

# Evaluation of Vitamin D Deficiency in Patients with Chronic Liver Disease and its Clinical Significance

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## ABSTRACT

**Introduction:** Vitamin D deficiency has been reported highly prevalent in Chronic liver disease (CLD) and there is an emerging interest to explore the relationship of vitamin D deficiency and severity of various types of CLD.

**Aim:** To evaluate vitamin D level in patients with various type of CLD and clinical significance of its deficiency.

**Materials and Methods:** Serum vitamin D levels were measured by ELFA in 100 patients (91 male and 9 female) suffering from CLD. The degree of liver dysfunction was estimated by Child Pugh criteria and Model for End stage Liver Disease (MELD) score.

**Results:** Prevalence of vitamin D deficiency and insufficiency were 43% and 42% respectively among CLD patients. Low levels of vitamin D were associated with leucopenia or leucocytosis indicating increased risk of infection. On Linear regression vitamin D level showed significant negative correlation with Child Pugh score ( $r = -0.7382$ ,  $p < 0.0001$ ) and MELD score ( $r = -0.6673$ ,  $p < 0.0001$ ). Our study shows low vitamin D level was associated with poor outcome (mean vitamin D level  $10.38 \pm 2.35$  who died vs  $23.14 \pm 6.68$  who survived and discharged).

**Conclusion:** CLD is associated with a significantly low level of vitamin D which was independent to patient's gender, BMI, residence and education level. The lower level of vitamin D is associated with severity of CLD, mortality and increased risk for infections. Awareness of serum vitamin D level in patients with CLD is important to improve outcome.

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## Introduction

Chronic liver disease (CLD)<sup>1</sup> is major sources of morbidity and mortality all over world, it accounts for about 2% of all deaths in Europe (170,000/year) with increasing mortality rates in several countries.<sup>2</sup> Alcohol abuse and infections with hepatotropic viruses, especially hepatitis C virus (HCV) infection, have been the predominant risk factors for cirrhosis, however, recent study on multiethnic cohort at United States has shown Nonalcoholic fatty liver disease (NAFLD) is the most common cause of CLD in all ethnic groups combined (52%), followed by alcoholic liver disease.<sup>3</sup> Other causes of CLD are chronic hepatitis C, chronic hepatitis B, autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis, haemochromatosis and Wilson's disease.<sup>4,5</sup> In a study conducted (unpublished data) at our area (West zone of Rajasthan, India), Alcohol induced CLD still remain most common cause.<sup>6</sup>

Vitamin D is best known for its function in calcium homeostasis and bone mineralization.<sup>7</sup> However, it has numerous additional roles such as regulation of cellular proliferation, apoptosis, differentiation and inflammation.<sup>8,9</sup> Vitamin D generation is a multi-step process involving the skin, the liver and the kidneys. Cholecalciferol is hydroxylated to the bioactive 25-hydroxyvitamin D<sub>3</sub> (25(OH)D) in the liver and is bound to the vitamin D binding protein (DBP).<sup>9</sup> 1 $\alpha$ -hydroxylase converts 25(OH)D to 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)D) mainly in the kidneys. 1,25(OH)D known as calcitriol, is the most bioactive form.<sup>9</sup> Recently, an increased incidence of 25(OH)D deficiency has been found in individuals suffering from liver diseases and the severity of 25(OH)D deficiency in the patients correlated with the severity of liver dysfunction.<sup>10,11,12</sup> Liver disease is accompanied by activation of the innate immune system and vitamin D levels inversely correlate with the expression of toll like receptors (TLRs) in monocytes, indicating an inverse

correlation between vitamin D levels and systemic inflammation.<sup>10</sup> Strong inflammatory conditions displayed by high levels of C-reactive protein (CRP) or soluble CD163 (sCD163) are associated with an unfavorable prognosis in patients with cirrhosis.<sup>13,14</sup> Observations in cirrhotic patients showed a poor prognosis in individuals with low 25(OH)D levels.<sup>15,16</sup>

## Material and Methods

This study was carried out in the Department of Medicine, S.P. Medical Collage Bikaner on 100 patients suffering from chronic liver disease admitted in medical wards, attending medical outdoor and gastroenterology outdoor, during the period of June 2014 to November 2015.

**INCLUSION CRITERIA:** (1) Patients suffering from CLD. (2) Patients given consent to participate in the study.

**EXCLUSION CRITERIA:** (1) Patient suffering from other co-morbid conditions like diabetes mellitus, Ischemic heart disease, malignancy or other chronic conditions. (2) Patient already on vitamin D<sub>3</sub> therapy. (3) Patients who are not giving consent for the study.

All patients were subjected to clinical and laboratory evaluation as per proforma. Complete blood count, blood sugar, liver function test, renal function test, PT-INR, HBsAg, ANTI-HCV, Ultrasonography abdomen, and upper GI endoscopy was done in all the patients. Severity of CLD was defined as per Child Pugh criteria<sup>17</sup> and MELD system<sup>18</sup>. MELD system provides a more objective means of assessing disease severity and has less center-to-center variation than the Child-Pugh score as well as wider range of values.

