Vitamin D Profile : Can it Affect the Response to Standard Hepatitis C Treatment in Egyptian Patients ?

Mohamed E Farghaly[1], Hala IM Husseein[1], Noha A Shaheen[1], Nagla A Abdel-Wahab[1], Heba F Pasha[2]

[1]*Tropical Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*
[2]*Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

Corresponding Author: ***Mohamed E. Farghaly***

E mail: rana4emo90@yahoo.com

Key words:vitamen D, HCV, treatment, Egyptian

Background and study aim : Vitamin D is a potent immunomodulator. It is reported to be related to the severity of fibrosis and responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C (CHC), so we aimed to evaluate if there is an association between vitamin D metabolism related genes and vitamin D level with the degree of liver damage and the response to treatment of CHC in our locality.

Patients and methods : Two hundred and forty five Egyptian patients (123 patients with sustained virological response and 122 patients with treatment failure) were included. They were subjected to routine investigation needed for treatment, in addition to estimation of 25-OH vitamin D level in serum by ELISA and CYP27B1-1260 gene polymorphism by PCR-RFLP method.

Results: We found that serum levels of vitamin D showed statistically significant increase in responders in comparison with non responders. The distribution of CYP27B1-1260 AC and CC genotypes were significantly presented in the non responders in comparison with the responders. CYP27B1-1260 AC and CC genotypes carriers had a high risk for treatment failure, (OR= 14.7, 95% CI= 2.3-20.1, P<0.001; OR = 20.4, 95% CI= 2.9-21.3, P<0.005 respectively). Serum levels of vitamin D showed statistically significant negative correlations with the activity and fibrosis of the liver in both responders and non responders. Also, there was negative correlation between vitamin D level and viral load in non responder patients (r= -.232, P=0.01). As regard the value of serum vitamin D level in discriminating responders from non responders; area under the ROC curve was 0.708 (95% CI 0.643-0.774). At a cutoff value of 19 ng/dL of serum vitamin D yielded sensitivity 79%, specificity 58%, positive predictive value (PPV) 65%, and negative predictive value (NPV) 73%.

Conclusion: Vitamin D serum level and CYB27B1 -1260 genotype could be used as a predictor to anti HCV treatment response in our locality.

INTRODUCTION

Egypt reports the highest prevalence of hepatitis C virus (HCV) world wide, ranging from 60% to more than 40% among regions and demographic group **[1]**. The recommended therapy for chronic hepatitis C, is pegylated interferon and ribavirin for 24 or 48 weeks **[2]**. Sustained virological response (SVR), defined as undetection of HCV RNA in patient's serum for 6 months after end of treatment, is ranging from 42.9% to 69% in patients with genotype 4 **[3-4]**. Several factors are associated with treatment failure including host and viral predictors such as body weight, ethnicity, liver histology, genotype, viral load and metabolic factors such as elevated fasting glucose **[5,6,7]**.

Vitamin D was initially identified as a calcium homeostatic hormone. Vitamin D is now known to have pleiotropic functions, dealing with both innate and adaptative immunity. Calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), which is expressed not only by intestine, bone and kidney but also on cell membranes of T lymphocytes, B lymphocytes, dendritic cells and macrophages responsiveness**.** Immunomodulatory actions of vitamin D are elicited through its direct action on T-cell antigen-presenting cell function **[8]**.

Moreover vitamin D improves insulin sensitivity, suppresses proinflammatory cytokines, increases anti-inflammatory cytokines, and improves CD4 T cell hyper-responsiveness **[9,10]**.

Vitamin D deficiency is very common among patients with chronic liver disease (92%), and at least one-third suffer from severe vitamin D deficiency (<12 ng/ml) **[11]**. Serum vitamin D deficiency and the CYP27B1-1260 promoter polymorphism are more prevalent in patients with chronic hepatitis C and related to more fibrosis, and that they are associated with a lower response rate to interferon-alfa based therapy in genotype 1 chronic hepatitis C (CHC) **[12]**.

Our aim is to evaluate the relationship between vitamin D metabolism-related genes and vitamin D level and the response to standard care of treatment for chronic hepatitis C infection in our locality, as genotype 4 is the predominant.

