JOURNAL OF INTERFERON & CYTOKINE RESEARCH
Volume 35, Number 1, 2015

DOI: 10.1089/jir.2014.0060

Impact of Vitamin D Supplementation on Sustained Virological Response in Chronic Hepatitis C Genotype 4 Patients Treated by Pegylated Interferon/Ribavirin

Gamal Esmat,[1] Maissa El Raziky,[1] Aisha Elsharkawy,[1] Dina Sabry,[2] Mohamed Hassany,[3] Amal Ahmed,[4] Noha Assem,[5] Mohamad El Kassas,[3] and Wahid Doss[3]

The current standard of care therapy (SOC) for chronic HCV is pegylated interferon/ribavirin (Peg-IFN/RBV). Many reports showed the possible role of vitamin D supplementation in augmenting the response to SOC. The aim of this study was to assess the role of vitamin D supplementation on the response to treatment in chronic HCV genotype 4 patients. One hundred and one chronic HCV patients were classified into two groups (Group 1): 51 patients received the SOC therapy consisting of Peg-interferon alfa-2b plus ribavirin, (Group 2): 50 patients received the SOC therapy + vitamin D3 (Cholecalciferol) in a dose of 15,000 IU/week during the treatment course. Vitamin D deficiency was found in 95% of patients. No correlation was found between vitamin D levels and stage of fibrosis in the whole population. Vitamin D supplementation had no positive impact on treatment outcome where sustained virological response (SVR) was achieved in 51.2% in group 2 and 71.4% in group 1 by per-protocol analysis and in 44% in group 2 and in 68.6% in group 1 by intention to treat analysis (*P* value 0.22 and 0.220 respectively). Despite its role in other genotypes, vitamin D supplementation has no significant impact on SVR in HCV Genotype 4 patient. No correlation was found between vitamin D levels and stage of liver fibrosis.

Introduction

The combination of pegylated interferon alfa (Peg-IFN) plus ribavirin (Peg-IFN/RBV) for 48 weeks is the recommended treatment for patients with HCV genotype 4. The sustained virological response (SVR) approaches 50% based on various retrospective and prospective trials (Alfaleh and others 2004; Kamal and others 2005). Furthermore, there is a large burden on both the hospital and the patient because of the frequent hospital visits and prolonged course of treatment (Llovet and others 2003). Various options have been considered in attempting to improve the virological response rates, including extended treatment duration, higher dose Peg-IFN/RBV therapy, and addition of other drugs (Abu-Mouch and others 2011).

A large number of studies have examined the relationship between the vitamin D status of patients with chronic hepatitis C and disease outcome (Gutierrez and others 2011; Cholongitas and others 2012; Kitson and Roberts 2012). The majority found that HCV-positive patients have depressed 25(OH) D levels. (Petta and others 2010) Several association studies demonstrated that vitamin D levels inversely correlate with the stage of liver fibrosis and that there was a positive correlation between 25(OH) D levels and the likelihood of achieving SVR (Gutierrez and others 2011; Cholongitas and others 2012).

A retrospective review of patients in Italy who were treated with Peg-IFN/RBV for recurrent HCV post-transplantation found that concurrent treatment with vitamin D (for bone disease) resulted in significantly higher SVR rate (Bitetto and others 2011). In addition, two randomized clinical trials showed that vitamin D supplementation (2,000 IU/day) significantly enhanced the SVR rates of treatment-naïve patients infected with HCV genotype 1 (Abu-Mouch and others 2011; Nimer and Mouch, 2012).

As previous studies were conducted in a population where genotype 1 was more prevalent, it would be pertinent to examine whether the same effectiveness of vitamin D can be replicated in HCV genotype 4 (HCV4) patients.

Even with the appearance of the new emerging antiviral drugs, Peg-IFN/RBV may remain the standard of care (SOC) for the next few years especially with the expected high costs and the limited number of patients who will be eligible for the new treatment. So every effort is made to improve the performance of the SOC.

Departments of [1]Endemic Medicine and Hepatology and [2]Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt.
Departments of [3]Tropical Medicine and [4]Biochemistry, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.
[5]Department of Community Medicine, Cairo University, Cairo, Egypt.

