

Original Research Article

Relationship of serum vitamin D with hepatic fibrosis in patients with chronic hepatitis C

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ABSTRACT

Background: Serum vitamin D concentration is proposed to have an important role on outcome in patients with chronic hepatitis C virus (HCV) infection. A few studies have shown an inverse association of vitamin D level with stage of fibrosis. The aim of the present study was to verify whether serum vitamin D level is an independent predictor of significant hepatic fibrosis.

Methods: Seventy-two treatment naive chronic HCV subjects and 40 healthy age and sex matched controls were included in the study. A serum vitamin D level was assessed in both HCV subjects and controls, and liver biopsy was performed in all HCV subjects to assess for stage of fibrosis.

Results: Serum vitamin D levels were significantly lower HCV patients in comparison to age and sex matched controls (18.04 ± 6.92 versus 21.53 ± 8.2 , $p < 0.01$). Most common genotype in HCV patients was genotype 3 (62.5%) and blood transfusion was the most common mode of transmission (28%) followed by intravenous drug user (IVDU) (17%). The HCV patients with vitamin D level < 20 ng/ml had higher metavir score as compared to vitamin D ≥ 20 ng/ml (1.67 ± 0.66 versus 2.5 ± 0.67 , $p < 0.001$). Both univariate and multivariate analysis performed using logistic regression revealed that vitamin D < 20 ng/dl is a significant negative predictor of liver fibrosis ($p < 0.05$).

Conclusions: Chronic HCV patients had significantly lower vitamin D levels as compared to healthy controls. Serum vitamin D was a negative predictor of stage of fibrosis in patients with chronic hepatitis C.

Keywords: Vitamin D, Liver fibrosis, Chronic hepatitis C

INTRODUCTION

Chronic hepatitis C owes a serious threat to global health with 170 million people affected worldwide that accounts for about 3% of the world's population. Chronically infected hepatitis C individuals often develop severe liver inflammation and fibrosis, bearing an increased risk of liver cirrhosis and hepatocellular carcinoma (HCC) if left untreated. Hepatitis C virus (HCV) continues to be a leading cause of end stage liver disease with 28% cases of

cirrhosis and 26% cases of liver cancer globally.^{1,2} Approximately 12-18 million people are infected with HCV in India, a significant share of the global HCV prevalence. In northern part of India, HCV accounts for 20% of the total chronic hepatitis patients.^{3,4}

Serum vitamin D concentration, is of great clinical interest because it is easily modifiable by dietary supplementation. Vitamin D is a potent immunomodulator. 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*. Low serum level of 25-hydroxy vitamin D (<20 ng/ml) prevents macrophages from initiating this innate immune re-sponse.^{5,6} Multiple proinflammatory markers like tumor necrosis factor- α (TNF- α) and chemokine interferon- γ inducible protein 10 (CXCL10) are strongly associated with chronic HCV infection, correlating with extent of hepatic inflammation and treatment outcome.^{7,8} Moreover, vitamin D improves suppresses these pro-inflammatory cytokines, increases anti-inflammatory cytokines, and improves cluster of differentiation 4 (CD4) T cell hyper-responsiveness.⁹

A large number of studies have shown the relationship of low vitamin D level with poor outcome in HCV patients. The majority of studies have found low 25(OH)D levels in patients with chronic HCV infection and severity of liver disease correlated with extent of vitamin D deficiency.¹⁰⁻¹² Chronic HCV infection can result in excessive accumulation of extracellular matrix resulting in liver fibrosis. Studies have shown that, age, male sex, duration of infection, diabetes, alcohol consumption and co-infection with human immunodeficiency virus (HIV) are associated with progression of liver fibrosis.¹³ There is an inverse correlation of vitamin D level with stage of hepatic fibrosis, as shown in multiple studies. This effect is probably due to anti-inflammatory effect of vitamin D in causing less hepatic inflammation and fibrosis.^{10,11} There is a few studies from India in available literature depicting the association of vitamin D levels with liver fibrosis.

The aims of the present study were to ascertain whether vitamin D deficiency has any role in promoting hepatic fibrosis in patients chronically infected with HCV and to compare vitamin D levels with age and sex matched controls.

METHODS

This cross-sectional study was conducted from September 2013 to September 2016 in the Department of Gastroenterology, Institute of Medical Sciences (IMS), Banaras Hindu University, Varanasi, Uttar Pradesh. The cases were included from Gastroenterology/Hepatology outpatient and inpatient clinics of Sir Sunderlal Hospital, IMS, Banaras Hindu University.

