL28B SNP, and the type of IL28B SNP were not adopted in this study for several reasons. First, the study was totally and completely self-funded by the authors which was on obstacle against using further investigations. Most importantly that, the authors were aiming in this study is to come out with a simple, practical and economical method that can be used in the Egyptian settings to establish a relationship between the HCV treatment and the response, where vitamin D was proven to be able to provide this in a third world country like Egypt.

Conclusion

The present study suggests a possible role for the serum vitamin D level in predicting the outcome of antiviral therapy in HCV infected patients with genotype 4. Vitamin D deficiency predicts an unfavorable response to antiviral treatment of HCV.

Our current study lacks monitoring whether vitamin D supplementation would enhance virological clearance by IFN, which has been previously suggested (38,39), and further investigations are encouraged to detect the effect of this supplementation on HCV patients with genotype 4 and on the probability of achieving a SVR following antiviral treatment.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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