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# The Clinical Utility of Vitamin D Levels in Management of Treatment Naive Chronic HCV Infection

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**Abstract:** ***Background**: Hepatitis C virus (HCV) is a major cause of chronic hepatitis and the leading cause of end stage liver disease including liver cirrhosis and hepatocellular carcinoma. The best predictor of long-term response for chronic hepatitis C (CHC) to treatment is sustained virological response (SVR). Two major predictors of SVR are genotypes and viral load[4]. Recently emerged predictor of response to antiviral treatment is serum vitamin D concentration. Vitamin D plays an emerging role in inflammatory and metabolic liver diseases, including infection with hepatitis C virus (HCV).Patients with chronic hepatitis C (CHC) frequently suffer from severe vitamin D deficiency. **Aims and Objectives**: To evaluate the clinical utility of vitamin D levels in management of treatment naive HCV infection. To correlate between serum vitamin D levels and HCV RNA viral load in patients with chronic hepatitis C infection. **Materials and Methods**: Study was conducted in the department of Gastroenterology, Yashoda Hospital, Hyderabad from November 2014 to March 2016. Informed consent of the study participants was obtained in all cases. The study had approval of local Ethical Committee. We screened 65 patients for the study and a total of 50 treatment naïve chronic hepatitis C (CHC) patients were included in this study. Data was collected prospectively from consecutive patients from both outdoor and indoor patients based on clinical interview and review of records. Detailed history and systemic examination was done. Blood samples were obtained for complete blood count, renal function test, liver function test, Thyroid function tests, and serum calcium levels. Blood samples were taken for vitamin D levels, quantitative HCV RNA levels for CHC patients. **Results**: A total off 50 patients were selected in hepatitis C group according to the inclusion criteria. Out of 50 patients 33 were males and 17 were females with mean age of 52.7 ±8.5) years. Mean serum vitamin D concentrations of entire cohort was 21.1±15.2) ng/ml. Mean HCV RNA of the entire cohort was 3.4±2.2) log10IU/ml. Of the 55 patients of the entire cohort, 09 (18%) had severe vitamin D deficiency [25(OH)D <10ng/ml], 29 (58%) had insufficient vitamin D levels [25(OH)D ≥10 and <20ng/ml] and 12 (24%) had optimal vitamin D levels [25(OH)D ≥ 20ng/ml] (p <0.0001). Patients with vitamin D levels below 20 ng/m had significantly high viral loads as compared to patients with vitamin D levels ≥ 20ng/ml (p<0.001).Hence vitamin D deficiency and insufficiency was highly prevalent in CHC patients. Patients with high viral loads had mean serum vitamin D levels of 12.5±4.7 versus patients with low viral loads 24.5±16.6 and this difference was statistically significant (p=0.01).In both univariate and multivariate analyses, HCV RNA was the major determinant factor of low 25(OH)D levels (r=-0.431,p=0.002).The mean vitamin D serum concentrations in autumn-winter and spring-summer months were 14.6±3.3 and 18.5±3.1ng/ml, respectively (p < 0.0001). **Conclusion**: 1) HCV RNA viral loads appears to be the strongest determinant of low 25(OH)D serum levels in treatment naïve CHC patients. 2) HCV RNA viral loads appear to have an inverse relationship with serum vitamin D levels. 3) Serum Vitamin D levels appears to have significant seasonal fluctuations.*

**Keywords:** Hepatitis C virus, Vitamin D, Hepatitis C virus (HCV) RNA

## 1. Introduction

Hepatitis C virus (HCV) is a major cause of chronic hepatitis and the leading cause of end stage liver disease including liver cirrhosis and hepatocellular carcinoma[1]. It is a major global health challenge affecting an estimated 2.7 million people worldwide[2]. HCV variability, which facilitates rapid development of antiviral resistance, provides a strong rationale for the development and implementation of antiviral combination therapies[3].

The best predictor of long-term response for chronic hepatitis C (CHC) to treatment is sustained virological response (SVR), defined as undetectable serum HCV RNA by PCR assay at 24 weeks after cessation of therapy[4].Two major predictors of SVR are genotypes and viral load[4]. Recently, emerged predictor of response to antiviral treatment is serum vitamin D concentration. [5,6].

