

Serum 25-hydroxyvitamin D level in patients with chronic liver disease and its correlation with hepatic encephalopathy: A cross-sectional study

Priyadarshi Kumar¹, Sumita Chaudhry¹, Nishanth Dev¹, Rahul Kumar¹, Gaurav Singh¹

¹Department of Medicine, VMMC and Safdarjung Hospital, Delhi, India

ABSTRACT

Background: Hepatic encephalopathy (HE) is an established clinical manifestation in chronic liver disease (CLD). It is associated with various factors including gastrointestinal bleed, constipation, and dyselectrolyemia. Recently 25-hydroxyvitamin D (25-OHD) deficiency has been identified as one of the factors associated with the development of HE. The current study was aimed to assess the level of 25-OHD in patients with CLD and hepatic encephalopathy and the relationship between 25-OHD deficiency and hepatic encephalopathy. **Materials and Methods:** This cross-sectional study included 100 subjects of either sex between 18 and 60 years of age, diagnosed as CLD on the basis of ultrasonography with hepatic encephalopathy and 50 age, sex-matched CLD subjects without encephalopathy. Hemogram, hepatic and renal functions, serum electrolytes, coagulation profile, and serum 25-hydroxyvitamin D levels were recorded. **Results:** The baseline variables were matched for age, sex, hepatic and kidney function, and coagulation profiles. The hemoglobin ($P = 0.002$) and platelet count ($P = 0.0003$) were significantly lower in subjects with HE. The mean level of 25-OHD was significantly lower in subjects with HE as compared to the control group (25.62 ± 21.94 nmol/L vs 37.44 ± 18.61 nmol/L, $P < 0.001$). The mean 25-OHD level was 30.64 ± 21.64 nmol/L in grade 1 HE, 12.03 ± 11.05 nmol/L in grade 3 with $P < 0.0001$, and 18.8 ± 16.88 nmol/L in grade 4 with $P < 0.0001$ when compared to grade 1. Moderate and severe deficiency of 25-OHD level was significantly associated with higher grades of HE, i.e. grades 3 and 4 ($P < 0.0001$). There was a significant negative correlation between 25-OHD levels and worsening grades of hepatic encephalopathy (person's correlation coefficient $r = -0.354$; $P = 0.0003$). **Conclusion:** In this cohort of North Indian population, serum 25-OHD level was significantly lower in patients with CLD and HE. The levels of 25-OHD showed a significant negative correlation with hepatic encephalopathy.

Keywords: Chronic liver disease, encephalopathy, Vitamin D

Introduction

Patients with chronic liver disease (CLD) manifest various nutritional abnormalities which are associated with progression and severity of liver disease. The manifestation is believed to be due to alteration in absorption of nutrients and altered metabolism as

well as alteration in synthetic function of liver. Increasing severity and progression of disease is also associated with worsening of these alterations.^[1] A manifestation of progression of disease manifests in the form of hepatic encephalopathy (HE) which is described as a complex amalgam of neurological symptoms which include impairment of cognitive and motor functions. It is seen in up to 55% of chronic liver disease patients.^[2] Pathogenesis of HE is multifactorial, and its exact mechanism has not been clearly understood yet. For decades the pathogenesis of HE has been believed to be related to high levels of ammonia, because of

Address for correspondence: Dr. Nishanth Dev,
Department of Medicine, VMMC and Safdarjung
Hospital, Delhi, India.

E-mail: devnishant@gmail.com

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impaired detoxification in the liver, as a result of liver cirrhosis/failure or due to portosystemic shunts in patients with cirrhosis. The manifestation of hepatic encephalopathy is associated with various factors including upper gastrointestinal bleed, electrolyte imbalance, and spontaneous bacterial peritonitis.^[3-6] One of the various factors which has been identified recently to be associated with hepatic encephalopathy is 25-hydroxyvitamin D deficiency.^[7]

25-hydroxyvitamin D (25-OHD) is a group of fat-soluble Seco-steroids associated with regulation of calcium, magnesium, and phosphate metabolism and absorption. Apart from these activities, it is also associated with various other biological effects with roles suggested as an antiinflammatory and immunomodulatory agent.^[8] Its role has been demonstrated to be associated with various neurological deficits like loss of cognitive function, dementia, and Alzheimer's disease.^[9,10] 25-OHD deficiency is associated with a number of psychiatric conditions such as autistic spectrum disorder and data indicating 25-OHD deficiency in early life affects neuronal differentiation, axonal connectivity, and brain structure and function. Studies have shown that a low level of 25-hydroxyvitamin D level was associated with greater risk of cognitive impairment in older as well as younger adults by stepwise linear regression and Pearson's correlation.^[11]