Inclusion criteria

Age more than or equal to 18 years.

Patients with chronic hepatitis C with compensated cirrhosis with Child Pugh A: serum total bilirubin ≤ 2.5 mg/dl, serum albumin level ≥ 3 g/dl, and international normalized ratio ≤ 1.5 .

Exclusion criteria

Patients with advanced cirrhosis (Child-Pugh B or C).

Patients with hepatocellular carcinoma.

Human immunodeficiency (HIV) and/or hepatitis B virus (HBV) co-infection.

Concomitant use of medications, which are known to affect serum vitamin D metabolism.

Patients with concomitant alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), diabetes mellitus, chronic kidney disease, bone disease and thyroid disease.

Patients with active intravenous drug addiction.

Patients who did not give consent for liver biopsy.

One hundred twenty patients of chronic hepatitis C patients were screened for the study. After exclusion, a total of seventy-two compensated chronic HCV patients were enrolled in the study. Consecutive 40 healthy individuals without any active disease and who had normal liver on trans-abdominal sonography were included as the controls for the study. A detailed clinical history, family history of liver diseases, history of ascites, jaundice, encephalopathy and gastrointestinal bleeding were taken. Presence of concomitant medical conditions including alcoholism, renal failure and diabetes were recorded. Drug history was elicited to identify use of hepatotoxic drugs. A detailed clinical examination was done to look for the signs of liver failure and associated co-morbidities. The study was approved by institutional review board and informed consent were taken from the participants.

Laboratory measurement

An overnight fasting blood sample was drawn to determine baseline blood tests, including HCV ribonucleic acid (RNA) quantification using real-time polymerase chain reaction (COBAS TaqMan, Roche), and HCV genotype using the InnoLipa genotyping kit (Innogenetics, Zwijndrecht, Belgium).

Vitamin D assay and homeostatic model assessment (HOMA) score

Serum 25-OH vitamin D levels were measured using a chemiluminescent immunoassay (Abbott, USA). The values were expressed as nanograms per millilitre (ng/ml). The serum samples were also used to measure glucose and insulin levels. The insulin level was determined using an electro-chemiluminescence immunoassay (Immulite 1000, Siemens, Gwynedd, UK). The homeostasis model assessment score was calculated as described by Bonara et al.¹⁴

Histology

Seventy-two patients underwent liver biopsy as per standard percutaneous technique. The biopsy specimens were fixed in 10% formalin and stained with hematoxylin

and eosin (H&E) and reticulin stains. A senior pathologist blinded to patient's identity coded and read the slides. Minimum length of 15 mm of biopsy specimen or presence of at least 10 portal tracts was required. Stage of fibrosis was scored according to the metavir scoring system.

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences version 16 (SPSS 16 Inc., Chicago, IL, USA). Continuous variables are presented as the mean±standard deviation and categorical variables are presented as frequencies (%). The association between categorical variables were evaluated using a Pearson chi-squared test and, when appropriate, a chi squared test for linear trend. Stepwise logistic regression analysis was performed to identify independent predictors of sustained virological response (SVR). For all statistical tests, a two-sided p value of less than 0.05 was considered significant.

RESULTS

Baseline characteristics of HCV patients and control group were depicted in Table 1. Mean age of HCV subjects was 44.99±9.47 years and of controls was 41.15±10.05 years (p=0.07). There was slight male preponderance in our study population, and this was not statistically significant. Body mass index (BMI) of HCV patients and controls were similar (p=0.86). Total platelet count (173.77±44.3 versus 208.1±29.57, p<0.001), serum albumin (3.67±0.33 versus 4.04±0.22, p<0.001) was significantly higher in chronic HCV patients as compared to controls. Serum

vitamin D (18.04±6.92 versus 21.53±8.2, p<0.01) was significantly lower and serum alanine aminotransferase (ALT) (83.28±29.64 versus 33.88±4.59, p<0.001) was significantly higher in HCV patients than the age and sex matched controls (Figure 1). Most common genotype of HCV was genotype 3 (46%) followed by genotype 1 (34.7%). Genotype 2 was detected in 12.3% and genotype 4 in 7% of HCV patients. Among the risk factors for HCV acquisition, most common mode of acquiring HCV infection was blood transfusion (28%) followed by intravenous drug use (IVDU) (17%), sexual promiscuity (15%) and iatrogenic procedures (14%). The mode of transmission could not be attributed to any cause in 26% of patients.