Cholecalciferol is the precursor of the bioactive vitamin D metabolite, calcitriol[7]. The bioactive vitamin D metabolite, $1,25(OH)_2D_3$, which is also called calcitriol, exerts its biological functions predominantly by signaling through a nuclear vitamin D receptor (VDR), which serves as a ligand-activated transcription factor[7]. Importantly, clinical assays to quantify calcitriol are generally characterized by poor reliability[8].Therefore, the stable, easy-to-quantify metabolite, $25(OH)D_3$, is usually measured in clinical routine to assess a patient's vitamin D status[8]. By induction or repression of expression of hundreds of genes, calcitriol serves as an important modulator of numerous signaling pathways related to both innate and adaptive immunity[8,9-11].

In patients with tuberculosis, a therapeutic value of the immunomodulatory effect of vitamin D has already been proven in randomized, controlled clinical trials[12,13]. Importantly, vitamin D supplementation in these studies

resulted in an increased activity of intrinsic interferon-alpha (IFN-α) signaling[14].Vitamin D plays an emerging role in inflammatory and metabolic liver diseases, including infection with hepatitis C virus (HCV)[15-18]. For example, it was shown that patients with chronic hepatitis C (CHC) frequently suffer from severe vitamin D deficiency[15,17-19]. Although the stage of liver fibrosis was a determinant of vitamin D deficiency in CHC patients, even patients without any relevant degree of liver fibrosis had a significantly higher risk of vitamin D deficiency, compared to healthy controls[17].Further there is lack of correlation between HCV viral load and vitamin D serum levels[20].

In contrast to these well-documented findings, it currently remains conflicting whether 25(OH)$D_3$ serum levels can be considered as a predictor of treatment outcome in patients with CHC[15,17-19].

The main purpose of this study is to evaluate the correlation of vitamin D levels with virological parameters in patients with treatment naive HCV infection.

## 2. Aims and Objectives

**Aim:**
The aim of this study is to evaluate the clinical utility of vitamin D levels in management of treatment naive HCV infections.

**Objectives:**
To correlate between serum vitamin D levels and HCV RNA viral load in patients with chronic hepatitis C infection.

## 3. Materials and Methods

Study was conducted in the department of Gastroenterology, Yashoda Hospital, Hyderabad from November 2014 t0 March 2016. Informed consent of the study participants was obtained in all cases. The study had approval of local Ethical Committee. It was a prospective study. We screened 65 treatment naïve chronic hepatitis C (CHC) patients for the study and a total of 50 patients were included in this study.

### 3.1 Inclusion and Exclusion Criteria

**Inclusion Criteria**
1) Treatment naive chronic HCV patients (CHC) defined as detectable anti HCV and HCV RNA ≥ 6 months.
2) Age ≥18 years

**Exclusion Criteria**
1) Coinfection with HCV/HBV, human immunodeficiency virus (HIV).
2) Excessive alcohol consumption (>40 g/day).
3) Malignancy including HCC.
4) Chronic renal failure (serum creatinine > 1.25mg/dl).
5) Thyroid disorders.
6) History of calcium or Vitamin supplements within previous 3 months.

### 3.2 Methodology

Data was collected prospectively from consecutive patients from both outdoor and indoor patients based on clinical interview and review of records. Detailed history taken and systemic examination was done. Blood samples were obtained for complete blood count, renal function test, liver function test, Thyroid function tests, serum calcium levels etc. Blood samples were taken for vitamin D levels, quantitative HCV RNA levels for CHC patients.

Quantitative HCV RNA levels were also done by ABI real time PCR using Taqman Chemistry in which 'Viral Load 1 IU = ~2.7 COPIES', linearity 37 – 74074074 IU/mL or 100 – 200000000 copies /mL HCV genotyping was done by PCR and sequencing method.

25-Hydroxy Vitamin D 25(OH)D levels were done by EIA and values were measured in ng/L. Patients were classified into three groups as per their vitamin D levels as vitamin D deficient (≤10 ng/ml),insufficient (11-20 ng/ml) and optimal (>20 ng/ml).