25-hydroxyvitamin D also regulates cell proliferation and differentiation, and has immunomodulatory, antiinflammatory, and antifibrotic properties. These effects are relevant in the pathogenesis and treatment of many causes of chronic liver disease.^[12,13] 25-OHD deficiency is frequently present in chronic liver disease and may predict nonresponse to antiviral therapy in chronic hepatitis C. Small studies suggest that 25-OHD supplementation improves sustained viral response rates, while 1 α -hydroxylase polymorphisms and 25-OHD-binding protein are also implicated in therapeutic outcomes.^[14] 25-OHD deficiency also closely relates to the severity of nonalcoholic fatty liver disease (NAFLD) and is implicated in the pathogenesis of insulin resistance, a key factor in the development of NAFLD.^[15,16]

Few studies have also demonstrated worsening of 25-OHD deficiency to be associated with progression of disease. Patients with hepatic encephalopathy have been shown to have significantly lower vitamin D levels than nonencephalopathic patients.^[17] This correlation suggests that vitamin D deficiency may have an unrecognized role in the development of HE. However, there are very few studies which have examined the role of 25-OHD deficiency in hepatic encephalopathy. Thus, this study was planned to assess the levels of 25-OHD in patients with hepatic encephalopathy and to find its correlation with various grades of HE.

Materials and Methods

The primary objective of the study was to assess the level of 25-hydroxyvitamin D in patients with hepatic encephalopathy in cirrhosis of liver. The secondary objective of the study was

to assess the correlation between 25-hydroxyvitamin D level and grades of hepatic encephalopathy (HE) in cirrhosis of liver.

The study was conducted in the Department of Medicine at a teaching tertiary care institute in North India. For the study, persons of either sex between 18 and 60 years of age and diagnosed as a case of chronic liver disease on the basis of ultrasonography with hepatic encephalopathy diagnosed based on West haven criteria were included.^[1] Age and sex-matched individuals with chronic liver disease without hepatic encephalopathy constituted the control group.

Patients with chronic kidney disease, acute fulminant hepatic failure, recent onset cerebrovascular accidents, hepatocellular carcinoma, sepsis, and other causes of neurological deficits surgery were excluded.

The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000 and approved by the institutional review board, and informed consent was obtained from all study subjects.

Based on the previous studies, the sample size was calculated with 80% power and a 5% level of significance. We enrolled 100 patients in the study group and 50 in the control group.

A detailed record of clinical history and physical examination was done with special attention in the relevance of liver disease for all the enrolled subjects fulfilling the inclusion and exclusion criteria. All enrolled subjects underwent investigations for complete hemogram, liver function test, kidney function test, serum electrolytes, coagulation profile, and serum 25-hydroxyvitamin D levels. 25-hydroxyvitamin D level was measured by solid-phase ELISA, CALBIOTECH 25(OH) vitamin D ELISA. Results were expressed in nmol/L. Whole blood samples were collected by venipuncture and allowed to clot prior to processing.

25-hydroxyvitamin D level was defined as sufficient (>75 nmol/L), insufficient (50–75 nmol/L), mild deficiency (25–50 nmol/L), moderate deficiency (12.5–25 nmol/L), and severe deficiency (<12.5 nmol/L).^[18]

Statistical analysis categorical variables were presented in number and percentage (%) and continuous variables were presented as mean \pm SD and median. The normality of data was tested by the Kolmogorov-Smirnov test. Quantitative variables were compared using the unpaired *t*-test. Qualitative variables were compared using the Chi-square test. A correlation was assessed using Pearson's correlation coefficient. A *P* value of < 0.05 was considered statistically significant. All analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