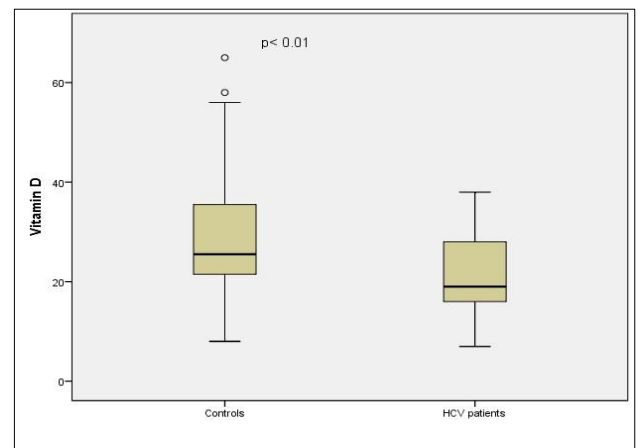


Figure 1: Box plot analysis showing vitamin D levels in HCV subjects and controls.

Table 1: Demographic profile and biochemical features of CHC patients and controls.

Parameters	HCV patients (n=72)	Controls (n=40)	P value
Age in years	44.99±9.47	41.55±10.05	0.07
Male (sex)	40 (55%)	28 (70%)	0.16
BMI (kg/m ²)	24.51±3.15	24.40±3.39	0.86
Platelet count (10 ³ /ml)	173.77±44.3	208.1±29.57	0.001*
Serum ALT (mg/dl)	83.28±29.64	33.88±4.59	0.001
Serum albumin (g/dl)	3.67±0.33	4.04±0.22	0.001
Vitamin D (ng/dl)	18.04±6.92	21.53±8.2	0.01*
Genotype	Genotype 3- 45 (62.5%)		
	Genotype 1- 17 (23.6%)		
	Genotype 2- 6 (8.4%)		
	Genotype 4 - 4 (5.5%)		

HCV RNA: hepatitis C RNA; HOMA-IR: homeostatic model assessment; ALT: alanine transaminase; BMI: body mass index; IVDU: intravenous drug users

Relationship of vitamin D status with different variables in CHC patients is shown in Table 2. The HCV patients with vitamin D level <20 ng/ml had higher metavir score as compared to vitamin D ≥20 ng/ml (1.67±0.66 versus 2.5±0.67, p<0.001). The other parameters like age of the patient, male sex, baseline serum ALT, HCV RNA, HOMA IR and Serum albumin had no relationship with serum vitamin D levels (p>0.05).

The factors predictive of higher grade of hepatic fibrosis is depicted in Table 3. Univariate analysis performed using stepwise logistic regression showed that vitamin D and age >50 years were significant predictors of liver fibrosis. However, HOMA IR (OR=1.23, 95%CI: 0.92-1.63, p=0.59), age, sex and HCV RNA levels were not found to be predictors of liver fibrosis in community health center (CHC) patients. Multivariate analysis also found serum

vitamin D and age >50 years (OR-0.75, 95% CI 0.57-0.99, $p < 0.04$ and OR-1.88, 95% CI 1.29-2.73, $p < 0.01$) as

significant predictors of hepatic fibrosis in patients with chronic hepatitis C.

Table 2: Demographic profile and biochemical features of CHC patients and controls.

	Vitamin D level <20 ng/ml (n=42)	Vitamin D level \geq 20 ng/ml (n=30)	P value
Age >50 years	39.12 \pm 11.96	43.30.8 \pm 11.45	0.14
Male (sex)	25 (60%)	15 (50%)	0.36
Platelets ($10^3/\text{ml}^3$)	177.2 \pm 42.01	169.43 \pm 46.17	0.45
Serum ALT	87.40 \pm 31.40	77.5 \pm 26.09	0.16
HCV RNA >6 lac U/ml	89.11 \pm 159.71	62.04 \pm 181.73	0.49
Albumin	3.62 \pm 0.31	3.73 \pm 0.35	0.15
Metavir score	2.5 \pm 0.67	1.67 \pm 0.66	0.001**
HOMA-IR	2.93 \pm 2.24	2.37 \pm 2.09	0.28

HCV RNA: hepatitis C RNA; HOMA-IR: homeostatic model assessment; ALT: alanine transaminase

Table 3: Logistic regression analysis showing factors predicting higher grade of hepatic fibrosis (>F2).

