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Vitamin D and the response of hepatitis C virus to Interferon and Ribavirin in Egyptian patients.

Hanan M El-Tokhy[1]*, Doaa S.E. Zaky[1], Samiha A.E. Abd Rabo[1], Nagwa Abd-El-Ghaffar Mohammed[2], Samy Zaky El-Sayed[3], and Hala S. Kotb[1].

[1]Department of Internal Medicine, Faculty of Medicine for Girls Al-Azhar University.
[2]Clinical and Chemical Pathology, National Research Centre, Egypt.
[3]Tropical Medicine, Cairo, Egypt.

ABSTRACT

Vitamin D has an immunomodulatory action as it has a direct action on antigen-presenting T-cells. In chronic hepatitis C patients T-cell responses, including production of interferon- γ, are severely suppressed. Vitamin D deficiency may be associated with lack of response to antiviral therapy in chronic HCV patients. To assess the role of vitamin D in chronic hepatitis C patients and its relation to the response for treatment. 25-hydroxyvitamin D (25(OH) D) level was determined by electro-chemiluminescence binding assay in 60 patients with chronic HCV infection on combined pegylated-interferon/ribavirin therapy (Group I). They were subdivided according to their early viral response into responders (Group Ia) and non-responders (Group Ib). Twenty age and sex matched healthy individuals were recruited as controls (Group II). There was a highly statistically significant decrease of vitamin D in group I compared to group II, p value (<0.01). Also there was a highly statistical significant increase in vitamin D level in responders compared to non-responders. Serum concentration of 25-OH vitamin D is inversely related to viral load & early virologic response in combined Peg-IFN & RBV therapy in patients with HCV.

Keywords: Vitamin D, HCV.

Corresponding author

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INTRODUCTION

300 million persons worldwide are infected by chronic hepatitis C viral (HCV) infection which causes a major health problem. Egypt has the highest prevalence of the infection in the world, amounting to 14–20% [1].

One of the main causes of chronic liver disease is chronic hepatitis C (CHC) all over the world and its history varies from minimal changes, fibrosis, and cirrhosis with its complications, such as hepatocellular carcinoma [2].

The aim of treatment is to cure hepatitis C virus infection and prevent its liver complications, extra-hepatic manifestations and death [3].

Sustained viral response (SVR) is the endpoint of therapy and is defined by undetection of HCV RNA in the serum at 24 weeks (SVR24) after the end of therapy. A SVR corresponds to definitive cure of HCV infection [4].

Vitamin D is the most important hormone which regulates calcium phosphate homeostasis and mineral bone metabolism. The discovery that many tissues can express vitamin D receptor (VDR) has opened new ways of research related to the vitamin, and its different biological effects [5].

Vitamin D is considered biologically inactive and it is hydroxylated in the liver to 25-hydroxy vitamin D [25(OH)D] which is used for classification of the vitamin [6]. It is converted in the kidney to 1, 25-dihydroxy vitamin D [1,25$(OH)_2$D] by 1-alpha-hydroxylase, but this conversion can occur in tissues outside the kidnys as the liver. 1,25$(OH)_2$D then binds to the vitamin D receptor (VDR) [7]. The immue modulator effect of vitamin D is exerted through its direct action on the antigen-presenting cell. T helper cell type 1 (TH1) response is intensified when vitamin D is low or when signals through VDR are weak favoring a pro-inflammatory response which may decrease interferon and insulin signaling, causing decrease in the viral response [8].

We designed this work to study the role of vitamin D in chronic HCV infection in a sample of Egyptian patients and its relation to interferon and ribavirin response rate.

PATIENTS AND METHODS

The study consisted of 60 individuals with HCV infection as well as 20 age and sex matched healthy controls. The patients were selected from outpatient clinic of Al-Fatemeia Cairo hospital after obtaining oral consent to be participated in the study. To be eligible for the study, patients had to be older than 18 years, be chronically infected with HCV, not taking any vitamin or mineral supplementation. The patients received combined pegylated interferon and ribavirin therapy. Patients with diabetes mellitus, decompensated liver disease, co-infection with HBV, malignancy, autoimmune diseases, pregnancy, breast feeding, clinically significant retinal abnormalities, or severe pre-existing psychiatric conditions were excluded from the study. Approval of the ethical committee of faculty of medicine, Al-Azhar University was also obtained. Also written consent was obtained from all subjects in the study.

