DOES VITAMIN D CONCENTRATION IS CORRELATED TO CHRONIC HEPATITIS C TREATMENT FAILURE: A CASE CONTROL STUDY

BY

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Background

Egypt has the highest prevalence rate of Hepatitis C virus (HCV) in the world, where, chronic hepatits C (CHC) is considered a major health problem. The standard treatment of CHC is combination therapy of pegylated interferon and ribavirin. Successful treatment and sustained virological response (SVR) are only achieved in 30% of patients. Major adverse effects and high cost of the treatment makes predicting the treatment output is an important approach. The aim of this study to find an association between Vitamin D concentration with achieving SVR. **Patients and methods**: In this study; 250 patients were selected and divided into 3 groups (100 CHC patients who achieved SVR, 100 CHC patients who did not achieve SVR, and 50 patients as control). Blood samples were collected to measure vitamin D concentration and routine liver function tests. **Results**: Vitamin D concentration was found significantly higher in the responders more than non responders. **Conclusion**: Vitamin D concentration is valid predector to the response of CHC patients to combination of pegylated interferon and ribvirin thrapy

Keywords

HCV genotype 4, Interferon and ribavirin therapy, vitamin D concentration

Introduction

Hepatitis C virus genotype 4 (HCV-4) is the most common variant of hepatitis C virus (HCV) in the Middle East and Africa, particularly Egypt. This region has the highest prevalence of HCV worldwide, with > 90% of infections due to HCV-4, which is considered a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma (HCC), and liver transplantation in the country[1]. HCV-4 has recently spread beyond its strongholds in Africa and the Middle East to several western countries, particularly in Europe, due to variations in population structure, immigration and routes of transmission. However, the features of this genotype and management strategies for patients infected with this genotype are not well developed [2].

HCV is remarkably efficient at establishing persistent infections. This suggests that HCV has evolved one or more strategies to evade the host immune response, among which are effects upon T-lymphocyte responses, including interferon (IFN)-γ production; documented by the severely suppressed T-lymphocyte responses in patients with chronic HCV infections [3].

Vitamin D (Vit D) is a critical regulator of immunity, playing a role in both innate and cell-mediated immune responses [4]. Vit D suppresses production of T-helper (Th)-1 lymphocyte type cytokines, such as IFN-γ and interleukin (IL)-2, and consequently leads to an enhanced production of Th-2 cytokines, such as IL-4 and IL-5, thereby promoting humoral immune responses. Vit D also endorses innate immunity by directly inducing gene expression of antimicrobial peptides, such as cathelicidin and β-defensin 2, in various human cell types [5]. Vit D deficiency has been shown to associate several immune-mediated diseases, as well as to increase susceptibility to both infections and cancer. Specifically, a 25(OH)-Vit D concentration < 50 nmol/L (i.e., 20 ng/mL) is accepted as a marker of deficiency, whereas a concentration of 51-74 nmol/L (21-29 ng/mL) indicates insufficiency [6].

Combination therapy with pegylated interferon plus ribavirin is the successful treatment of chronic hepatitis C (CHC), however, the success rate of this treatment is influenced by other non drug factors. Some of these factors depend on the HCV, such as viral genotype, changes in critical regions of the viral genome and viral load. Other are host-related, either genetic (gender and race) or acquired (insulin resistance, obesity, liver steatosis, iron overload and liver fibrosis stage) [7]. Therefore, by combining the influence of those non drug factors as baseline before treatment makes it possible to predict probability of achieving sustained virologic response (SVR, defined as non-detectable HCV RNA in serum 6 months after the end of therapy). Discovery of new anti-HCV drugs is important, on the other hand, improving the ability to predict the response to interferon plus ribavirin therapy is inevitable.

In the present study we aim to evaluate vitamin D concentration as baseline biomarkers of response to interferon-ribavirin therapy in patients with CHC.