Results

On the assessment of baseline variables, [Table 1] the age (years) of the enrolled subjects was comparable in the

study and control groups (which included both covert and overt HE) (46.77 ± 13.76 vs 43.64 ± 13.89 ; $P = 0.54$). The control group had slightly higher hemoglobin levels (g%) (8.64 ± 1.76 vs 7.076 ± 1.6 ; $P = 0.002$). The mean platelet levels (per cu mm) also showed higher numbers in control group (97846 ± 38379.99 vs 88622 ± 91194.23 ; $P = 0.0003$). The total leucocyte count, serum bilirubin, SGOT and SGPT, serum creatinine, PT, and INR were comparable in both groups. There were significantly higher levels of ALP (IU/L) in the study group (160.85 ± 48.65 vs 112.78 ± 38.65 ; $P = 0.0001$). There were significant differences in the electrolyte's levels in the study group as compared to the control group. Serum sodium levels (mEq/L) were 136.95 ± 5.1 in study against 134.6 ± 4.92 with $P = 0.017$. Similarly, serum potassium levels (mEq/L) were higher in study group (4.2 ± 0.77 vs 3.88 ± 0.65 ; $P = 0.001$). The levels of serum 25-OHD (nmol/L) were significantly lower in the study group (25.62 ± 21.94 vs 37.44 ± 18.61 ; $P < 0.0001$). The differences in the various baseline parameters between the study and the control group highlight the fact that there are variable factors associated with the development of hepatic encephalopathy.

Alcohol consumption was found to be the most common cause for chronic liver disease (62% in study vs 58% in control). Viral hepatitis and unknown causes were responsible for CLD in remaining subjects. [Table 2]

Majority of the patients in the study group had overt hepatic encephalopathy (grade 2,3,4 HE) seen in 80% of the patients. Covert HE (grade 1) comprised of the remaining 20% in the study group. [Table 3]

Serum 25-OHD levels were found to be sufficient only in 4% in study and 2% in control groups. Severe deficiency was seen in 38% in study group as compared to only 6% in the control group [Table 4]. The mean levels of serum 25-hydroxyvitamin D in various grades of hepatic encephalopathy showed significant difference. Whereas the level was 30.64 ± 21.64 in grade 1 HE, it was as low as 12.03 ± 11.05 in grade 3 HE and 18.8 ± 16.88 in grade 4 with a $P < 0.0001$ [Table 5, Figure 1].

Correlation between serum 25-OHD level and hepatic encephalopathy was assessed by Pearson's correlation coefficient and a negative correlation was found. A lower level of serum 25-OHD was associated with increasing presence of hepatic encephalopathy ($r = -0.354$; $P = 0.003$) [Figure 2].

Discussion

The current study was aimed to study the levels of 25-hydroxyvitamin D in various grades of hepatic encephalopathy and compare it with a group of subjects with chronic liver disease without hepatic encephalopathy.

The mean age of the enrolled subjects in the study group was found to be 46.77 ± 13.76 years. The age of presentation is lower

Table 1: Baseline variable in the study and control groups

Baseline Variables	Study Group (n=100)	Control Group (n=50)	P
Age (years)	46.77±13.76	43.64±13.89	0.054
Hb (g%)	7.76±1.6	8.64±1.76	0.002
TLC (per cu mm)	6462.4±3486.72	7114.8±3797.35	0.255
Platelet (per cu mm)	88622±91194.23	97846±38379.94	0.0003
Bilirubin (mg/dl)	3.6±2.32	3.86±3.77	0.06
SGOT (IU/L)	84.33±70.4	93.82±71.42	0.063
SGPT (IU/L)	73.98±49.73	75.66±37.48	0.172
ALP (IU/L)	160.85±48.65	112.78±38.65	<0.0001
Creatinine (mg/dl)	1.76±0.75	1.55±0.61	0.158
Sodium (mg/dl)	136.95±5.12	134.6±4.92	0.017
Potassium (mEq/L)	4.2±0.77	3.88±0.65	0.001
PT	16.47±2.94	15.72±2.41	0.108
INR	1.7±0.49	1.81±0.45	0.096
25-Hydroxyvitamin D Level (nmol/L)	25.62±21.94	37.44±18.61	<0.0001

Data expressed in Mean±SD unless specified, n: number, Hb: Hemoglobin, TLC: Total leucocyte count, SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, ALP: Alkaline phosphatase, PT: Prothrombin time, INR: international normalized ratio