The patients with chronic HCV infection were subdivided according to their early virologic response (EVR) after 12 weeks from the starting therapy into two groups: **Group Ia:** Responders to combined PEG-IFN and RBV therapy (51 patients). They were 19 females (37.25%) and 32 males (62.75%), their age ranged from 21 to 58 years, with mean age± SD (40.549 ± 10.076) years. **Group Ib:** Non responders to combined PEG-IFN and RBV therapy (9 patients). They were 2 females (22.22%) and 7 males (77.78%), their age ranged from 21 to 60 years, with mean age± SD (41.556 ± 11.980) years. **The control (Group II)**: they were 10 females (50%) and 10 males (50 %), their age ranged from 25 to 46 years, with mean age± SD (36.350±6.218) years.

Patients and controls included in the study underwent a standard procedure of detailed history taking and a complete physical examination including calculation of BMI.

Laboratory investigations

- Blood picture.
- Fasting blood glucose.
- Liver function tests (serum bilirubin, serum albumin, prothrombin time (PT) and concentration, INR, ALT and AST).
- Kidney function tests (urea, creatinine).
- HCV antibodies, HbsAg and alpha fetoprotein.
- RT-PCR.
- Anti-DNA.
- 25-OH vitamin D.

Five ml of fasting (6-8 hours) venous blood samples were taken from each subject participating in the study and divided into 2 parts: The 1st part was 2ml of blood and was added to a tube containing EDTA for blood picture determination. The 2nd part (3ml) was left to clot and centrifuged at 3000 x *g* for 10 minutes and the separated serum was then stored at-20°C for determination of liver & kidney function tests, hepatitis markers, alpha fetoprotein, RT-PCR, anti-DNA and 25-OH vitamin D. While fasting blood glucose was determined immediately after serum separation.

Blood picture was performed on Coulter Counter T890 (Coulter Counter, Harpenden, UK). Liver functions, fasting blood glucose, kidney functions were determined calorimetrically on Hitachi 912 auto analyzer (Hitachi 912, Hitachi, Japan). HCV Antibodies were detected using a third generation enzyme linked immunosorbent assay (ELISA) technique [9]. HbsAg was determined using non–competitive sandwich assay based on (ELISA) technique [10].. Alpha fetoprotein was determined using ELISA kit [11]. The RT-PCR test was done by Automated Amplicor System (version 2.0) supplied by Roche Diagnostic's detection *(Roche Diagnostics, Branchburg, New Jersey, USA)*. The real time PCR test which was done by Stratagene Mx3000P instrument (Stratagene Mx3000PQPCR Systems, La Jolla, CA 92037, USA) [12]. Anti-ANA were determined using indirect immuno fluorescence assay on mouth kidney and stomach slides and crithidialuciliae slides (Immco Diagnostics Inc, 640 Ellicott street, New York, USA). The slides were analyzed with Nikon epi fluorescence microscope (Nikon Inc., USA).

Vitamin D (25(OH) vitamin D) was determined using electro-chemiluminescence binding assay performed on a Cobas e411 immunoassay analyzer (Roche Diagnostics GmbH). Vitamin D deficiency was defined as ≤20 ng/ml while vitamin D insufficiency is recognized as 21–29 ng/ml. The recommended level for vitamin D is ≥30 ng/ml [13].

Imaging: Abdominal ultrasound was done.

***Liver biopsy*:** An ultrasonographic-guided liver biopsy was performed for all patients using a true-cut needle. Biopsy specimens were fixed in formalin, embedded in paraffin, and finally the slides obtained were stained by hematoxylin and eosin (H&E), perls, and orcein for routine histopathological evaluation.

***Statistical analysis*:** IBM SPSS Statistics (version 21.0, 2012; IBM Corp., USA) was used for data analysis. Data were expressed as mean ± SD for quantitative parametric measurements in addition to median percentiles for quantitative nonparametric measurements, and both number and percentage for categorized data. The following tests were carried out: (i) comparison between two independent mean groups for parametric data using Student's t-test; (ii) Pearson's correlation test to study the possible association between both the variables among each group for parametric data. The P of error of 0.05 was considered significant, whereas that of 0.01 and 0.001were considered highly significant.

RESULTS

The results and data were collected and analyzed in tables [1-3] and Figures [1-7]:

As regard the result of personal data of the studied groups:

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Table (1): Comparison between group I & group II as regard demographic & laboratory data (Mean ± SD).