Patients and methods

Patients

This study included 250 caucasian patients divided into 3 groups; 100 HCV patients who received interferon/ribavirin therapy for 48 weaks who reached early virological response (Viral clearance after 3 months), 100 HCV patients who received interferon/ribavirin therapy and did not reached SVR, and 50 apparently healthy individuals as control. All HCV patients have HCV genotype 4 for more than 6 months with no other liver diseases, HIV, kidney diseases, parathyroid diseases. Only patients who completed a full course of therapy or who had stopped therapy due to therapeutic failure were included in the study. To participate in the study, written consent was obtained from each subject in accordance with the Declaration of Helsinki and following the guidelines of the ethical committee of Misr international university.

Laboratory Methods

Serum samples were separated and stored at -20 °C until analysis. Routine hematological and biochemical tests were performed as follows.

Biochemical markers assessment

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total bilirubin, serum albumin and Alkaline phosphatase (ALP) were assessed using (Randox laboratories limited, Country Antrim, UK) kits.

Vitamin D assay

Circulating 25-hydroxyvitamin D levels were measured using 25(OH) Vitamin D ELISA Kit (enzo life sciences, USA) [8].Data were expressed in ng/ml.

Assessment of HCV levels

Quantitative reverse transcription polymerase chain reaction (RT-PCR) for HCV was done using TaqMan technology according to the method of Scott and Gretch [9] , and only HCV-4-infected patients were included in the study. Typically, an RT-PCR has a limit of quantification (LOQ) of 25 IU/mL and a limit of detection (LOD) of 10-15 IU/mL; in the assays used here for HCV-RNA testing, the LOQ was 24 IU/mL and the LOD 12 IU/mL.

For genotyping, HCV type-specific primers designed by Okamoto et al [10] were utilized. Assessments of genotype burdens required three steps: (1) RNA virus was extracted from patient samples using a Tripure Method (Roche, Mannheim, Germany); (2) isolated RNA was converted to cDNA using random hexamers and Moloney Murine Leukemia Virus Reverse Transcriptase enzymes from Promega (Madison, WI, United States); and (3) the product cDNA was amplified using an allele-specific PCR method. The PCR program was set for 1 cycle at 96 °C for 6 min, then for 40 cycles at 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, and a final extension cycle of 72 °C for 10 min. For each patient, two vials containing primer specific for virus types 1a/1b and for

2a and 3a were used. A positive control for each genotype (supplied by the kit manufacturer) was also run in parallel with each set of samples. In addition, HCV viral load was also determined using an Artus Real Art PCR Kit (Qiagen, Valencia, CA, United States). For both assays, a LightCycler® 480 real-time PCR System (Roche) was used. The slope of each reaction was between 3.2 and 3.4, and the error < 0.002. All results of the quantitative HCV analyses were expressed as IU/mL.

Statistical analysis

All data are expressed as mean ± SD. All analyses utilized graphpad prism 5 for Windows version 5. Analysis of variance was employed for comparisons of means of the different parameters, with independent T test, Anova one way test with Tukey's post hoc test. $P < 0.05$ was accepted as statistically significant.

Results

Liver functions

AST, ALT activities, and albumin concentrations were measured in serum samples of all patients. ALP activity and total bilirubin concentration were measured in responders and non-responders group only. All mentioned parameters showed significance difference between tested groups except for total bilirubin that showed no significance difference between the tested groups (Figures 1,2,3,4,5).