Blood samples for vitamin 25(OH)D estimation were taken in dark room and estimation was done by Enzyme Linked Fluorescent Assay (ELFA) technique by using kit VIDAS® 25 OH Vitamin D TOTAL (VITD) and VIDAS® automated immunoanalyzer.

## Results

In our study, we enrolled 100 patients. Out of them, 91 (91%) were males and remaining 9 (9%) were females. The mean age was  $46 \pm 10$  (males) and  $45 \pm 19$  (females). 77 (77%) of the cases were suffering from alcoholic CLD (76 males and 1 female) and in this 11 males belonged to age group 18-35years, 54 to 36-55yrs and 11 were >55 years old. The only female was 45 years old. Two patients were suffering from Hepatitis B related CLD (male=1, female=1), 6 patients Hepatitis C (male=5, female=1), 3 with autoimmune CLD (male=0, female=3) and 12 were suffering from cryptogenic CLD (male=9, female=3). This showed alcohol was the most common cause of CLD in males and autoimmune CLD was common in females. The distribution of vitamin D levels is shown in table 1.

The mean level of vitamin D was  $23.38 \pm 8.36$  in the age group of 18 – 35 years (n=17),  $22.58 \pm 7.38$  in 36 – 55 years (n=65) and  $17.81 \pm 5.53$  in  $\geq 55$  years old (n=18). This shows that lower levels of vitamin D level was associated with increasing age ( $p < 0.05$ ). The mean vitamin D was  $21.73 \pm 6.10$  in patient with BMI <18.5 (n=8),  $22.18 \pm 7.83$  in patient with BMI 18.5 – 24.99 (n=83),  $19.30 \pm 3.00$  in patients with BMI 25 – 29.99 (n=7) and  $18.25 \pm 8.13$  in patient with BMI  $\geq 30$  (n=2) ( $p > 0.05$ ). We found inverse relationship of mean vitamin D level and BMI, least mean level of vitamin D was found in obese patients.

Out of 77 patients with alcoholic CLD, the consumption of alcohol was >200gm/day in 26 (33.77%) patients, 150-200 gm/day in 21(27.27%) patients, 100-150 gm/day in 18 (23.37%) patients and <100 in 12 (15.58%) patients. The mean levels of vitamin D were  $19.21 \pm 8.38$ ,  $21.31 \pm 7.47$ ,  $23.67 \pm 7.18$  and  $25.27 \pm 6.74$  respectively. We observed that vitamin D level decreases with increasing consumption of alcohol although it was not statistically significant ( $p = 0.093$ ).

The mean vitamin D level was  $25.58 \pm 7.19$  with bilirubin level <2 mg/dl (n=35),  $21.99 \pm 4.08$  with bilirubin level 2 – 3 mg/dl (n=16) and  $19.16 \pm 7.42$  with

bilirubin level >3 mg/dl (n=49). The mean vitamin D level decreases with increase in bilirubin level ( $p < 0.05$ ).

The mean vitamin D level was  $34.57 \pm 2.22$  with MELD score <9 (n=3),  $24.61 \pm 5.96$  with MELD score 10 – 19 (n=51),  $20.08 \pm 6.47$  with MELD score 20 – 24 (n=34),  $14.70 \pm 3.82$  with MELD score 30 – 39 (n=7) and  $8.40 \pm 0.547$  with MELD score  $\geq 40$  (n=5). Low levels of vitamin D were significantly related with higher score of MELD ( $p < 0.05$ ) table 2. The mean vitamin D level was  $32.90 \pm 4.06$  with Child-Pugh Class A (n=12),  $23.02 \pm 5.37$  with Child Pugh Class B (n=57) and  $15.46 \pm 5.44$  with Child-Pugh Class C (n=31) table 3. There was consistent trend towards lower vitamin D level with increasing severity of cirrhosis ( $p < 0.05$ ). On Linear regression vitamin D level showed significant negative correlation with Child Pugh score ( $r = -0.7382$ ,  $p < 0.0001$ ) and MELD score ( $r = -0.6673$ ,  $p < 0.0001$ ).

Out of 100 cases, 90(90%) patients were discharged and death occurred in 10 (10%) patients. The mean vitamin D level was  $10.38 \pm 2.35$  in died patients and  $23.14 \pm 6.68$  in discharged patients. This shows low vitamin D level was associated with poor outcome. On applying unpaired T-test the difference of mean vitamin D level is statistically significant ( $P < 0.05$ ).

## Discussion

In our study, we enrolled 100 patients with mean age  $46.05 \pm 11.31$  years. Out of them, 91 (91%) were male and remaining 9 (9%) were female. In our study, most of male patients belong to age group 36-55 years (n=65), because alcoholism is common in this age group. Whereas most of the females was in age group 18-35 years, because autoimmune diseases commonly manifest in this age group.