Moreover, since approximately 20% of the 175 million global cases of HCV are caused by HCV genotype 4 (El-Zayadi and others 2005), effective individually tailored treatment approaches are crucial.

The aim of this study is to assess the role of vitamin D supplementation on the response to treatment in chronic HCV 4 patients and its possible relation to the stage of hepatic fibrosis.

Patients and Methods

Subjects

The study was conducted on 101 chronic HCV4 patients recruited from the hepatology outpatient clinic in the National Hepatology and Tropical Medicine Research Institute, Cairo. The patients were categorized according to treatment received into two main groups:

Group 1 (Control Group): received the standard of care therapy (SOC) consisting of Peg-IFN/RBV (51 patients).

Group 2 (Cases Group): received the SOC therapy + vitamin D3 (50 patients) concomitant therapy.

Inclusion criteria: age 18–60 years old, chronic HCV infection genotype 4 for >6 months by detectable serum quantitative HCV-RNA, naïve to treatment, compensated liver disease with the following minimum hematologic and biochemical criteria (Hemoglobin ≥12 g/dL for males and ≥11 g/dL for females, WBC >3,500/mm^3; Granulocyte count >1,500/mm^3, Platelet count >75,000/mm^3, Albumin and thyroid function tests within normal limit, and ANA ≤1:80).

Ultrasound guided liver biopsy within 12 months prior to study entry, using a semiautomatic true-cut needle (16G). Liver biopsy was fixed in formalin and embedded in paraffin and all biopsy specimens were analyzed by an experienced pathologist. All biopsy specimens were at least 15 mm lengths and contain 6 portal tracts. Liver fibrosis staging was evaluated according to the METAVIR scoring system and those confirming a histological diagnosis consistent with HCV and fibrosis score ≥F1 according to METAVIR score were included (Bedossa and Poynard 1996).

Patients with other liver diseases, decompensated liver cirrhosis, hepatocellular carcinoma, liver biopsy contraindication, those who were not fit for combined interferon and ribavirin treatment due to persistant hematological abnormalities, those who are receiving medications known to affect vitamin D3 level or metabolism (calcium, vitamin D supplementation, estrogen, alendronate, isoniazid, thiazide diuretics, long-term antacids, calcium channel blockers, cholestyramine, anticonvulsants, and orlistat), those with clinically evident osteomalacia (waddling gait, bone pain, and pathological fractures), those with renal diseases or parathyroid diseases, and those with BMI >35 were excluded.

The study was conducted according to the principles of the Declaration of Helsinki. Institutional Review Board (IRB) study approval was obtained through the (NHTMRI) IRB office prior to commencement of the study and signed informed consent was obtained from all study patients at the point of recruitment and before randomization.

Study design

This was a prospective, randomized controlled, open-labeled, two group assignment study.

Sample size

This study had a power of 80% to show that the response rate for 48 weeks treatment in the triple therapy arm (Peg-INF/RBV combination with vitamin D) was greater than the response rate for active control (48 weeks treatment in the Peg-INF/RBV combination). This assumed that the response rates for the active control and the new treatment arm were precisely unequal to (80%), that a difference of 20% or more is important, that the sample size in the two groups was 50 each, and that alpha (one-tailed) was set at 0.05. The sample size also took into account a 5% drop out rate from either arm. Here, the null hypothesis was that the response rate for the triple therapy arm was 20% higher than the response rate for active control and the study had a power of 80% to reject the null hypothesis. Equivalently, the likelihood was 80% that the 95% confidence interval for the difference in response rates excluded a 20% difference in favor of the active control.

Randomization

All patients, after screening, meeting study criteria, and signing the informed consent were randomized by using concealed previously labeled envelops in a 1:1 ratio.

Study procedure

Patients were subjected to thorough history taking, clinical examination and pretreatment laboratory work-up. Quantitative reverse transcription–polymerase chain reaction (PCR) for detection of HCV-RNA (RT-PCR) using Abbott Real-Time HCV kit, with a detection limit of 12 IU/mL was performed.