	Group I No (60)			Group II No (20)			T-test	
	Mean	±	SD	Mean	±	SD	T	P-value
Age (years)	40.700	±	10.278	36.350	±	6.218	1.783	0.079
Weight (Kg)	77.467	±	11.879	79.900	±	14.312	-0.753	0.454
Height (m)	1.681	±	0.078	1.710	±	0.081	-1.441	0.154
BMI(Kg/m^2)	27.185	±	3.811	26.562	±	3.188	0.657	0.513
Glucose (mg/dl)	90.667	±	10.478	88.000	±	6.391	1.071	0.287
Albumin (g/dl)	4.177	±	0.438	4.125	±	0.388	0.474	0.637
AST (IU/l)	54.850	±	29.921	23.650	±	5.224	7.731	0.000*
ALT (IU/l)	62.350	±	39.232	25.350	±	6.532	7.019	0.000*
Total Bilirubin (mg/dl)	0.715	±	0.270	0.741	±	0.126	-0.579	0.565
Indirect Bilirubin (mg/dl)	0.496	±	0.242	0.520	±	0.115	-0.584	0.561
ALP (IU/l)	85.433	±	28.463	71.950	±	16.462	2.592	0.012*
PC (%)	87.902	±	11.708	96.600	±	3.102	-5.230	0.000*
PT (sec)	13.503	±	1.074	12.760	±	0.469	4.275	0.000*
Urea (mg/dl)	28.400	±	4.784	26.050	±	4.019	1.974	0.062
Creatinine (mg/dl)	0.832	±	0.188	0.755	±	0.188	1.581	0.124
Hemoglobin (g/dl)	14.105	±	1.994	13.415	±	0.945	1.488	0.141
WBC (10^3/ul)	6.740	±	1.925	6.475	±	1.443	0.651	0.519
Platelet (10^3/ul))	212.867	±	51.676	238.450	±	40.929	-2.011	0.048
HCV RNA (IU/ml)	1350092.933	±	2220017.899	.	±	.		

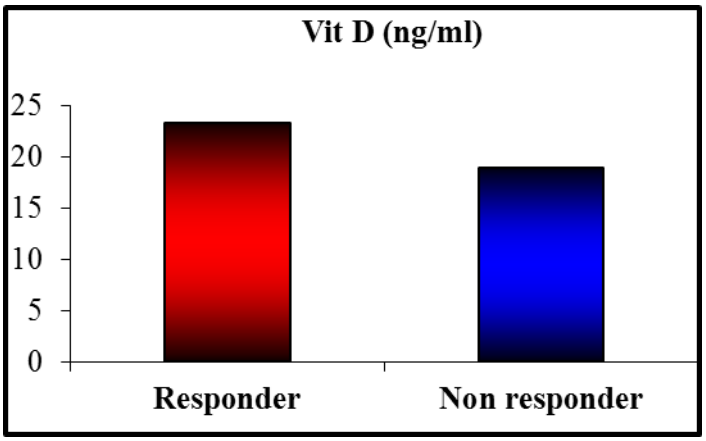

Figure (1): Comparison between group Ia & group Ib as regard vitamin D level.

Table (2): Comparison between vitamin D level in group Ia, Ib and control group II.

	Vitamin D (ng/ml)						ANOVA	
	Range			Mean	±	SD	F	P-value
Group Ia	13.300	-	27.400	23.324	±	3.303	232.227	<0.001*
Group Ib	17.200	-	20.700	19.000	±	0.995		
Group II	36.800	-	67.900	52.155	±	9.267		

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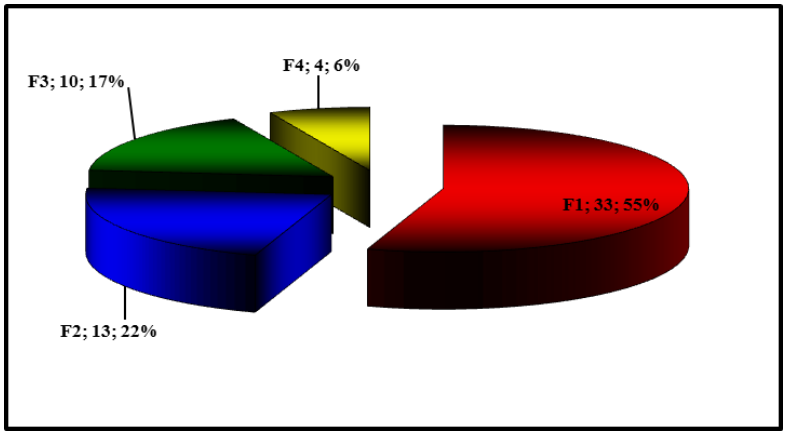

Figure (2): Hepatic fibrosis in liver biopsy in group I.