Table 2: Etiology of chronic liver disease in control and study groups

	Control n (%)	Study n (%)	P
Etiology			
Alcoholic cirrhosis	29 (58)	62 (62)	0.939
Hepatitis B virus	09 (18)	17 (17)	
Hepatitis C virus	06 (12)	09 (09)	
Unknown	06 (12)	12 (12)	

n: number, HE: hepatic encephalopathy

Table 3: Various grades of hepatic encephalopathy in study group

HE grades in study group	n (%)
0	0 (0)
1	20 (20)
2	30 (30)
3	20 (20)
4	30 (30)

n: number, HE: hepatic encephalopathy

Table 4: Serum 25-hydroxyvitamin D levels in study and control groups

25-Hydroxyvitamin D Level (nmol/L)	Control n (%)	Study n (%)	P
Severe deficiency	03 (06)	38 (38)	<0.0001
Moderate deficiency	11 (22)	26 (26)	<0.0001
Mild deficiency	20 (40)	17 (17)	<0.0001
Insufficiency	15 (30)	15 (15)	<0.0001
Sufficient	01 (02)	04 (04)	0.121

n: number, HE: hepatic encephalopathy

than those enrolled in an Australian cohort by Vidot *et al.* in which the mean age was 53 ± 8 years.^[7] The lower age of presentation may be due to differences in the genetic, racial makeup as well tolerability and alcohol metabolism.

Table 5: Serum 25-hydroxyvitamin D levels in various grades of hepatic encephalopathy in study group

25-Hydroxyvitamin D Level (nmol/L)	HE grade 1	HE grade 2	HE grade 3	HE grade 4	P
Mean±SD	30.64±21.64	38.16±24.83	12.03±11.05	18.8±16.88	<.0001
Median	21.7	36.5	9.65	12.45	
Min-Max	5.6-78	3.49-105	3-52.5	3.97-66.4	
Interquartile Range	13-53	15.200-56	5.700-12.850	8-24	

HE: Hepatic encephalopathy, SD: Standard deviation

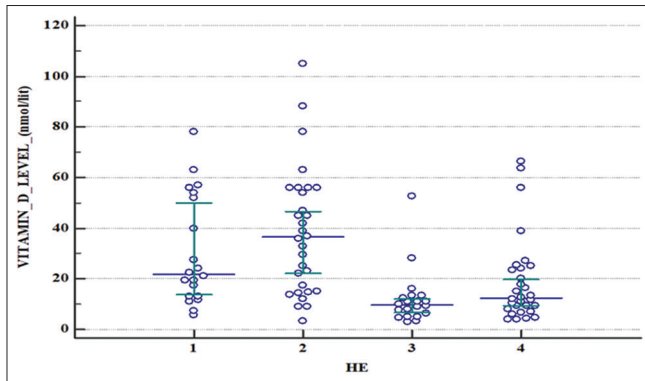


Figure 1: Whisker plot showing 25-hydroxyvitamin D levels in various grades of Hepatic encephalopathy

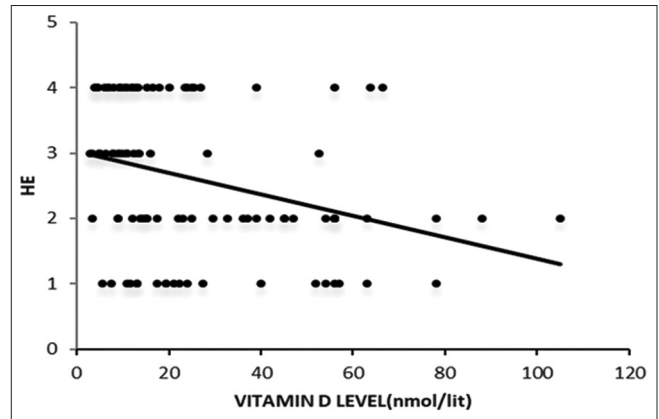


Figure 2: Shows correlation of serum 25-hydroxyvitamin D levels with hepatic encephalopathy (HE)

Males constituted the majority of the enrolled subjects which was 80% in the study group. The figure is similar to those enrolled in other studies, in which males constituted about 78% of the enrolled subjects.^[7]

There was significant difference noted in the etiology of chronic liver disease in our study as compared to other studies. Whereas alcohol intake was associated with CLD in 62% in our study, viral hepatitis was found to be the major cause of CLD in the Australian cohort, which constituted almost 55% cases.^[7] The difference may again be attributed to differences in the genetic, racial make-up as well tolerability and alcohol metabolism.