## 4. Statistical Methods

HCV RNA and vitamin D levels are numeric variables, so the mean and standard deviation was calculated. After analyzing normal or non-normal distribution of the continuous variables, continuous data was examined using the student t test (if normally distributed), Mann–Whitney test (if non-normally distributed), and categorical variables were examined by chi square test. The relationship and comparison between viral loads and vitamin D levels was assessed using Pearson correlation coefficient.

Associations between dichotomic was assessed by logistic regression models and linear (e.g., HCV RNA concentration) variables and 25(OH)$D_3$ serum levels were assessed by linear regression models. After univariate analyses, multivariate analyses was performed for significant associations. Multivariate models was obtained by backward selection, using a *P* value >0.15 for removal from the model. Group differences were assessed by means of chi-square contingency tables or Wilcoxon-Mann-Whitney's U tests, as appropriate.

## 5. Results

**Table1:** Demographic, clinical and biochemical features of HCV patients

| Parameters | Total Patients (N=50) |
|---|---|
| Age(yrs) | 52.7 (8.5) |
| Sex(M/F) | 33/17 |
| BMI (kg/m2) | 24(1.9) |
| HB (g/dl) | 11.8(1.1) |
| TLC (/µl) | 5.8(1.5) |
| Platelet($10^9$/L) | 1.9(0.8) |
| Bilirubin (mg/dl) | 1.2(0.7) |
| SGOT (U/L) | 56.7 (25.8) |
| SGPT (U/L | 62.4(32.6) |
| ALP (U/L) | 95.1(36.5) |
| Total Protein (g/dl) | 6.2(0.8) |
| Albumin (g/dl) | 3.5(0.4) |

| | |
|---|---|
| Prothrombin Time (seconds) | 15.3(2.0) |
| Serum Creatinine (mg/dl) | 0.8(0.1) |
| Serum Calcium (mg/dl) | 8.7(0.6) |
| TSH (µIU/ml) | 1.9(0.7) |
| 25(OH)D3 (ng/ml) | 21.1(15.2) |
| HCV RNA log10 (IU/ml) | 3.4(2.2) |
| Genotype | 3 |

Values are shown as Mean (SD)

A total off 50 patients were selected in hepatitis C group according to the inclusion criteria. Out of 50 patients 33 were males and 17 were females with mean age of 52.7 ±8.5) years. All the patients had genotype 3. Mean serum vitamin D concentrations of entire cohort was 21.1±15.2) ng/ml. Mean HCV RNA of the entire cohort was 3.4±2.2) log10IU/ml.

**Table 2:** Demographic, clinical and biochemical characteristics in patients with chronic hepatitis C according to 25(OH)D levels

| | 25(OH)D (ng/ml) | | | |
|---|---|---|---|---|
| | >20 | 10-20 | <10 | p value |
| Number (%) | 12(24) | 29(58) | 09(18) | 0.0001 |
| 25(OH)D3 (ng/ml) | 44(15.2) | 15.7(2.5) | 8.1(1.3) | |
| Age(yrs) | 49(5.9) | 53.6(8.9) | 54.4(9.4) | 0.22 |
| Sex (M/F) | 06/06 | 20/09 | 07/02 | |
| BMI(kg/m$^2$) | 25.2(1.2) | 25.3(0.4) | 25.6(1.5) | 0.37 |
| Hb(g/dl) | 11.7(0.9) | 12(1.2) | 11.5(1.05) | 0.81 |
| TLC (/µl) | 5.6(1.4) | 5.7(1.4) | 6.1(1.8) | 0.33 |
| Platelet(10$^{9/}$L | 2.3(1.08) | 1.9(0.7) | 1.4(0.6) | 0.04 |
| Bilirubin (mg/dl) | 0.9(0.4) | 1.2(0.8) | 1.8(0.7) | 0.02 |
| SGOT (U/L) | 43.5(17) | 55.4(26.7) | 62.8(43.4) | 0.28 |
| SGPT (U/L | 46.1(20.3) | 58.9(30.8) | 88.4(42.9) | 0.01 |
| ALP (U/L) | 81.8(12.7) | 92.6(32.9) | 121.1(55.9) | 0.58 |
| Total Protein (g/dl) | 6.5(0.7) | 6.3(0.6) | 5.9(0.9) | 0.14 |
| Albumin (g/dl) | 3.7(0.3) | 3.6(0.4) | 3.2(0.4) | 0.01 |
| Prothrombin Time (seconds) | 14.5(1.67) | 15.07(1.9) | 17.1(1.9) | 0.02 |
| S. Creatinine (mg/dl) | 0.8(0.09) | 0.8(0.1) | 0.9(0.1) | 0.03 |
| S. Calcium (mg/dl) | 8.6(0.6) | 8.8(0.6) | 8.6(0.7) | 0.53 |
| TSH (µIU/ml) | 1.9(0.9) | 1.8(0.7) | 1.7(0.9) | 0.84 |
| HCV RNA log10 (IU/mL) | 2.08(1.3) | 3.4(2.1) | 5.45(2.43) | **0.001** |