Table (3): Correlation between vitamin D concentration& demographic, laboratory and histopathologic data in group I.

Correlation	Vitamin D (ng/ml)	
	R	P-value
Age	-0.203	0.120
BMI	-0.088	0.502
Glucose	0.105	0.424
Albumin	-0.093	0.478
AST	-0.263	0.042*
ALT	-0.234	0.072
Total Bilirubin	-0.052	0.692
Urea	-0.097	0.461
Creatinine	-0.175	0.181
Hemoglobin	-0.140	0.288
WBC	-0.135	0.303
Platelets	0.301	0.020*
HCV RNA	-0.268	0.039*
AFP	-0.248	0.05*
Fibrosis	-0.328	0.010*

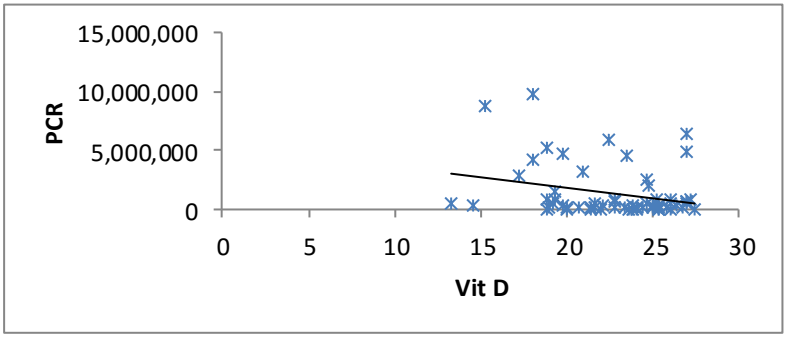

Figure (3): Correlation between vitamin D concentration & PCR in group I (r value= -0.268, p value < 0.05).

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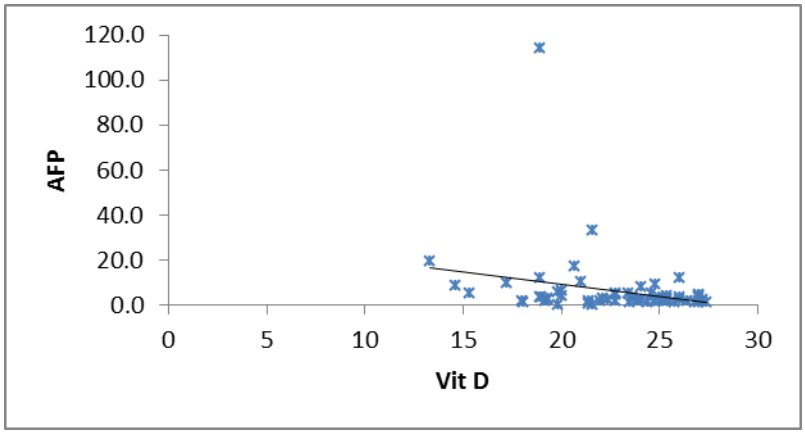

Figure (4): correlation between vitamin D and AFP in group I (r value > -0.248, p value = 0.05).

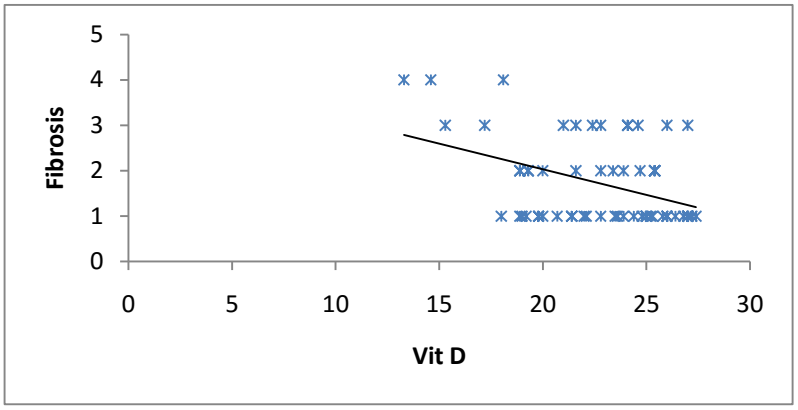

Figure (5): Correlation between vitamin D concentration & degree of fibrosis in group I (r value= -0.328, p value < 0.01).