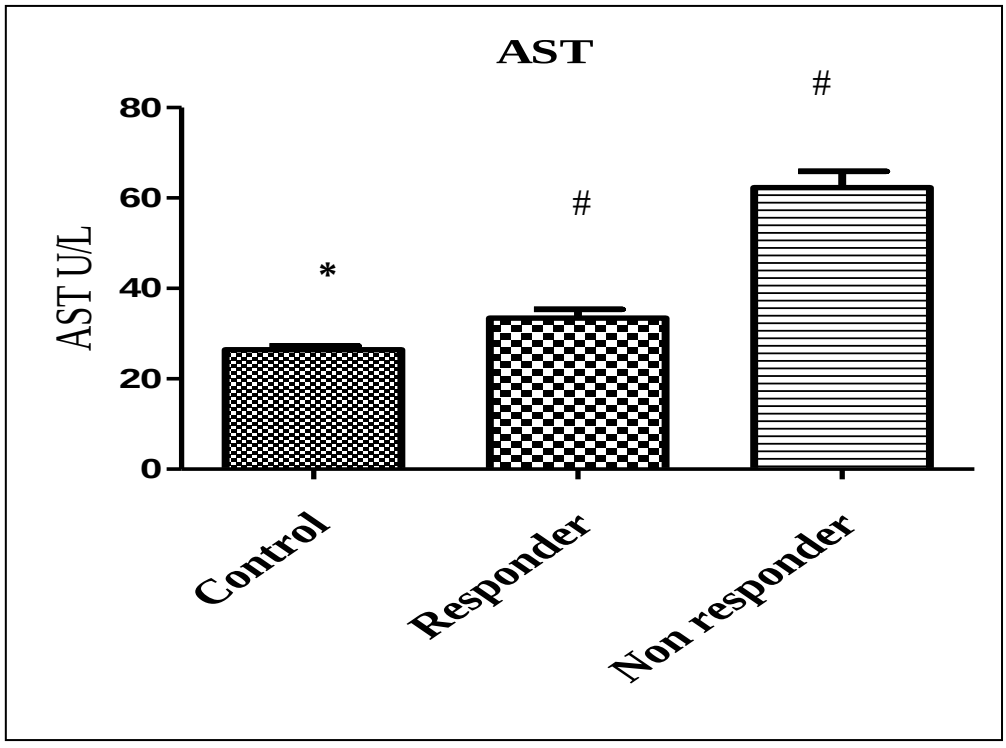

Figure 1: Mean values of AST of patients in tested groups. Values are expressed as means ± standard deviation. P < 0.00001, using Anova test. Tuky's post hock test reveals that the means of both (control, non responders)* and (responders, non responders)# are significantly different.

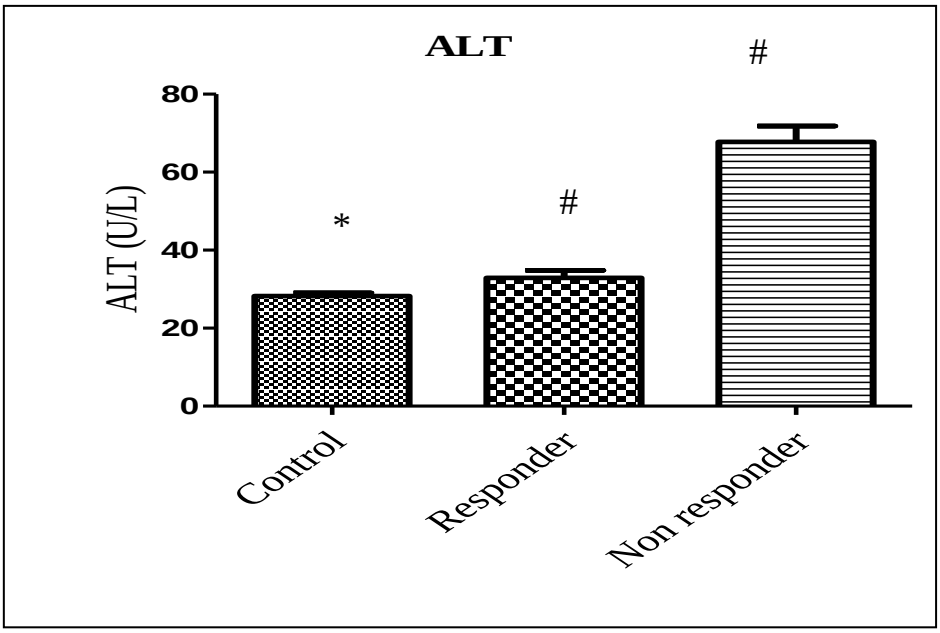

Figure 2: Mean values of ALT of patients in tested groups. Values are expressed as means ± standard deviation. P < 0.00001, using Anova test. Tuky's post hock test reveals that the means of both (control, non-responders)* and (responders, non-responders)# are significantly different.