Values are shown as Mean (SD)

The comparison of demographic, clinical and biochemical characteristics was done in patients with chronic hepatitis C according to 25(OH)D levels (**Table 2**).

Significant differences were observed in serum vitamin D levels, platelet counts, serum bilirubin, SGPT, serum albumin, Prothrombin time, serum creatinine and HCV RNA in relation to 25(OH)D levels.

Of the 55 patients of the entire cohort, 09 (18%) had severe vitamin D deficiency [25(OH)D <10ng/ml], 29 (58%) had insufficient vitamin D levels [25(OH)D ≥10 and <20ng/ml] and 12 (24%) had optimal vitamin D levels [25(OH)D ≥ 20ng/ml] (p <0.0001). Patients with vitamin D levels below 20 ng/m had significantly high viral loads as compared to patients with vitamin D levels ≥ 20ng/ml (p<0.001).Hence vitamin D deficiency and insufficiency was highly prevalent in CHC patients.

In order to define ‘ high ’ and ‘ low ’ viral load we used the cut-off value of 400,000 IU/mL (5.6 log 10 IU/ mL), proposed by Mangia[90]. Patients with high viral loads had mean serum vitamin D levels of 12.5±4.7 versus patients with low viral loads 24.5±16.6 and this difference was statistically significant (p=0.01) (**Table 3**).

**Table 3:** Comparison of 25(OH)D levels according to HCV RNA (IU/ml).

| HCV RNA (IU/ml) | 25(OH)D (ng/ml) | P value |
|---|---|---|
| <400000 | 24.5 (16.6) | 0.01 |
| >400000 | 12.5 (4.7) | |