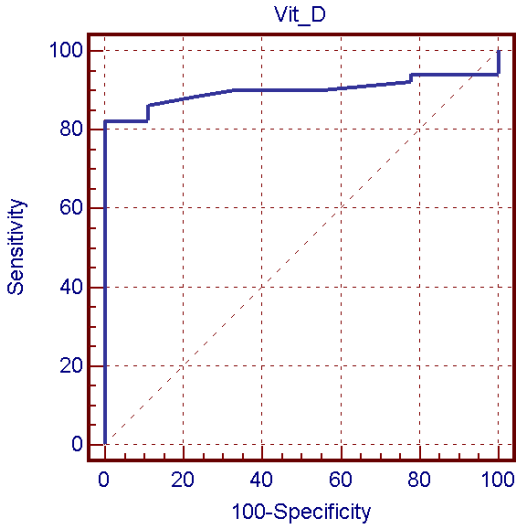

Figure (6): Roc curve to determine sensitivity &specificity of the cutoff value for vitamin D.

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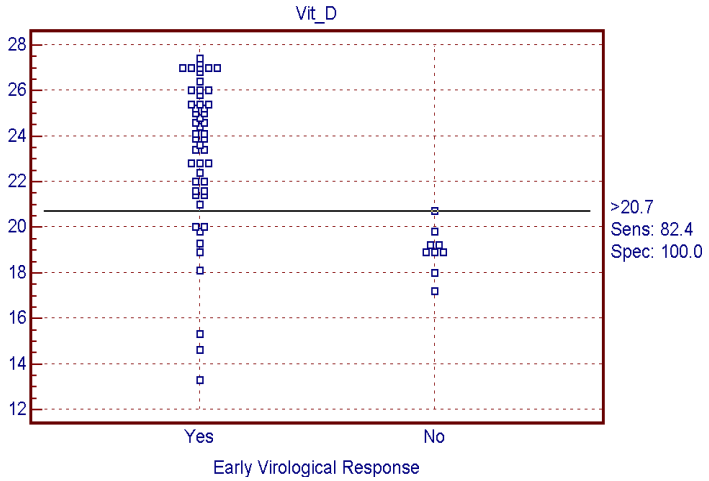

Figure (7): The cutoff value for vitamin D as a predictor of EVR to combined Peg-IFN & RBV therapy.

Comparison between 60 patients with HCV candidate for therapy and 20 healthy control subjects as regard demographic & laboratory data showed a highly statistically significant increase in group I compared to group II as regard AST, ALT, ALP & PT (p value < 0.01). There was a highly statistically significant decrease in PC (p value < 0.01) and significant decrease in platelet counts in group I compared to group II. While there was insignificant statistical difference between group I and group II as regard demographic data, fasting blood glucose, albumin, total & indirect bilirubin**,** renal functions, hemoglobin & WBCs (p value > 0.05). The HCV virologic load by quantitative RNA was (1350092.933 ± 2220017.899) in group I (table 1).

By using Anova test to compare vitamin D level in the three studied groups Ia, Ib ,and group II there was a highly statistically significant decrease of vitamin D in group Ia & group Ib compared to group II p value (<0.01 &<0.01) respectively (table 2).

There was highly significant increase in mean serum vitamin D in responders compared with non-responders (figure 1).

To detect stage of fibrosis, liver biopsy was done for group I. Among the 60 patients there were 33(55%), 13 (22%), 10 (17%) and 4 (7%) have F1- F4 respectively according to Metavir score (figure 2).

There was a statistical significant negative correlation between vitamin D and HCV PCR (r = -0.268, p < 0.05) (Figure 3), and between vitamin D and AFP (Figure 4) (r = -0.248, p < 0.05) while there was a highly statistically significant negative correlation between vitamin D and degree of fibrosis (r value= -0.328 p value < 0.01) (figure 5).