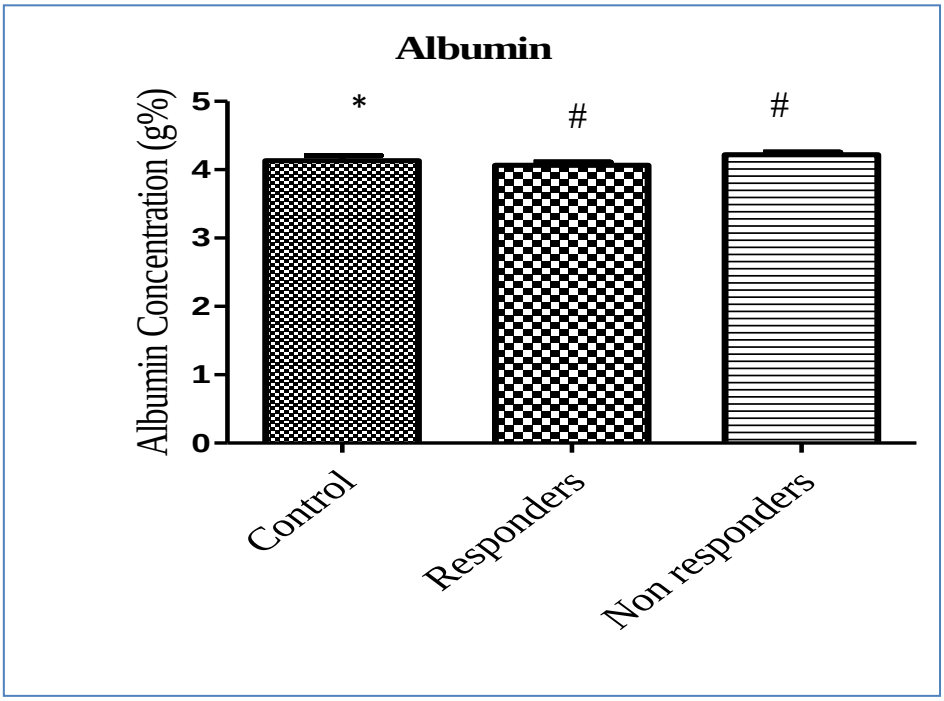

Figure 3: Mean values of Albumin concentration of patients in tested groups. Values are expressed as means ± standard deviation. P= 0.0441, using Anova test. Tuky's post hock test reveals that the means of responders and non responders is significantly different (#).

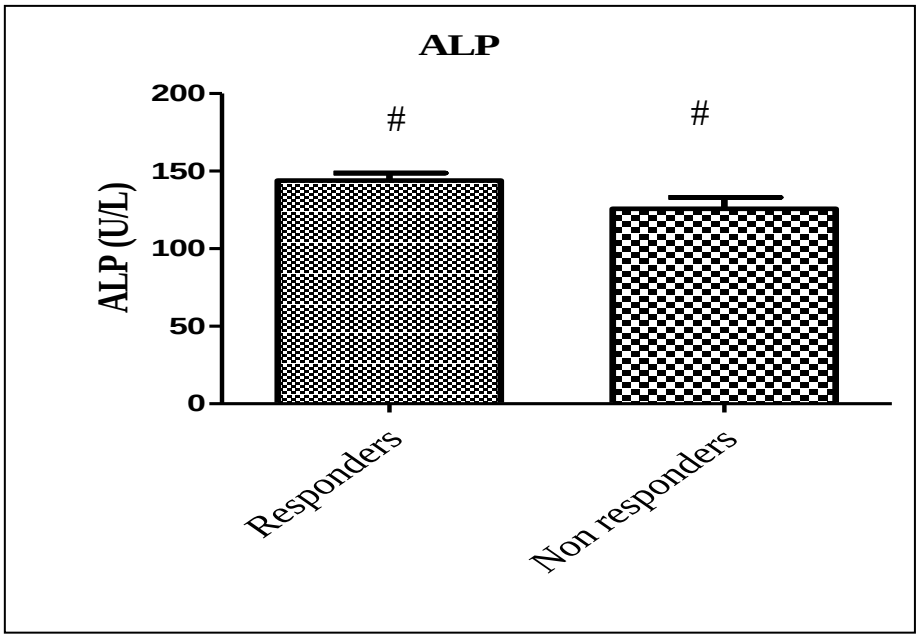

Figure 4: Mean values of ALP of patients in tested groups. Values are expressed as means ± standard deviation. P= 0.039, using T test that reveals, the means of responders and non responders is significantly different (#).