	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	AOR	95% CI	P value
Age >50 years	1.93	1.38-2.69	0.001	1.88	1.29-2.73	0.01
Male (sex)	0.53	0.18-1.38	0.18			
HCV RNA >6 lac U/ml	0.42	0.15-1.17	0.9			
HOMA-IR	1.01	0.8-1.2	0.87			
Vitamin D	0.79	0.69-0.89	0.001	0.75	0.57-0.99	0.04

HCV RNA: hepatitis C RNA; HOMA-IR: homeostatic model assessment; OR: odd's ratio; AOR: adjusted odd's ratio; CI: confidence interval

DISCUSSION

Vitamin D is now recognized for its pleiotropic actions in addition to maintenance of bone homeostasis.¹⁵ Furthermore, vitamin D has a major role in regulation of immune responses and multiple studies have shown that vitamin D deficiency is associated with wide spectrum of diseases, including autoimmune diseases, bronchial asthma, several malignancies and osteoporosis.¹⁶ Though vitamin D deficiency is more frequent in healthy individuals, it is more pronounced in patients with liver diseases. Vitamin D deficiency may increase hepatic fibrosis, hepatic decompensation and liver related mortality. And also, liver disease may increase the risk of vitamin D deficiency.¹⁷

In our study, patients with chronic hepatitis C had significantly lower vitamin D levels than age and sex, matched controls. A study from Silicy found that vitamin D levels were significantly lower in HCV patients as compared to healthy age and sex matched controls, similar to findings of our study.¹⁸ The possible mechanism of low vitamin D is alteration of lipid metabolism by HCV, thus reducing the synthesis of vitamin D level. Another study revealed that HCV reduces production of 7 dehydrocholesterol, a precursor of vitamin D causing low vitamin D levels in these patients.¹⁹

Present study revealed that vitamin D level was independently associated with presence of severe hepatic fibrosis in HCV patients. Petta et al in their cross-sectional

study also found a negative correlation between vitamin D status with severe necro inflammatory activity. Few association studies also showed inverse association of vitamin D levels with stage of liver fibrosis.^{10,11} Kitson et al found that vitamin D deficiency was more prevalent among higher stage of fibrosis in HCV patients.²⁰ One of our previous study from Varanasi also revealed the negative association of vitamin D level with stage of fibrosis.²¹ The relation of vitamin D with stage of fibrosis could be attributable to anti-inflammatory activity of vitamin D, recruitment of less inflammatory cells leading to less fibrosis.²²

Among the risk factors for HCV acquisition, blood transfusion was the most common occurring in one third of patients followed by IVDU and iatrogenic procedures. An Indian study by Gupta et al depicted blood transfusion a most common risk factor for HCV acquisition.²³ Few western studies had identified intravenous drug use as the leading risk factor for HCV.²⁴ Our study depicted that genotype 3 was the most common genotype (62.5%) in patients with hepatitis C virus infection followed by genotype 1.

In a study from Varanasi, genotype 3 was seen in 70% and genotype 1 in 10%.⁴ Abraham et al from India reported higher percentage (77%) of genotype 3 in their study population.²⁵ Singh et al from New Delhi, India also found genotype 3 as the most common genotype in 61% of patients.²⁶ The genotypic distribution of HCV our study is at par with other Indian studies.

Although a promising study, this study has some limitations. First, relatively lower number of patients enrolled in the study. Second, it is a cross sectional study, hence unable to analyze the temporal relationship of vitamin D status with stage of fibrosis. Moreover, whether stage of fibrosis regresses with vitamin D supplementation, needs to be ascertained in longitudinal studies. Another limitation of this study is lack of data on confounding factors like exposure to sunlight and prevalence of osteoporosis in HCV patients which can affect serum vitamin D levels. The strength of the study is that metavir score, assessed by liver biopsy correlated with vitamin D levels. A significant correlation of vitamin D level with stage of fibrosis was found in our study, further studies on effect of vitamin D supplementation on regression of liver fibrosis may be undertaken in future to validate our finding.

CONCLUSION

The present study confirms that chronic HCV patients had significantly lower vitamin D levels as compared to age and sex matched healthy controls. Serum vitamin D was an independent predictor of stage of fibrosis in chronic hepatitis C patients, hence opening a new area of research on the effect of vitamin D supplementation on stage of liver fibrosis.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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