**Mean values of serum vitamin D in patients with low and high viral load are depicted in following bar diagram:**

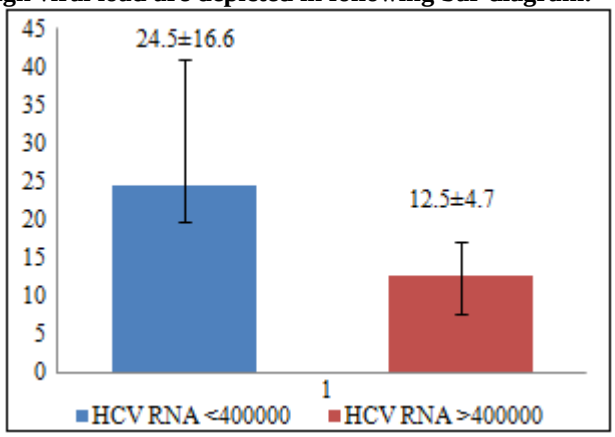



**Table 4**: Logistic multivariate regression analysis of determinant factors associated with 25(OH)D in CHC patients.

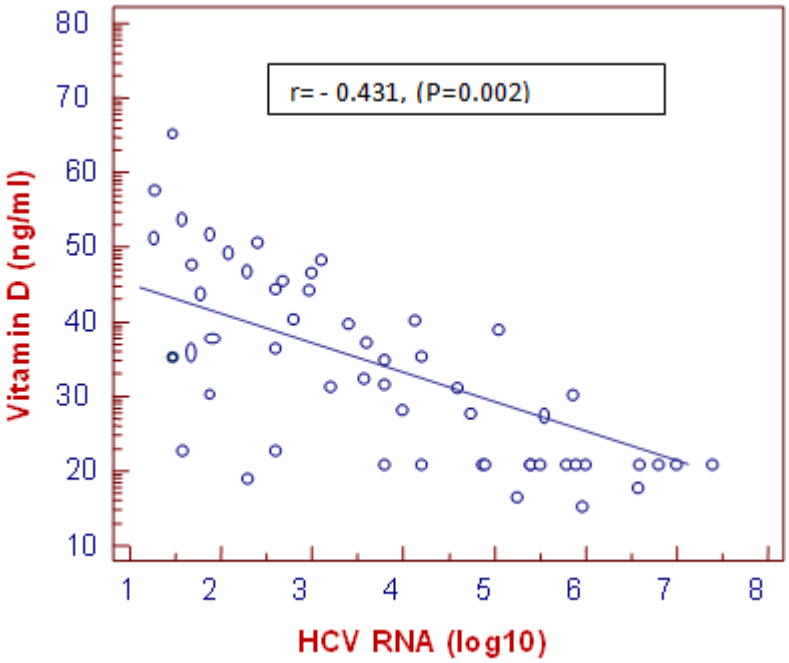
| Variable | p value (Univariate) | p value (Multivariate) |
|---|---|---|
| Age(yrs) | 0.044 | 0.184 |
| BMI (kg/m$^2$) | 0.484 | 0.908 |
| HB (g/dl) | 0.356 | 0.172 |
| Platelets(10$^{9/l}$) | 0.013 | 0.372 |
| Creat. (mg/dl) | 0.021 | 0.265 |
| Bilirubin (mg/dl) | 0.034 | 0.661 |
| AST (mg/dl) | 0.017 | 0.942 |
| ALT(mg/dl) | 0.007 | 0.765 |
| Albumin (g/dl) | 0.177 | 0.887 |
| Prothrombin Time (seconds) | 0.055 | 0.70 |
| TSH (µIU/ml) | 0.281 | 0.467 |
| HCV RNA log10 (IU/mL) | 0.001 | 0.002 |

In both univariate and multivariate analyses, HCV RNA was the major determinant factor of low 25(OH)D levels (r=-0.431, p=0.002).

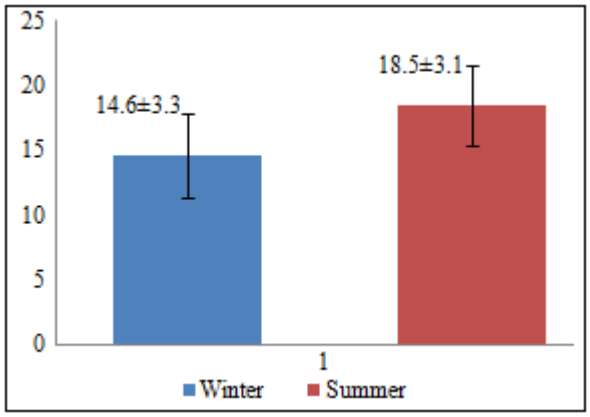
**Table 5:** Pearson Correlation between HCV RNA and 25(OH)D levels:

| | |
|---|---|
| Correlation coefficient r | - 0.431 |
| Significance level | 0.002 |

**Scatter plot depicting negative correlation between vitamin D levels and HCV RNA levels:**




The mean vitamin D serum concentrations in autumn-winter and spring-summer months were 14.6±3.3 and 18.5±3.1ng/ml, respectively. The difference between seasons was statistically significant ($p < 0.0001$).




Bar chart depicting the seasonal variation of vitamin D levels:

## 6. Discussion

Recently, it has been recognized that vitamin D has other functions in addition to its role in bone metabolism[21]. It has been demonstrated that vitamin D deficiency may play a role in the development of autoimmune diseases, inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis, diabetes, certain cancer types, cardiac failure, stroke and infectious diseases such as tuberculosis and pneumonia, and that vitamin D supplementation is efficacious in these patients[22-26].