Vitamin D assay was performed at week 0 only for group 1 because we had no ethical justification to take extra samples from this control group and it was expected to remain low because this group didn't receive supplementation. For group 2 patients, it was measured at weeks 0, 6, 12, 24, and 48. Human 25-hydroxyvitamin D [25-(OH) D]assay (Glory Science): The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of [25-(OH) D] in samples.(Heijboer and others 2012). Assay range: The range of the kit is 0.5–150 ng/mL. Vitamin D level is considered deficient <20 ng/mL, insufficient 20–30 ng/mL, and normal >30 ng/mL (Hollis 2008).

Study medications

Peg-interferon alfa-2b (Peg-Intron-MSD®)–in a dose of 1.5 μg/kg subcutaneous injection once/week and ribavirin (Rebetol, MSD®) (SOC); dose determined by patient weight [<75 kg = 1,000 mg/day; ≥75 kg = 1,200 mg/day in two separate oral doses after meals in the morning and at night] for 48 weeks.

Vitamin D3 (Cholecalciferol) (Vidrop, MUP) in a dose of 15,000 IU/week (5.3 mL), given as oral solution with juice once weekly for the course of treatment duration.

Treatment response follow-up (efficacy assessment)

Patients with complete early virological response (EVR) or partial EVR at week 12 continued treatment until week 24 (Ferenci and others 2005).

Table 1. Baseline Characteristics for the Studied Population

	Group 1 N = *51 (mean ± SD, %)*	*Group 2* N = *50 (mean ± SD, %)*	P *value*
Age (years)	39.69 ± 8.98	39.74 ± 9.76	0.973
BMI (kg/m^2)	26.73 ± 3.05	26.01 ± 3.72	0.375
Male	76.5%	74%	0.774
AST (IU/L)	55.57 ± 32.12	60.12 ± 27.9	0.125
ALT (IU/L)	69.39 ± 38.61	71.88 ± 36.73	0.59
T.Bilirubin (mg/dL)	0.78 ± 0.32	0.66 ± 0.26	0.068
AFP (ng/mL)	8.34 ± 21.91	10.06 ± 14.48	0.023*
HCV RNA (IU/m)	552937 ± 677729	1598680 ± 3146656	0.132
Serum [25(OH)D] (ng/mL)	5.54 ± 4.63	11.02 ± 15.97	0.029*

*P value ≤ 0.05.

Patients with undetectable HCV viremia at week 24 continued their treatment till 48 weeks. Patients with end of treatment response entered a follow-up period until 72 weeks (Ghany and others 2009).

Patients who had negative HCV RNA at week 72 were considered to achieve sustained virologic response (SVR) (Sarrazin and others 2010).

Every effort was done to ensure that compliance is maximized and that patients continued to take the allocated treatments. Good compliance is defined as the completion of 80% of the prescribed treatment doses (McHutchison and others 2002). Yet, 7 patients were not regular during follow-up from group 2 and 2 patients from group 1. None of the missed patients had stopped the treatment due to adverse events, 5 of the 9 patients stopped treatment due to social problems, 2 refused to continue follow-up after treatment completion, and 2 had completed their treatment outside our center. In the rest of patients, there were no observed side effects.

Statistical analysis

Analysis of data was performed using SPSS 17 (Statistical Package for Scientific Studies) for Windows. Description of quantitative variables was in the form of mean, standard deviation (SD), median, 25th and 75th percentiles, minimum and maximum. Description of qualitative variables was in the form of numbers (No.) and percents (%). The results indicated that data were not normally distributed so nonparametric tests were used for comparisons. Comparison between quantitative variables was carried out by Mann–Whitney U. Comparison between qualitative variables was carried out by Chi-Square test (χ^2). Fisher exact test was used instead of Chi-square test when one expected cell or more were ≤ 5. Binary correlation was carried out by Spearman correlation test. Results were expressed in the form of correlation coefficient (R) and P values, which are significant when P value ≤ 0.05.

Results

There were no significant differences between the two groups in terms of age, sex, BMI, and baseline laboratory values except AFP being higher in group 2 patients and the baseline serum [25(OH) D] level being lower in group 1 with P value < 0.05 as shown in (Table 1). All patients in group 1 had severe baseline vitamin D deficiency < 20 ng/mL, regarding group 2 patients, 90% had severe vitamin D deficiency, 2% had insufficient level 20–30 ng/mL, and 8% had normal vitamin D level > 30 ng/mL.

No significant difference was observed between [25(OH) D] levels in different fibrosis stages with $P = 0.255$ as shown in (Fig. 1), also no correlation between [25(OH) D] levels and fibrosis stage at baseline assessment with ''R'' value (0.113) and P value (0.259).

Mean [25(OH) D] level in group 2 patients during the study period is shown in Table 2. Follow-up of [25(OH) D] assay showed at week 6 (44.8% had vitamin D deficiency; 22.5% had insufficient levels, and 32.7% had normal levels), at week 12 (89.6% had vitamin D deficiency; 10.4% had normal levels), and at weeks 24 and 48 of treatment 100% of the patients that continued their treatment had normal vitamin D levels as shown in Fig. 2.

The treatment outcome by per-protocol analysis is shown in (Fig. 3). All results in different treatment weeks are statistically not significant between both study groups.

The treatment outcome by intention to treat analysis was statistically not significant between both study groups (Fig. 4).

Discussion

The long duration of treatment, considerable side effects, and lack of satisfactory treatment results make it necessary to find complementary treatments with reasonable price especially with the expected very high costs for the new Directly Acting Antiviral Drugs (Esmat and others 2012).

Many studies showed reasonable results of the possible role of vitamin D supplementation in augmenting the

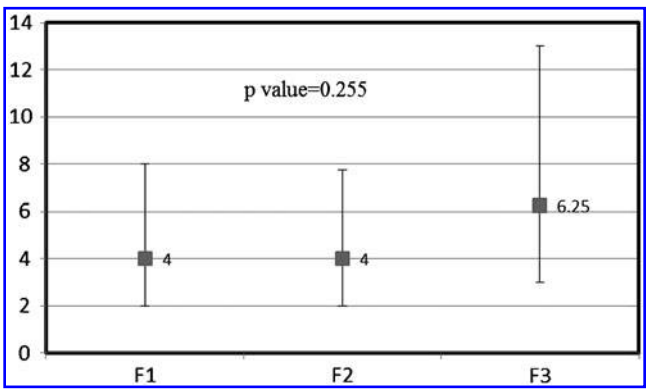

FIG. 1. Median baseline 25 (OH) D levels at different fibrosis stages.

Table 2. Classification of Serum [25(OH) D] Levels in Group 2 Patients Throughout the Study Period

Treatment week	Deficiency < 20 ng/mL		Insufficiency 20–30 ng/mL		Normal > 30 ng/mL		Missed patients
	No.	%	No.	%	No.	%	
Week 6	22	44.8	11	22.5	16	32.7	1
Week 12	43	89.6	0	0	5	10.4	2
Week 24	0	0	0	0	39	100	11
Week 48	0	0	0	0	29	100	21

response to SOC therapy of HCV (Yano and others 2007; Abu-Mouch and others 2011).

In the current study 95% of patients showed marked deficiency in [25-(OH) D] levels. At baseline (week 0 of treatment), vitamin D deficiency was found in all group 1 patients and in 90% of group 2 patients. These data match with previously published trials that showed a remarkable vitamin D deficiency in their studied populations (Petta and others 2010; Abu-Mouch and others 2011; Terrier and others 2011; Ladero and others 2013).

Egypt has a problem of vitamin D deficiency, which seems to be on nationwide base and might be not directly related to HCV or other diseases; yet, recent published data suggest many possible contributing factors including dark skin color, dietary calcium deficiency, and inadequate sun exposure especially in females who remain fully covered for religious or social reasons (Meguid and others 2010; Hamza and others 2011; Amr and others 2012) After vitamin D supplementation in group 2, which was started from day 0 of treatment till the last dose of Peg IFN/RBV, vitamin D levels started to show an upward movement at week 6. Vitamin D level recurred to drop again at week 12 with re-increased number of deficiency groups reaching 89.6%. This may be attributed to the eagerness for tissue satisfaction that make it difficult to have high level of vitamin D in serum. Then, a final plateau rise in the level of vitamin D was obtained at weeks 24 and 48 when 100% of patients had normal vitamin D levels.

Variable regimens of vitamin D supplementation were used in similar trials to obtain satisfactory plasma levels,

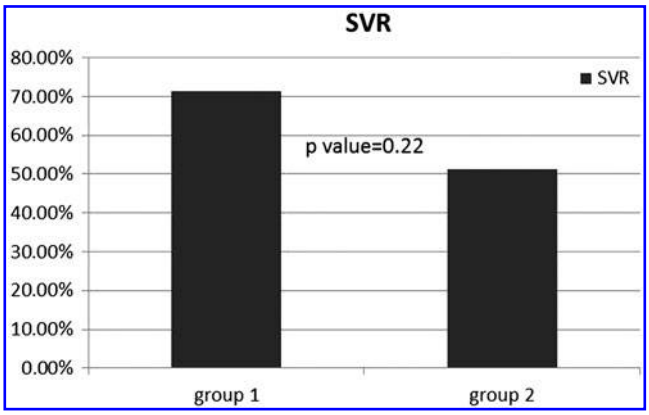

FIG. 3. Treatment outcomes by efficacy subset analysis (per-protocol analysis) at week 72 (SVR). SVR, sustained virological response.

Ladero and others (2013) studied a daily vitamin D supplementation in 41 chronic HCV patients treated with pegylated interferon in a dose of 2,000–4,000 IU/day, depending on the serum levels, and he was able to correct the mean vitamin D level from 18.39 ± 5.68 ng/mL at baseline levels to 59.66 ± 25.93 ng/mL at end of treatment period. In another trial on 72 chronic HCV patients, the patients went to a lead in phase (4 weeks prior to therapy) with daily dose of 2,000 IU/day and the dose was increased to reach the serum level of > 32 ng/mL. Then, the patients were maintained on 2,000 IU/day till the end of therapy (Abu-Mouch and others 2011).

In our study, no correlation was found between [25-(OH) D] levels and fibrosis stage at baseline assessment of the whole 101 studied population and no significant difference was observed. These data run parallel to that reported earlier (Ladero and others 2013; Kitson and others 2013), who found that baseline [25-(OH) D] level is not associated with fibrosis stage. But, another study (Petta and others 2010) declared strong negative association between serum [25-(OH) D] levels and fibrosis scores.

Although good correction of serum vitamin D level was achieved in this study, no positive impact was observed on treatment outcome in terms of SVR by per-protocol analysis, or by intention to treat analysis with no statistically

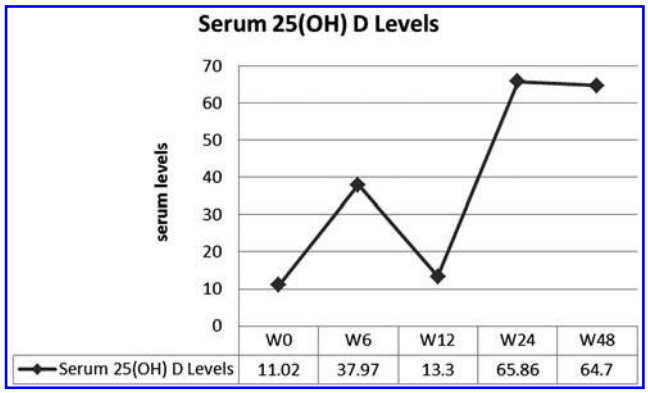

FIG. 2. Mean serums [25(OH) D] in group 2 through the study period (baseline and weeks 6, 12, 24, and 48).

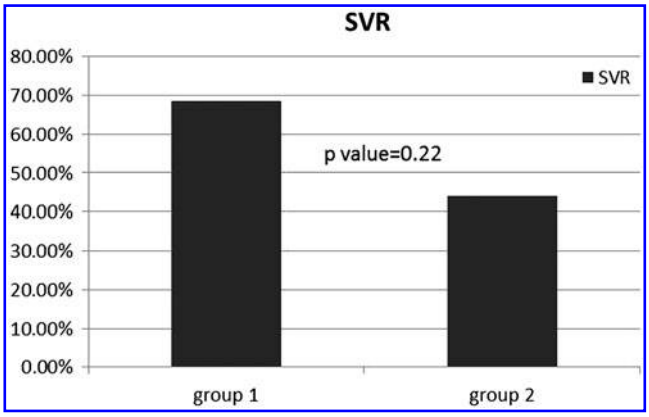

FIG. 4. Treatment outcomes by intention to treat analysis at week 72 (SVR).

significant difference between both groups. Discordant data described the relation between vitamin D level and response to SOC for HCV; our results were in agreement with those reported earlier (Kitson and others 2013), who studied 274 chronic HCV genotype 1 patients treated up to 48 weeks with pegylated interferon alfa-2a plus ribavirin, no association between [25-(OH) D] status and SVR was found. These data were strengthened by Ladero and others (2013) when 108 chronic HCV patients of variable genotypes were studied. Deficiency of vitamin D (< 20 ng/mL) and suboptimal levels (20–30 ng/mL) were detected in 36.1% and 40.9% of patients, respectively. Terrier and others 2011 studied 189 HIV-HCV co-infected patients, who received ≥ 80% of interferon (IFN) and no correlation was found between [25-(OH) D]levels and HCV sustained virologic response to IFN-based therapy.

On the other hand, our study runs in a different way rather than that reported earlier (Petta and others 2010). It was reported that [25-(OH) D] serum levels were significantly lower in CHC than in controls and by multivariate analysis, and that [25-(OH) D] levels were independently associated with no SVR and linked to decreased responsiveness on interferon (IFN)-based therapy.

The previous data (Petta and others 2010) were affirmed (Abu-Mouch and others 2011), 72 chronic HCV genotype 1 patients randomized into two groups with similar clinical characteristics were studied and concluded that adding vitamin D to conventional Peg-α-2b/ribavirin therapy for treatment-naïve patients with chronic HCV genotype 1 infection significantly improves the viral response.

Differences between our results and others regarding SVR may be attributed to several factors. First, the regimen of vitamin D supplementation, some went to a lead in phase 4 weeks prior to SOC therapy and continued till the end of therapy and others started administration of vitamin D from week 0. Second, our population is genotype 4, which is different from other genotypes with regard to the SVR, especially genotypes 2 and 3 who have better response than genotype 4.

Conclusion

Despite its role in other genotypes, vitamin D supplementation has no significant impact on SVR in HCV Genotype 4 patient. No correlation was found between vitamin D levels and stage of liver fibrosis

Author Disclosure statement

No competing financial interests exist.

References

Abu-Mouch S, Fireman Z, Jarchovsky J, Zeina AR, Assy N. 2011. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. World J Gastroenterol 17(47):5184–5190.

Alfaleh FZ, Hadad Q, Khuroo MS, Aljumah A, Algamedi A, Alashgar H, Al-Ahdal MN, Mayet I, Khan MQ, Kessie G. 2004. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C in Saudi patients commonly infected with genotype 4. Liver Int 24(6):568–574.

Amr N, Hamid A, Sheta M, Elsedfy H. 2012. Vitamin D status in healthy Egyptian adolescent girls. Georgian Med News (210):65–71.

Bedossa P, Poynard T. 1996. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 24(2):289–293.

Bitetto D, Fabris C, Fornasiere E, Pipan C, Fumolo E, Cussigh A, Bignulin S, Cmet S, Fontanini E, Falleti E, Martinella R, Pirisi M, Toniutto P. 2011. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. Transplant Int 24(1):43–50.

Cholongitas E, Theocharidou E, Goulis J, Tsochatzis E, Akriviadis E, Burroughs K. 2012. Review article: the extra-skeletal effects of vitamin D in chronic hepatitis C infection. Aliment Pharmacol Ther 35(6):634–646.

El-Zayadi AR, Attia M, Barakat EM, Badran HM, Hamdy H, El-Tawil A, El-Nakeeb A, Selim O, Saied A. 2005. Response of hepatitis C genotype-4 naïve patients to 24 weeks of Peg-interferon-alpha2b/ribavirin or induction-dose interferon-alpha2b/ribavirin/amantadine: a non-randomized controlled study. Am J Gastroenterol 100(11):2447–2452.

Esmat G, El Raziky M, El Kassas M, Hassany M, Gamil ME. 2012. The future for the treatment of genotype 4 chronic hepatitis C. Liver Int 32 (Suppl. 1):146–150.

Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxì A, Chaneac M, Reddy KR. 2005. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. J Hepatol 43(3):425–433.

Ghany MG, Strader DB, Thomas DL, Seeff LB. 2009. American Association for the Study of Liver Diseases AASLD PRACTICE GUIDELINES Diagnosis, Management, and Treatment of Hepatitis C: An Update. Hepatology 49(4):1335–1374.

Gutierrez JA, Parikh N, Branch AD. 2011. Classical and emerging roles of vitamin D in hepatitis C virus infection. Semin Liver Dis 31(4):387–398.

Hamza RT, Awwad KS, Ali MK, Hamed AI. 2011. Reduced serum concentrations of 25-hydroxy vitamin D in Egyptian patients with systemic lupus erythematosus: relation to disease activity. Med Sci Monitor 17(12):CR711–CR718.

Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. 2012. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. Clin Chem 58(3): 543–548.

Hollis BW. 2008. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr 88(2): 507S–510S.

Kamal SM, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SA, Saleh WA, Ismail A, Aziz AA, Madwar MA. 2005. Peginterferon {alpha}-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. Gut 54(6):858–866.

Kitson MT, Dore GJ, George J, Button P, McCaughan GW, Crawford DH, Sievert W, Weltman MD, Cheng WS, Roberts SK. 2013. Vitamin D status does not predict sustained virologic response or fibrosis stage in chronic hepatitis C genotype 1 infection. J Hepatol 58(3):467–472.

Kitson MT, Roberts SK. 2012. D-livering the message: the importance of vitamin D status in chronic liver disease. J Hepatol 57(4):897–909.

Ladero JM, Torrejón MJ, Sánchez-Pobre P, Suárez A, Cuenca F, de la Orden V, Devesa MJ, Rodrigo M, Estrada V, López-Alonso G, Agúndez JA. 2013. Vitamin D deficiency and vitamin D therapy in chronic hepatitis C. Ann Hepatol 12(2): 199–204.

Llovet JM, Burroughs A, Bruix J. 2003. Hepatocellular carcinoma. Lancet 362(9399):1907–1917.

McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. 2002. International Hepatitis Interventional Therapy Group. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 123(4):1061–1069.

Meguid NA, Hashish AF, Anwar M, Sidhom G. 2010. Reduced serum levels of 25-hydroxy and 1,25-dihydroxy vitamin D in Egyptian children with autism. J Altern Complement Med 16(6):641–645.

Nimer A, Mouch A. 2012. Vitamin D improves viral response in hepatitis C genotype 2–3 naïve patients. World J Gastroenterol 18(8):800–805.

Petta S, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, Cabibi D, Licata G, Porcasi R, Marchesini G, Craxí A. 2010. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology 51(4):1158–1167.

Sarrazin C, Shiffman ML, Hadziyannis SJ, Lin A, Colucci G, Ishida H, Zeuzem S. 2010. Definition of rapid virologic response with a highly sensitive real-time PCR-based HCV RNA assay in peginterferon alfa-2a plus ribavirin response-guided therapy. J Hepatol 52(6):832–838.

Terrier B, Carrat F, Geri G, Pol S, Piroth L, Halfon P, Poynard T, Souberbielle JC, Cacoub P. 2011. Low 25-OH vitamin D serum levels correlate with severe fibrosis in HIV-HCV co-infected patients with chronic hepatitis. J Hepatol 55(4):756–761.

Yano M, Ikeda M, Abe K, Dansako H, Ohkoshi S, Aoyagi Y, Kato N. 2007. Comprehensive analysis of the effects of ordinary nutrients on hepatitis C virus RNA replication in cell culture. Antimicrob Agents Chemother 51(6):2016–2027.

Address correspondence to:
Dr. Aisha Elsharkawy
Department of Endemic Medicine and Hepatology
Faculty of Medicine
Cairo University
Cairo 11562
Egypt

E mail: a_m_sharkawy@yahoo.com

Received 3 April 2014/Accepted 19 May 2014