The mean level of serum 25-hydroxyvitamin D was found to be 25.62 ± 21.94 nmol/l in our study in comparison to 36 ± 15 nmol/l in the study in Australian Cohort.^[7] The lower levels in our study may be due to racial difference, the difference in body structure, poor nutrition as well as genetic make of patients. However, the levels were significantly higher than the levels in a German cohort, in which the level of 25-hydroxyvitamin D was found to be 9.2 nmol/L.^[17] 96% of the enrolled subjects in the study group were found to have some deficiency of 25-OHD in our study. The figure is similar to other studies by Vidot *et al.* where the figure was almost 98% and 92.4% in the study by Arteh *et al.* but in contrast to a figure of 61% in the study by Kubesch *et al.*^[7,17,19] Moderate deficiency of 25-OHD level was found in 26% case in our study. This finding is in accordance with various other studies.^[7] The percentage of subjects with severe deficiency of 25-OHD was slightly higher in our study and was seen in 38% of cases. The similar figure was 29.5% in the study by Arteh *et al.*^[19]

Moderate and severe deficiency of 25-OHD was significantly associated with higher grades of hepatic encephalopathy,

i.e. grade 3 and 4 with a $P < 0.0001$. The finding is similar to the Australian study where moderate deficiency was found in 49 Patients. 36 had grade 1-2 HE compared with 13 who did not have HE ($P < 0.0001$). There was significant correlation between severity of Vitamin D deficiency and severity of liver disease ($P < 0.0001$) and significant correlation between severity of liver disease and the presence of HE ($P < 0.0001$). Individuals are more likely to have Vitamin D deficiency if they had HE compared to patients without HE ($P < 0.0001$).^[7]

The study also assessed the correlation of 25-OHD levels with various grades of hepatic encephalopathy. Our results showed a strong negative correlation between 25-OHD levels and worsening grades of hepatic encephalopathy (person's correlation coefficient $r = -0.354$; $P = 0.0003$). A similar result was observed in a study assessing the correlation between the 25-hydroxyvitamin D levels and Model for end-stage liver disease with $P < 0.0001$. A similar finding was demonstrated by Kubesch *et al.* on-25-hydroxyvitamin D deficiency and its association with hepatic decompensation and inflammation in patients with liver cirrhosis also concluded that 25-hydroxyvitamin D levels were inversely associated with liver cirrhosis-related systemic inflammation and the risk of hepatic decompensation.^[17]

Although there seems a significant association between 25-OHD levels and hepatic encephalopathy, it still remains unclear whether there is a threshold level of 25-OHD beyond which there is a steady reduction in the cognitive performance of the subjects with CLD and hepatic encephalopathy. In addition, it does not prove the causal association of 25-OHD deficiency with HE. To demonstrate a causative relationship of 25-OHD with HE, it is

necessary to examine the effects of 25-OHD supplementation on encephalopathy syndrome in patients with CLD.^[20]

The current study is able to demonstrate significantly lower levels of 25-hydroxyvitamin D in patients with CLD and hepatic encephalopathy. The study also demonstrates a significant negative correlation between the levels of 25-OHD and presence of hepatic encephalopathy in CLD patients. The high prevalence of 25-OHD deficiency in patients with chronic liver disease can easily be addressed with necessary dietary modifications as well as through its supplementation. It paves the way for interventional studies on the effect of supplementation of 25-OHD in various grades of hepatic encephalopathy. Since the diagnostic facility for Vitamin D deficiency is widely available now and the primary contact of these individuals are with the primary care physicians, the role of primary care physicians becomes more important to identify such modifiable deficiencies which can be easily corrected if identified early.

The current study had certain limitations. Although the study showed a significant negative correlation between 25-OHD deficiency and HE, a causal effect can not be attributed. We did not use the severity scores of chronic liver disease to assess the relation between the severity of CLD with 25-OHD levels. A follow-up was also not done to assess for associated mortality. Cause effect and benefit of 25-OHD supplementation in HE after adjusting for various other causative factors may be undertaken in a larger group.

Conclusion

25-hydroxyvitamin D deficiency is very common in patients with chronic liver disease and there is a significant negative correlation between its level and worsening grades of hepatic encephalopathy.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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