PATIENTS AND METHODS

This cross-sectional study was carried out in Tropical Medicine Department and Medical Biochemistry Department, Zagazig University Hospital, 245 Egyptian patients with compensated liver function out of 270 patients with chronic HCV, their ages ranged from 18 to 65 years, were enrolled in this study during the period from January 2013 to January 2014. All patients were receiving Peg interferon-á-2b (1.5 ug/kg per week) plus ribavirin (1000-1200 mg/d). the studied population included 123 patients with sustained virological response defined as undetectable HCV RNA at 24 weeks post treatment and another 122 patients with treatment failure," a non-responder is someone who does not have disappearance of the HCV RNA, does not ever have a 2-log drop in hepatitis C viral load at 12 weeks, and if HCV RNA was still detectable at week 24 in those patients in whom HCV RNA dropped more than 2 log at 12 week **[13]**. Both groups were matched for age, sex and body mass index**.**

Exclusion criteria :
The patients were excluded from the study if their WBCs less than 4000/mm^3, absolute neutrophil count of <1500 per mm^3, a platelet count of <90 000 per mm^3, hemoglobin level was abnormal, or if they had increased serum bilirubin more than 2 mg/dl **[14]**. Also patients with hepatitis B, auto immune hepatitis, metabolic liver disease, hepato-cellular carcinoma, renal failure and heart failure, decompansated chronic liver disease, or those who had any problem necessities stoppage or interruption of treatment were excluded **[15]**. In addition to those who are previously treated or those were receiving adjuvant medication with immunemodulatory effect.

An informed consent was taken from each patient. All patients were subjected to complete history taking, full clinical examination, ultrasonographic and histopathological evaluation according to Metavair score **[16]**.

Laboratory investigation :
All subjects were subjected to routine laboratory investigation including complete blood picture, liver function test and renal function test. Viral markers including hepatitis C virus antibodies (HCV Abs), hepatitis B surface antigen (HBsAg) and hepatitis B core antibodies (HBcAbs) were tested using ELISA. HCV RNA levels were determined by using the Real time PCR (Step One Real-time PCR System, Applied Biosystem), performed strictly in accordance with the manufacturer's instructions. Serum alpha-feto protein, thyroid hormone (triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH), anti nuclear antibodies (ANA Abs) and 25-OH vitamin D level in serum were measured using ELISA kits (kits provided by Biosource Europe S.A, Belguim).

Isolation of DNA :
Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol (QI Aamp Blood Kit; Qiagen GmbH, Hilden, Germany).

Genetic polymorphism detection of the CYP27B1 gene :
The -1260C>A polymorphism (rs10877012) of the CYP27B1 gene was analyzed by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method described by Jennings et al. **[17]**. The 1260 C was amplified in a single product using the primers forward 5-GTGTTCCCTAAGTGTTGTCTC-3 and reverse 5-GCTGACTCGGTCTCCTCTG-3. Fragments were amplified in 50µl reaction mixtures containing 10 µl genomic DNA, 30 µl one step PCR mixture (1 unit Taq polymerase, 10 mM KcL, 10 mM $(NH_4)_2$ SO_4, 20mM Tris Hcl (PH 8.75), 0.1% Triton X-100, 0.1 mg/ml BSA and 200 µm dTNPs) and 2 µl of each primer (BioBasic Inc., Ontario, Canada) and 8 µl DdH_2O. Reaction

conditions used with the thermal cycler (Biometra, Göttingem, Germany) were as follows: an initial incubation at 94ºC for 5 minutes followed by 30 cycles of incubation at 94ºC for 45 seconds, 58ºC for 45 seconds and 72ºC for 45 seconds with a final extension at 72ºC for 7 minutes. Subsequently, it was subjected to digestion with TfiI enzyme, which cleaved the A allele into two fragments of 195 and 103 bp.

Statistical analysis :

Data were analyzed with SPSS for version 15.0 (statistical package for the Social Science, Chicago, IL). Quantitative data were expressed as mean ± standard deviation (SD), data were analyzed by independent sample t and One Way Analysis Of Variance (ANOVA). While qualitative data were expressed as number and percentage and were analyzed by Chi square (X^2) test. Correlation was done using Pearson correlation test. The receiver operating characteristic (ROC) curve and 95% confidence interval (CI) was performed to determine cutoff values for serum level of Vitamin D. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined. Odds ratios (ORs) and confidence intervals (CI) were calculated. P-value was considered significant if <0.05 and highly significant if <0.001.