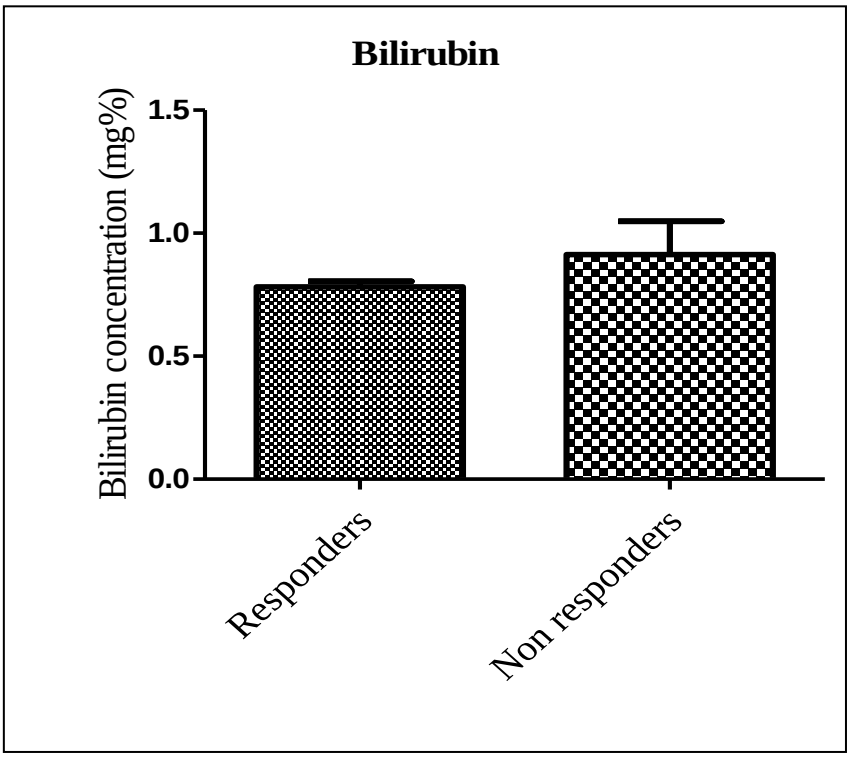

Figure 5: Mean values of total bilirubin of patients in tested groups. Values are expressed as means ± standard deviation. P= 0.394, using T test.

25-OH Vitamin D serum concentration is a association to response to pegylated interferon and ribavirin therapy:

25-OH vitamin D serum levels were measured in serum samples of all patients. 25-OH vitamin D levels were significantly lower in CHC patients who did not respond to treatment compared with CHC patients who respond to treatment and control patients ($p< 0.0001$). So, we can conclude that 25-OH vitamin D levels were significantly correlated to the patient expected response to pegylated interferon and ribavirin therapy (Figure 6).