There was a statistical significant negative correlation between vitamin D and AST (r value = -0.263, p value < 0.05). While there was insignificant correlation between vitamin D & ALT (r value = -0.234, p value > 0.05). There was also a statistical significant positive correlation between vitamin D and platelet counts (r value = 0.301, p value < 0.05). There was insignificant correlation between vitamin D & demographic data, glucose, liver functions other than AST, renal functions, Hb &WBCS (p value > 0.05) (table 3).

The cut off value for vitamin D was 20.7 ng/ml by sensitivity (82.4 %), specificity (100 %), Positive Predictive value: (100), Negative Predictive value : (50) & by accuracy (90%) as a predictor of EVR to peg-interferon & ribavirin therapy in 60 chronically hepatitis C infected patients (Figure 6 & 7).

DISCUSSION

Vitamin D is metabolized by the liver then converted in the kidney to its active form 1,25-dihydroxy-vitamin D3. The deficiency occurs in two thirds of CLD patients even without cirrhosis [14].

The actions of vitamin D include improvement of insulin sensitivity, suppressing the proinflammatory cytokines, enhancement of anti-inflammatory cytokines, and improvement of CD4 T cell hyper-responsiveness [8].

Inhibition of tumor necrosis factor-α and transforming growth factor–β production occurs through the anti-inflammatory and antifibrotic role of vitamin D in the liver [15].

There is an inverse relationship between the expression of vitamin D receptors in the liver and the degree of inflammation. These receptors are decreased in CHC [16].

The present work showed highly statistically significant decrease in the level of vitamin D in group I compared to group II. This is in agreement with [17] who found that vitamin D levels in HCV genotype 4 infected Egyptian patients were significantly lower than in healthy subjects. Also [18] found that in genotype 4-infected patients, significant decrease of the vitamin and its active metabolites occurs in comparison to healthy subjects

Our results showed a statistically significant negative correlation between vitamin D & degree of fibrosis in group I. This is in agreement with [19] who found that deficiency of vitamin D is common in those with severe liver fibrosis. They reported that 1, 25-(OH)2 D3 inhibits type I collagen formation in Hepatic stellate cells, and they reported that correction of vitamin D deficiency in patients with CLD is a potential therapy to inhibit progression of fibrosis

Also Abu-Mouch et al [20] concluded that severe fibrosis and a low SVR on interferon-based therapy are related to low vitamin D levels.

The results of our study showed that there was a highly statistically significant increase in vitamin D level in group Ia compared to group Ib which is in agreement with Mohamed et al., [17] who found that Vitamin D deficiency predicts an unfavorable response to IFN-based treatment of HCV in Egyptian patients infected with genotype 4. Also Villar et al [21] reported that in patients with high levels of vitamin D or in those receiving supplementation, a high sustained viral response were found.

Gal-Tanamy et al., [22] said that vitamin D inhibits HCV in dose dependent way so it may affects treatment response and the clinical outcomes of chronic HCV. Also our findings agree with Nimer and Abu Mouch [23] who found that in chronic HCV patients genotype 2-3 adding vitamin D to peg-interferon/ribavirin therapy improves significantly viral response.

Our results provide further support to Abu-Mouch et al. [8] who found that vitamin D supplementation to the standard treatment may improve the rate of rapid virologic response; early virologic response and sustained virologic response significantly in treatment of naïve genotype 1 HCV patients in comparison to the response rates with the standard therapy alone.

Contradictory Jazwinski et al., [24] found that in CHC-naïve patients genotype I receiving combined peg- interferon and ribavirin therapy there was no relation between vitamin D level and sustained virologic response.

Our results showed that there was a statistical significant negative correlation between AFP & EVR to combined Peg-IFN and ribavirin therapy which agreed with El Raziky et al., [25] who found that elevated levels of AFP were negatively associated with treatment response.

In our study, high baseline AFP levels were observed in non-responders, this agreed with Males et al [26], who had found negative association between AFP and the response for treatment in 100 Egyptian patients with CHC. As the patients with high AFP above the median value of (4.5 ng/ml) showed low SVR rate than the patients with AFP below that value.

In conclusion, serum concentration of 25-OH vitamin D in patients with HCV is inversely related to viral load & EVR on combined Peg-IFN & RBV therapy and its deficiency may had a role in the pathogenesis of HCV and may guide us for further investigations looking for if the vitamin has a role in prevention of HCV. We

recommend testing of the vitamin levels before starting peg- interferon and ribavirin treatment and to give vitamin D supplementation all through the course of treatment of CHC.

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