RESULTS

The study included 245 Egyptian patients (123 patients with sustained virological response and 122 patients with treatment failure), their clinical characteristics is shown in table (1). Serum levels of vitamin D showed statistically significant increase in responders in comparison with the non responders.

As regard distribution of of CYP27B1-1260 genotypes, AC and CC genotypes were significantly presented in the non responders in comparison with the responders. CYP27B1-1260 AC and CC genotypes carries had a high risk for treatment failure, (OR=14.7, 95% CI=2.3–20.1, P<0.001; OR= 20.4, 95% CI= 2.9-21.3, P<0.005 respectively).

Serum levels of vitamin D showed statistically significant decrease among those with advanced grading and staging, with a statistically significant negative correlation between vitamin D level and the activity and fibrosis of the liver in both responders and non responders.

Also, there was negative correlation between vitamin D level and viral load in non responder patients (r= -.232, P=0.01, data not shown). As regard the value of serum vitamin D level in discriminating responders from non responders; area under the ROC curve was 0.708 (95% CI 0.643-0.774), (Fig. 1). At a cutoff value of 19 ng/dl of serum vitamin D yielded sensitivity 79%, specificity 58%, positive predictive value (PPV) 65%, and negative predictive value (NPV) 73%.

Table (1) : Baseline patient's characteristics.

Parameter	Responders No=123	Non Responders No=122	T or X^2	P
Age (years)	40.08±9.5	41.37±10.5	0.992	0.322
Sex: Male Female	83 (67.4%) 40 (32.6%)	82 (67.2%) 40 (32.8%)	000	0.96
Viral load (Iu/ml)	598945.5±114	1632321±252	4.332	<0.001
HB (mg/dl)	14.15±1.1	13.93±1.4	2.29	0.13
WBCs/mm^3	6.55 ±1.47	5.13 ±1.32	7.103	0.14
Platelets/mm^3	187.08±49.32	170.54± 46.74	2.667	0.08
AST (Iu/ml)	59.75±34.80	66.29±35.00	1.452	0.15
ALT (Iu/ml)	66.66±35.52	80.58±48.35	2.541	0.12
Alkaline phosphatase (Iu/ml)	85.41±30.89	89.79±40.79	0.938	0.35
Albumin (g/l)	4.41±0.390	4.31±0.461	1.81	0.07
Creatinine (mg/dl)	0.81±0.15	0.87±0.18	1.52	0.13
Activity no (%) Grade 1 Grade 2 Grade 3	 46(37.4%) 62(50.4%) 15(12.2%)	 11(9.0%) 51(41.8%) 60(49.2%)	50.18	<0.001
Fibrosis no(%) Stage 1 Stage 2 Stage 3	 51(41.5%) 52(42.3%) 15(12.2%)	 12(9.8%) 32(26.2%) 78(64.0%)	71.11	<0.001

Table (2) : Vitamin D level in responders and non responders

Parameter	Responders No=123	Non Responders No=122	t	P
Vitamin D level (ng/ml)	30.26±13.89	20.38±10.80	6.151	<0.001

Table (3) : Distribution of CYP27B1-1260 genotypes among responders and non responders

CYP27B1-1260 genotypes	Responders No=123	Non Responders No=122	OR (95%)	P
AA No(%)	30(24.4%)	5(4.1%)		
AC No(%)	28(22.8%)	32(26.2%)	14.7(2.3-20.1)	<0.001
CC No(%)	65(52.8%)	85(69.7%)	20.4(2.9-21.3)	<0.001

Table (4) : Vitamin D level among different histopathological categories.