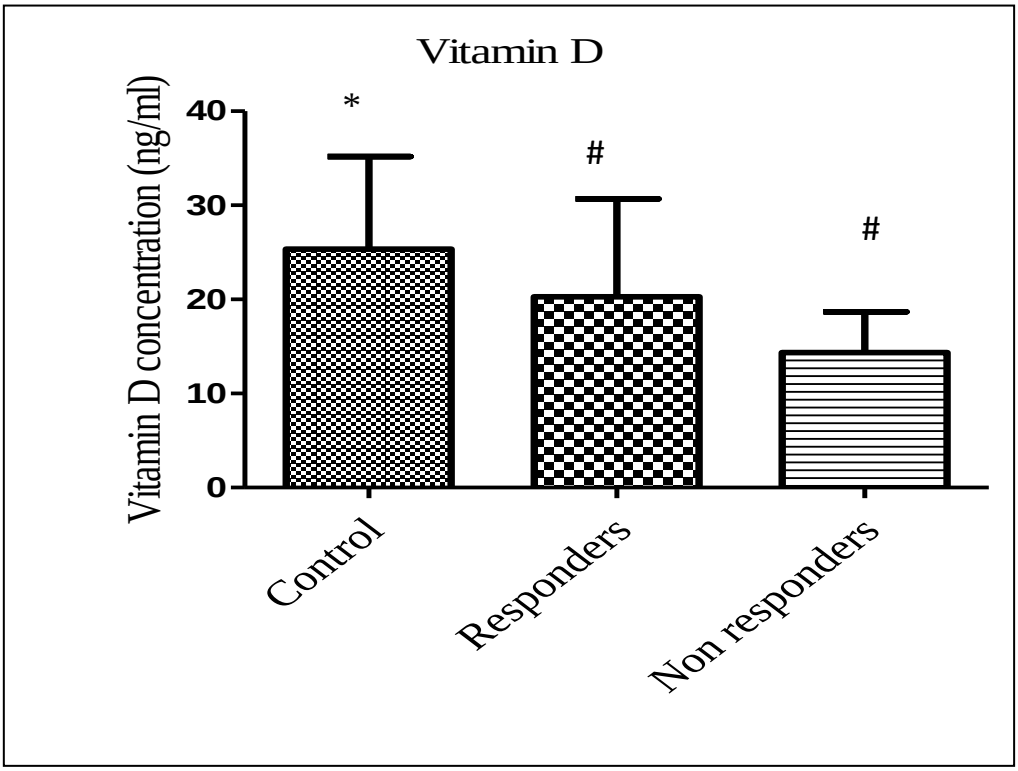

Figure 6: Mean values of vitamin D concentration of patients in tested groups. Values are expressed as means ± standard deviation. P< 0.00001, using Anova one way test. Tuky's post hock test reveals that the means of both (control, non responders)*, (control,responders)* and (responders, non responders)$^{\#}$ are significantly different.

Discussion:

Predicting the treatment output is an important aspect of research, especially when the treatment is expensive, has multiple adverse effects, and has low success rate. This study is aimed to assess the reliability of vitamin D concentration as being a mean to predict to interferon/ ribavirin therapeutic effect in CHC patients. Results revealed that vitamin D concentration is reliable predictors for the treatment output of interferon/ ribavirin in CHC patients.

Concerning vitamin D, there was a significant difference in serum vitamin D concentration between responders, non responders, and control group. Proving that low vitamin D serum level is related to low responsiveness to interferon/ ribavirin based therapy. Our results are bolstered by the results of ***petta, et al*** [11] who stated that low serum vitamin D level is associated with severe fibrosis and low response to interferon based therapy in HCV genotype1 patients. Similarely, ***Bitetto, et al*** [12] correlated vitamin D deficiency with the failure of interferon based therapy and they assumed that vitamin D levels might be assessed before starting antiviral therapy, which should be initiated only in the presence of normal serum vitamin D values; in the presence of vitamin D deficiency, it might be preferable to correct the deficiency before starting antiviral therapy.

The supposed relationship between the rapid slope of the HCV RNA level after therapy initiation and vitamin D suggests that the latter could amplify the immunological effect of IFN. In fact, beyond the classical actions related to calcium homeostasis and bone metabolism, vitamin D has emerged as a key regulator of the innate immunity response in humans [13].

Although the mechanisms underlying the role of vitamin D in clearing HCV are still unrevealed, two studies were performed in a try to resolve this enigma. ***Gal-Tanamy, et al*** [14] who demonstrated that vitamin D has a direct inhibitory effect on viral production. This inhibition may be partially attributed to augmentation of the innate immune response, as treatment of HCV-infected cells with vitamin D or calcitriol up regulate the expression of IFN-β, the immediate cellular response to viral infection. Moreover, we observed the downstream induction of the IFN-stimulated gene, which has been shown to directly inhibit viral production.