The high prevalence of vitamin D deficiency in patients with chronic liver illness occurs regardless of disease etiology. It was recently suggested that vitamin D may impact both clinical outcomes and clinical response in patients with CHC. Several in vitro studies have demonstrated that vitamin D inhibits HCV replication in a dose dependent manner[27,28].There are conflicting data in the literature regarding the relationship between baseline 25OHD levels and the attainment of SVR. A few studies did not found any association between vitamin D deficiency and the rates of viral clearance[29]. A meta-analysis by Villar LM et al demonstrated high prevalence of vitamin D deficiency and high SVR in individuals with higher serum vitamin D levels or receiving vitamin D supplementation[30].

There are a few papers discussing the possible links between low 25(OH)D levels and HCV virological parameters.

Aleksandra Berkan-Kawińska et al[31] assessed the relationship between HCV RNA and serum vitamin D levels. They found that patients having lower HCV RNA had higher serum vitamin D levels but this difference was not statistically significant. Ladero et al[32] also did not found any statistically significant relationship between serum vitamin D levels and HCV RNA levels even after normalization of serum vitamin D levels. In another study from china by Song Binbin et al[33], no significant difference for serum vitamin D levels between HCV RNA positive and HCV RNA negative groups were seen.

In a recent study by Gerova et al[30] significantly low serum vitamin D levels were observed in patients having high viral RNA loads ($p<0.01$).

In our study 60% of population was male and the mean age of population under study was 452.7 years. The mean serum vitamin D levels in our study population were 21.1±15.2 ng/ml. Our all patients were of genotype 3.The mean HCV RNA levels of the whole cohort were 3.4 ± 2.8 log10 IU/ml which is comparable to other studies.

Of the entire cohort, 18% had severe vitamin D deficiency [25(OH)D <10ng/ml], 58% had insufficient vitamin D levels [25(OH)D ≥10 and <20ng/ml] and 24% had optimal vitamin

D levels [25(OH)D ≥ 20ng/ml] (p <0.0001) which is almost comparable with the study by Gerova etal[30].

In our cohort, HCV RNA viral load appears to be the strongest determinant of low 25(OH)D serum levels which is in contrast with the previous studies[30,34,33]. Patients with HCV RNA viral load <400000 IU/ml, had substantially higher mean 25(OH)D serum levels, compared to patients with HCV RNA >4,00000 IU/mL (24.5 versus 12.5 ng/mL, respectively P<0.01) which is comparable with the study by Gerova. Moreover we observed a significant inverse relationship between serum vitamin D levels and HCV RNA levels (r=-0.431,p=0.002) which is in contrast to previous studies.

There are few studies in the literature taking into account and discussing seasonal variations in vitamin D status in the course of HCV infection. Bitetto et al[26] identified season (winter) and the age of over 50 years as an independent predictors of low vitamin D serum levels in patients with CHC. In the same study the authors concluded that the higher histology stage and season (winter or summer) were the only independent predictors of low vitamin D serum levels. Our results also demonstrated a significant seasonal variation in vitamin D levels for the entire studied HCV-cohort. Studies by Gerova et al and Aleksandra et al also showed the similar results.

The mean vitamin D level in HBV infected patients was lower than in HCV infected patients in our patients (20.4 ng/ml vs. 21.1.3 ng/ml; $p = 0.81$).

## 7. Conclusion

1) HCV RNA viral loads appears to be the strongest determinant of low 25(OH)D serum levels in treatment naïve CHC patients.
2) HCV RNA viral loads appear to have an inverse relationship with serum vitamin D levels.
3) Serum Vitamin D levels appears to have significant seasonal fluctuations.

## 8. Abbreviations

| | |
|---|---|
| HCV | Hepatitis C virus |
| CHC | Chronic Hepatitis C |
| SVR | Sustained Virological Response |
| 25(OH)D | 25-Hydroxy Vitamin D |
| VDR | Vitamin D Receptor |
| CLIA | Chemiluminescence Immunoassay |
| RBV | Ribavirin |
| PEG-IFNα | Pegylated Interferon α |

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