		Vitamin D Responders	F	P		Vitamin D Non responders	F	P
Activity (Grade)	Grade 1 N (46)	41.6±13.8*	39. 7	<0.001	Grade 1 N (11)	40.5±5.8*	29.1	<0.001
	Grade 2 N (62)	23.9±9.3			Grade 2 N (51)	20.1±10.5		
	Grade 3 N (15)	21.7±4.3			Grade 3 N (60)	17.3±7.8		
Fibrosis (Stage)	Stage 1 N (51)	38.9±14.1*	23.4	<0.001	Stage 1 N (12)	40.5±5.8*	28.4	<0.001
	Stage 2 |N (51)	24.7±10.9			Stage 2 N (32)	16.95±7.19		
	Stage 3 N (15)	21.7±4.3			Stage 3 N (78)	19.29±9.92		

Table (5) : Correlation between vitamin D level and histopathological findings.

Parameter	Responders N=123		Non responders N=122	
	r	p	r	P
Activity	-.581	<0.001	-.467(**)	<0.001
Fibrosis	-.501	<0.001	-.340(**)	<0.001

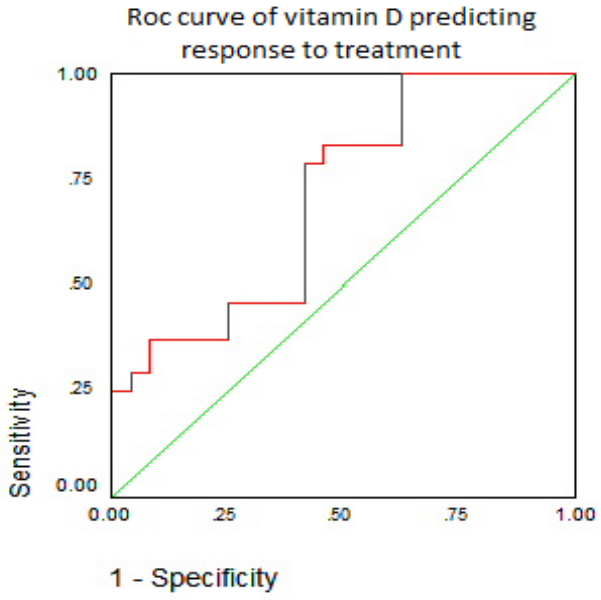

Fig. (1) : ROC curve of vitamin D as a predictor of response to treatment. At cut off level 19 ng/dl of vitamin D it has Sensitivity 79%, specificity 58%, PPV 65% and NPV 73%. Area under curve (AUC)= 0.708(0.643-0.774)

DISCUSSION

Hepatitis C virus genotype 4 (HCV-4) is the most common type of HCV in the Middle East and Africa, in particular Egypt, the response of HCV-4 to the standard regimen of treatment (pegylated interferon/ribavirin) lags behind other genotypes and has become the most resistant type to treat. The development of therapeutic strategies for all patients with HCV-4, have experienced a virological breakthrough **[18]**. Vitamin D is a potent immunomodulator that favour innate immunity and cell differentiation. Increased production of 1,25-dihydroxy vitamin D3 results in the synthesis of cathelicidin, a peptide capable of destroying many viral infectious agents as well as Mycobacterium tuberculosis **[19,20],** so thought that evaluation of vitamin D metabolism related genes and vitamin D level could help in predicting the degree of liver damage and the response to treatment of chronic hepatitis C in our locality.

In this study non responder patients showed statistically significant decrease in vitamin D levels in comparison with the levels of the responders. It was reported that there is an association between vitamin D status and outcome of antiviral therapy in patients with chronic HCV viral infection **[12]**. Also, Bitetto and his colleagues **[21]** found that vitamin D supplementation improved the response to antiviral treatment for recurrent HCV in liver transplant recipients. It had been shown the beneficial effect of vitamin D supplementation on the outcome in patients with chronic HCV genotype 2-3 infection **[22]**.

Current analysis of the genotypes distribution of CYP27B1-1260 among HCV responders and non responders demonstrated significant increase in AC and CC genotypes in non responders. Genotype CC of CYP27B1-1260 impairs the expression of the 1α-hydroxylase, which results in reduced concentrations of bioactive vitamin D **[23,24]**. Thus, one may speculate that the "poor-response" CYP27B1-1260 CC genotype may result in lower local concentrations of calcitriol in the HCV-infected liver, resulting in reduced responsiveness to IFN-α or impaired adaptive immune responses. Consistently, the CC genotype of CYP27B1 is associated with poor response to interferon-α-based treatment of chronic hepatitis C **[25]**.