Vitamin D inhibited HCV production presumably through its active hormonal form calcitriol. The conversion to calcitriol, the second step in vitamin D bioactivation, occurs mainly in the kidney by the renal 1α-hydroxylase. However, there is substantial evidence for additional extrarenal sites of production of calcitriol, which primarily serves as an autocrine/paracrine factor with cell-specific functions. 1α-Hydroxylase has been reported in many cells and tissues including the skin, prostate, brain, breast, colon, lung, pancreatic islets, lymph nodes, monocytes, parathyroid, placenta, colonic

epithelial cells, and in adipose tissue [15]. As well as, hepatocyte used in *invitro* model was shown to express 1α-hydroxylase leading to the direct production of active 1,25 (OH)2 vitamin D in the liver cell [14]. The second study to explain the mechanism of vitamin D was performed by ***Matsumura, et al*** [16] and had identifies 25(OH) vitamin D but not 1, 25(OH) 2 vitamin D as an effective anti-HCV metabolite with the ability to suppress infectious virus production. They found that 25-(OH) vitamin D did not influence the steps of HCV entry and replication but rather selectively inhibited the virus assembly step.

However, other studies contradict our findings, ***Lang et al, 2012*** [17] found that patients with chronic hepatitis C patients have a high prevalence of vitamin D insufficiency. In this study, 25(OH) D3 serum levels were not associated with treatment outcome in a subgroup of 269 patients with available baseline serum samples before antiviral treatment. In fact, 25(OH)D3 serum levels were even somewhat lower in patients who subsequently achieved SVR as compared to those who failed to respond to treatment. In their previous analysis, they did not observe any significant association between 25(OH)D3 serum levels and SVR to IFN-a-based therapy in a cohort of 317 HCV genotype 1-infected patients, but a significant association in a cohort of 156 patients infected with genotype 2 or 3 [18]. The reasons for these discrepancies remain unclear at the moment, but apparently 25(OH)D3 serum levels cannot be considered as an established predictor of treatment outcome at the moment [17].

Importantly, it is well-known that 25(OH)D3 serum levels correlate poorly with calcitriol serum concentrations, and 25(OH)D3 serum levels are therefore not a suitable marker for bioactive vitamin D or vitamin D receptor signaling, especially not for local calcitriol levels during inflammatory conditions. Thus, the lacking lack of an association between 25(OH)D3 serum levels and SVR may simply reflect the limited biological relevance of 25(OH)D3 serum levels. Unfortunately, there are no reliable methods to quantify serum levels of the bioactive vitamin D metabolite calcitriol, and the majority of clinical trials assessing the vitamin D status of patients focus on the calcitriol precursor25(OH)D3 [19].

Conclusion

This study aimed to find the correlation between vitamin D concentration and successful treatment of CHC with pegylated interferon and ribavirin combination therapy. Final results showed that people with serum vitamin D concentration > 15 ng/ml have higher chances to achieve SVR.

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" هل توجد علاقة مابين تركيز فيتامين د و فشل علاج التهاب الكبد المزمن سي: دراسة متحكمة الحالة"

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الملخص:

الالتهاب الكبدي المزمن سي يعد من كبرى المشاكل الصحية في مصر و السبب الرئيسي لالتهاب الكبدي المزمن سي هو فيروس سي من الفئة الجينية ٤. العلاج المعتمد لالتهاب الكبدي المزمن سي هو الجمع ما بين الانترفيرون و الريبافيرين و لكن الاستجابة الفيروسية الدائمة فرصها ضئيلة و قد أجريت هذه الدراسة لتقييم امكانية استخدام فيتامين د لتوقع استجابة المرضى المصريين المصابين بلالتهاب الكبدي المزمن سي للعلاج بالانترفيرون و الريبافيرين. أجريت هذه الدراسة على ٢٥٠ شخص تم تقسيمهم الى ثلاث مجموعات. المجموعة المتحكمة و هم أشخاص أصحاء ظاهريا و ٢٠٠ شخص تم تشخيصهم كمرضى التهاب الكبدي المزمن سي و تم علاجهم بالانترفيرون و الريبافيرين لمدة ٤٨ أسبوع و منهم ١٠٠ مريض وصلوا للاستجابة الفيروسية الدائمة و صنفوا كمجموعة المستجيبين و لكن هناك ١٠٠ مريض لم يصلوا للاستجابة الفيروسية الدائمة و صنفوا كمجموعة غير المستجيبين. ووجد أن مستوى فيتامين د أعلى في مجموعة المستجيبين. و بذلك يمكن استخدام فيتامين د لتوقع استجابة المرضى المصريين المصابين بلالتهاب الكبدي المزمن سي للعلاج بالانترفيرون و الريبافيرين .