We found low prevalence of CLD in Females in contrast to earlier study done by Putz Bankuti C et al,<sup>19</sup> which consists of 51 males (68%) and 24 females (32%) with a mean age of  $58 \pm 11$  years (range: 25–89 years). This is because of most of cases in our study belonged

**Table 1 :** Distribution of vitamin D level

	Vitamin D Level			
	<10	10 – 20	21 – 30	>30
No. of patients (n=100) (%)	5 (5%)	38 (38%)	42 (42%)	15(15%)
<b>Sex</b>				
Male (n=91)	5 (5.49)	35 (38.46)	37 (40.66)	14 (15.38)
Female (n=9)	-	3 (33.33)	5 (55.56)	1 (11.11)
Mean age	46.8 ± 12.39	49.82 ± 11.97	43.57 ± 10.84	43.2 ± 8.44
<b>Age (years)</b>				
18-35 (n=17)	01	04	08	04
36-55 (n=65)	03	22	30	10
>55 (n=18)	01	12	04	01
Total	05	38	42	15
<b>Etiology of CLD</b>				
Alcoholic (n=77) (%)	5 (6.49)	29 (37.66)	31 (40.26)	12 (15.58)
HBV (n=2)	-	-	2 (100)	-
HCV (n=6)	-	4 (66.67)	1 (16.67)	1 (16.67)
Auto-immune (n=3)	-	-	3 (100)	-
Cryptogenic (n=12)	-	5 (41.67)	5 (41.67)	2 (16.66)

**Table 2 :** Comparison of mean value of vitamin D level in relation to MELD score

Parameter	MELD Score					p value	significance
	<9 (n=3)	10 - 19 (n=51)	20 - 24 (n=34)	30 - 39 (n=7)	>40 (n=5)		
vitamin D mean ± SD	34.57± 2.22	24.61 ± 5.96	20.08 ± 6.47	14.70 ± 3.82	8.40 ± 0.547	<0.0001	Yes

**Table 3 :** Comparison of mean value of vitamin D level in relation to Child-Pugh Score

Parameter	Child Pugh Score			p value	significance
	Class A (5 – 6) (n=12)	Class B (7 – 9) (n=57)	Class C (10 – 15) (n=31)		
vitamin D mean $\pm$ SD	32.90 $\pm$ 4.06	23.02 $\pm$ 5.37	15.46 $\pm$ 5.44	<0.0001	Yes

to alcoholic CLD and alcoholism is common in males but very rare in females in our area.

We found vitamin D deficiency (<20 ng/dl) in 43 (43%) patients, out of them 5(5%) suffered from severe vitamin D deficiency(<10 ng/dl).Vitamin-D insufficiency (21-29.9 ng/dl) was found in 42(42%). Thus, vitamin D levels were sub normal in 85 (85%) patients. Similar observations has been made by earlier workers.<sup>20-22</sup> Possible explanations of vitamin D deficiency in CLD could be because of decrease vitamin D hydroxylation and vitamin D binding protein (DBP) production<sup>21</sup>, inadequate sun exposure, insufficient food intake, steroid use, jaundice related deterioration of vitamin synthesis on the skin and decreased vitamin D absorption caused by intestinal edema secondary to portal hypertension or to cholestasis induced bile salt disruption.<sup>23</sup>

On Linear regression analysis of vitamin D level we found significant negative correlation with Child-Pugh score ( $r = -0.7382, P < 0.0001$ ) and MELD score ( $r = -0.6673, P < 0.0001$ ). So low 25(OH)D levels were associated with increased severity of liver disease. Similar observations has been made by Miroliaee A et al<sup>20</sup>, Putz-Bankuti C et al.<sup>19</sup> and Finkelmeier F et al.<sup>22</sup> We also found low vitamin D level was associated with poor outcome similar to Finkelmeier F et al.<sup>22</sup> Thus our results suggest that vitamin D might be both a biomarker of severity and a potential therapeutic target in CLD.

**Conclusion:**

Our study concludes that CLD is associated with a significantly low level of vitamin D which was

independent to gender, BMI, residence and education level. The lower level of vitamin D is associated with severity of CLD, mortality and increased risk for infections. The findings of our study suggest that awareness of serum vitamin-D level in patients with CLD is important. Further studies are required to validate the importance of vitamin D levels in CLD patients by comparing with control normal healthy subjects and by doing intervention in the form of supplementation of vitamin D in CLD subjects.

**Authors Contribution:** Designed the study: BKG. Drafted the manuscript: BKG, HRN and MLS. Approved the final version to be published: BKG. Carried out clinical assessment, data collection and review of literature: BKG, SLM, MLS, HRN, MS and JG. Evaluated and analyzed laboratory data and their interpretation: BKG, HRN and MLS. All authors read and approved the final manuscript. Guarantors of the paper: BKG and HRN.

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**Ethical Approval:** A prior approval has been taken from the Institutional Ethics Committee to carry out this work, and an informed consent was obtained from the subjects enrolled in this study.

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