The present study demonstrated that vitamin D levels significantly decreased among those with advanced grading and staging of the liver with a statistically significant negative correlation in both responders and non responders. Bioactive vitamin D is an important immune modulator, because T cells and macrophages depend on calcitriol in various conditions **[26,27,28]**. Importantly, 1α-hydroxylase is expressed in inflamed tissue and even in immune cells, where it serves as a local, inducible producer of calcitriol **[29]**, this explains why low serum vitamin D level to be related to necroinflammatory activity and progression of liver fibrosis in chronic HCV patients **[12]**. As persistent HCV infection modulates the balance between immune stimulatory and inhibitory cytokines which can prolong inflammation and lead to fibrosis and chronic liver diseases **[30]**.

The negative correlation between vitamin D and IL-23 and -17, at least in part, show how these cytokines might be involved with vitamin D in immune responses in HCV genotype IV-related liver disease and may explain how vitamin D deficiency plays a role in increasing liver fibrosis **[31]**. Another opinion highlighted that Vitamin D is metabolized by the liver and is converted to 1,25 dihydroxy vitamin D3, which is the active form of the vitamin**.** Those with chronic liver disease may have poor conversion from vitamin D3 or any of its other biologically active metabolites**.** Vitamin D deficiency is very common among patients with chronic liver disease [(92%), and at least one-third of them suffer from severe vitamin D deficiency (<12 ng/mL)**[11]**.

This study showed that there is negative correlation between vitamin D levels and viral load in non responders after treatment. Previous study had shown that vitamin D3 increases vitamin D receptors protein expression and inhibits viral replication in cell culture **[32]**. Also, vitamin D acts by improvement of insulin resistance or immune function by up regulation of toll-like receptors involved in the immune response in HCV-infected patients **[33]**.

As regard the value of serum vitamin D level in discriminating responders from non responders; we analyzed the receiving operating curve (ROC) and found that area under the ROC curve was 0.708 (95% CI 0.643-0.774). At a cutoff value of 19 ng/dL of serum vitamin D yielded sensitivity 79%, specificity 58%, positive predictive value (PPV) 65%, and negative predictive value (NPV) 73%. Future studies can combine vitamin D with

other predictors to improve its validity in prediction of treatment response. Vitamin D insufficiency (defined by a 25-hydroxyvitamin D [25(OH)D_3] serum concentration <20 ng/mL) has been proposed as a predictor of failure of treatment of chronic hepatitis C with PEG-IFN-α and ribavirin in others genotypes **[12]**. These findings may have important implications for the management of chronic hepatitis C, as vitamin D status is a potentially modifiable determinant of treatment outcome **[33]**. So, we concluded that vitamin D levels were decreased with the increase in disease severity. Vitamin D serum level concentration and CYB27B1 -1260 genotype could be used as a predictors to HCV treatment response in our locality.

REFERENCES

1. Lehman EM, Wilson ML. Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. *Journal of Viral Hepatitis* 2009; 16(9):650-658.
2. Hoofnagle JH, Seeff LB. Peginterferon and ribavirin therapy for chronic hepatitis C. *N Engl J Med* 2006; 355 : 2444-2451.
3. Alfaleh FZ, Hadad Q, Khuroo MS, Aljumah A, Algamedi A, Alashgar H, et al. Peginterferon a-2b plus ribavirin compared with interferon a-2b plus ribavirin for initial treatment of chronic hepatitis C in Saudi patients commonly infected with genotype 4. *Liver Int* 2004; 24 : 568-574.
4. Kamal SM, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SK, et al. Peginterferon alpha-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. *Gut* 2005; 54 : 858-866.
5. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.
6. Manns, MP, McHutchison, JG, Gordon, SC, Rustgi, VK, Shiffman, M, Reindollar, R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 2001; 358 : 958–965.
7. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J et al. Peg interferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; 361 : 580-593.
8. Sochorová K, Budinský V, Rozková D, Tobiasová Z, Dusilová-Sulková S, Spísek R, Bartůnková J. Paricalcitol (19-nor-1,25-dihydroxyvitamin D2) and calcitriol (1,25-dihydroxyvitamin D3) exert potent immunomodulatory effects on dendritic cells and inhibit induction of antigen-specific T cells. *Clin Immunol* 2009; 133 : 69-77.
9. Mahon BD, Wittke A, Weaver V, Cantorna MT The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. *J Cell Biochem* 2003; 89 : 922-932.
10. Alvarez JA and Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis . *Int J Endocrinol* 2010; 351-385.
11. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. *Dig Dis Sci.* 2010 ; 55(9) : 2624-2628.
12. Petta S, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* 2010; 51 : 1158-1167.
13. Stribling R, Sussman N, Vierling JM. Treatment of hepatitis C infection. *Gastroenterol Clin North Am* 2006; 35 : 463-86
14. Ghany MG, Strader DB, Thomas DL, Seeff LB; Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49 : 1335-74.
15. Patel K, Muir J , McHutchinson. Diagnosis and treatment of chronic hepatitis C infection. *BMJ* 2006; 332 : 1013-1017.
16. Crawford DH, Dore GJ, Sievert W, Cheng WS, Weltman M, McCaughan G et al. Early on-treatment viral load and baseline METAVIR score: improved prediction of sustained virological response in HCV genotype 1 patients. *Antivir Ther* 2012; 17(5) : 849-854.
17. Jennings CE, Owen CJ, Wilson V, Pearce SHS. A haplotype of the CYP27B1 promoter is associated with autoimmune Addison's disease but not with Graves' disease in a UK population. Journal of *Molecular Endocrinology* 2005; 34 : 859–863.
18. Esmat G, El Raziky M, El Kassas M, Hassany M, Gamil ME. The future for the treatment of genotype 4 chronic hepatitis C. *Liver Int* 2012; 321 : 146-150.
19. 19. DeLuca HF. Overview of general physiological features and functions of vitamin D. *Am J Clin Nutr* 2004; 80 : 1689-1696.

20. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol* 2005; 289 : 8-28.
21. Bitetto D, Fabris C, Fornasiere E, Pipan C, Fumolo E, Cussigh A et al. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. *Transpl Int* 2011; 24 : 43-50.
22. Southern P, El-Sayed P, Fenton L. influence of vitamin D supplementation on outcome in the treatment of chronic hepatitis C. *Gut* 2010;59: 41.
23. Clifton-Bligh RJ, Nguyen TV, Au A, Bullock M, Cameron I, Cumming R et al. Contribution of a common variant in the promoter of the 1-α-hydroxylase gene (CYP27B1) to fracture risk in the elderly. *Calcif Tissue Int* 2011; 88 : 109-116.
24. Lange CM, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J et al. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. *J Hepatol* 2011; 54 : 887-893.
25. Renn CN, Sanchez DJ, Ochoa MT, Legaspi AJ, Oh CK, Liu PT et al. TLR activation of Langerhans cell-like dendritic cells triggers an antiviral immune response. *J Immunol* 2006;177:298-305.
26. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006; 311 : 1770-1773.
27. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res* 2010; 20: 1352-1360.
28. von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Odum N, Geisler C. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. *Nat Immunol* 2010; 11 : 344-349.
29. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, Hewison M. Extra-renal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. *J Clin Endocrinol Metab*. 2001; 86 : 888-894.
30. Larrubia JR, Benito-Martínez S, Calvino M, Sanz-de-Villalobos E, Parra-Cid T. Role of chemokines and their receptors in viral persistence and liver damage during chronic hepatitis C virus infection. *World J Gastroenterol* 2008; 14 :7149-7159.
31. El Husseiny NM, Fahmy HM, Mohamed WA, Amin HH. Relationship between vitamin D and IL-23, IL-17 and macrophage chemoattractant protein-1 as markers of fibrosis in hepatitis C virus Egyptians. *World J Hepatol* 2012; 4:242-247.
32. Gutierrez JA, Jones KA, Fitzgerald RL. Vitamin D metabolites inhibit replication of the hepatitis C virus . *Hepatology* 2010; Supplement; A803.
33. Abu-Mouch S, Fireman Z, Jarchovsky J, Zeina AR, Assy N. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. *World J Gastro-enterol* 2011; 17 : 5184-5190.

Peer reviewer: Maysaa Saed, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.**Editor:Tarik I Zaher**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt