Original Article

The Concentration of 25-Hydroxy Vitamin D in Chronic Liver Disease and its' Correlation with Severity

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Abstract

Chronic Liver Disease (CLD) is a common disease all over the world and the major biological factors are Hepatitis B virus (HBV) and Hepatitis C virus (HCV) in Bangladesh and Alcoholic liver disease in the western world. Life expectancy of CLD patient is increasing now a days by available modern treatment; but the long term complications are now evident. Hepatic osteodystrophy is one of the most common complication which is associated with vitamin D deficiency. Vitamin D undergoes hepatic 25-hydroxylation, but as the hepatic parenchyma is jeopardized so the metabolic activation of this vitamin is impaired. The aim of the study was to measure the concentration of 25-hydroxy vitamin D 25(OH) D in CLD patient in different etiology and to find out the relationship of level of 25(OH) D in different stages of the disease according to Child-Pugh classification. This cross sectional study was carried out in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka during the period of April 2015 to March 2016. Patients attending the Gastroenterology Department with cirrhosis of liver and who fulfilled the inclusion criteria were initially enrolled for the study. Study objectives were explained and informed consent was taken from patients prior to record their clinical history, examination findings and initial investigation reports in the standard data sheet. The diagnosis of liver cirrhosis was made by combination of clinical features, blood profile and trans abdominal ultrasonography (T. USG). Endoscopy of the upper GIT was also done to see the presence of oesophageal or gastric varices which is a sign of increase portal pressure. Stages of CLD were assessed by Child-Pugh scoring system and level of 25(OH) D was measured from blood with the help of the Department of Bio-chemistry. Data was collected using a structured data sheet and analyzed by SPSS. Out of 85 patients, male-female ratio was 3:1 and mean age was 53.0 ± 10.7 year within the range of 25-70 years. Most of the patients had acites 92.9% and anorexia 90.6%; where four-fifth patients had weight loss and more than one third had Jaundice. Nearly half of the patients had abdominal pain and 42.4% had melaena. Four-fifth patients had history of blood transfusion and most of them had H/O hospitalization 94.8%, anaemia (97.6%) and Splenomegaly 92.9%. More than half (52.9%) of patients had bone pain; where jaundice 61.2% and Leukonychia 61.2% were detected in equal number of patients. Mean vitamin 25(OH) D was 16.29 ± 7.96 in 69 HBV patients and 20.14 ± 9.76 in 16 HCV patients. In this study, 28.2% patients were in child Pugh A, 36.4% in child Pugh B and 32.9% in child Pugh C stages. Mean vitamin 25(OH) D were 27.12 ± 6.11, 15.97 ± 5.40 and 9.57 ± 1.15 in Child-pugh A, Child-pugh B and Child-pugh C stages respectively. Mean vitamin 25(OH) D was observed at decreased level as the changes of stage from lower to higher. Vitamin D deficiency was highly prevalent in patients with CLD and inversely correlated with disease severity. In the case of chronic liver diseases, vitamin D seems to modulate the innate and adaptive immune system, which explains the association. This study suggest that these parameters may improve with vitamin D supplementation. Monitoring of S. 25(OH) D is reasonable in CLD patient.

Keywords: *Avitaminosis, parathyroid hormone, portal hypertension, gastric varices, child-pugh a, child-pugh b and child-pugh c.*

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INTRODUCTION

Cirrhosis may be defined as a phase of chronic liver disease or insult leads to the diffuse destruction of hepatic parenchymal cell by fibrosis and the formation of nodules,

which results in disorganization of the liver's lobular and vascular architecture. In western countries, common causes are alcohol and in developing countries, chronic hepatitis B or C virus infection.[1] Cirrhosis may be compensated or decompensated when complicated by jaundice, ascites, and hepatic encephalopathy or raised prothrombin time. It is insidious. Initially asymptomatic later present with complication.[2] The final stage of chronic inflammation in the liver is cirrhosis. Liver cirrhosis gives rise to portal hypertension and complications such as bleeding esophageal varices, ascites and encephalopathy.[3] Though the disease is progressive, indolent and having many complications, but with the development of modern treatment modalities of cirrhosis of liver life span is increased. With the effective treatment long term complications are now commonly encountered.[4] It is a common complication among individuals with long standing hepatic disease. One study was conducted in the University of Tennessee Hepatology clinic, where 118 cirrhosis patients with different aetiology were included and their 25-hydroxy vitamin D level were measured. Severity was graded as mild (20-32 ng/ml), moderate (7-19 ng/ml) or severe (<7 ng/ml), normal being > 32 ng/ml. 25(OH) D is the only vitamin D metabolite that is used to determine whether a patient is vitamin D deficient, sufficient or intoxicated. The major circulating form of vitamin D is 25(OH) D that has a half-life of approximately 2-3 weeks.[5] The result showed vitamin D deficiency is universal (92 %) among patients with cirrhosis and at least one third of them suffered from severe vitamin D deficiency. Vitamin D is a fat soluble vitamin and helps in absorption of calcium from the renal tubule and intestine. Up to 93% of patients with chronic liver disease have insufficient vitamin D levels, and almost one-third of these show severe deficiency.[6] Other studies, such as Petta et al showed 73% and Bitetto et al showed level of vitamin D deficiency was 73% and 46.4% respectively.[7] Rode et al of Australia works with 158 patient where cirrhosis (n= 65) and no cirrhosis (n= 93). They categorize the patients in Viral (60), NASH (23), Alcoholic (22), Autoimmune (12), Haemochromatosis (9), Wilson's (2) Cholestatic (5) and others (25). The study outcome also suggest that patient with cirrhosis were more likely to be deficient in 25(OH) D (75%, P= 0.028).[8] Miroliace el al. of Iran, works with CLD patient where HBV (n=26), HCV (n=28), AIH (n=19), Cryptogenic (n=17) with 40 healthy controls. The main outcome is significantly higher prevalence of vitamin D deficiency in cirrhotic versus non-cirrhotic patient.[9] There are several causes for the deficiency of vitamin D in chronic liver disease. The important potential mechanisms are reduced exogenous exposure, intestinal malabsorption, reduced endogenous production of vitamin DBP and albumin in the liver, impaired hepatic hydroxylation of vitamin D to 25(OH) D and increased catabolic removal of 25(OH) D.[10] Lange et al in 2012 also worked with 269 patient and found 74% of the cirrhotic patient with vitamin D deficiency. So, all the studies showed vitamin D deficiency with a significant P value. Osteoporosis in CLD mainly affects trabecular bone. The way in which liver failure affects osteoblasts and contributes to the development of osteoporosis is unclear. Numerous growth factors, some of which affect osteoblast function, such as IGF-1 and TGF-p are synthesized by the liver.[11] The pathogenesis of osteoporosis in CLD is complex and poorly understood. Advanced liver disease and cirrhosis are associated with an increased prevalence of osteoporosis.[12] 25(OH) D is a summation of both vitamin D intake and vitamin D that is produced from sun exposure, the biologically active form of vitamin D is 1, 25(OH) D. But this is not the ideal measure for vitamin D status due to several reasons. The circulating half-life of 1, 25(OH) D is only 4-6 hours and this is thousand fold less than 25(OH) D. Besides this, when a person become vitamin D deficient, calcium absorption from the intestine and renal tubule will be less. So, a vitamin D deficient patient may have normal or elevated levels of 1, 25(OH) D due to increase level of PTH. This makes the assay useless as a measure of vitamin D status. The only way to determine whether a person is vitamin D deficient or sufficient is to measure their circulating level of 25(OH) D. Vitamin D deficiency was defined as serum 25(OH) D levels less than 20 ng/ml (50 nmol/L) and insufficiency is defined when the level is 31-20 ng/ml, for the general population.[5, 13] The-important potential mechanism that reduce the level of vitamin D is multifactorial and van among different liver pathologies. Vitamin D3, is primarily acquired endogenously through the photochemical conversion of 7-dehydrocholestrol to previtamin D3 in the skin and transported to the liver. On the other hand, vitamin D2 reach the liver from venous circulation for hydroxylation. After hydroxylation, it is converted to 25- hydroxivitamin D (25(OH) D and secreted in the circulation again mostly bound to DBF. Further hydroxylation to 1, 25-dihydroxivitamin D in the kidney converts the vitamin into its active form.[17] The radioimmunoassay and competitive protein binding assays for 25(OH) D are useful in detecting vitamin D deficiency.

MATERIAL AND METHODS

This cross sectional study was carried out in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University, Dhaka during the period of April 2015 to March 2016 to assess the concentrations of 25-Hydroxy Vitamin D in chronic liver disease patient and also to find out relationship with etiology and different stage of cirrhosis. A total 85 patients with cirrhosis of liver attending the Gastroenterology Department who fulfill the inclusion criteria were initially enrolled for the study. Only adult patients with liver cirrhosis age greater or equal to 18 years were included in this study; but patients with hepatocellular carcinoma, ongoing pregnancy, severe life threatening infection, deformity or fracture in any part of body, female took hormonal contraception or in postmenopausal periods, secondary cause associated with osteoporosis or affect BMD, chronic kidney disease, diabetes mellitus, history of endocrinal disease, metastatic bone disease or other malignancies, receiving vitamin D or calcium supplements, hormone replacement therapy, corticosteroids or any drug known to affect bone density were excluded. Their clinical history, examination and initial investigation report were noted in the standard data sheet. Prior to data collection both verbal and written consent was taken from the respondents. Data were collected using a preformed data collection sheet (questionnaire). Base line information was collected from the patient after exploration of different complaints. All information regarding clinical features were recorded in a data collection sheet. S. 25-hydroxy vitamin D wow done in the Department of Bio-chemistry, Bangabandhu Sheikh Mujib Medical University Dhaka Bangladesh. The diagnosis of liver cirrhosis was made by combination of clinical features, blood profile and transabdominal ultrasound. Endoscopy of the upper GIT was done to detect the presence of oesophageal or gastric varices. Transabdominal ultrasound demonstrated a shrunken liver with increase echogenicity, with or without splenomegally and presence or absence of ascites. Stages of liver disease were assessed by Child-Pugh scoring system. Level of 25(OH) D was measured from blood from the Department of Bio-chemistry. Data was collected using a structered data sheet and analysed by using softwere SPSS.

Data processing and analysis: After collection of data, all data were checked and cleaned. After cleaning the data, statistical analysis was done by using Statistical Packages for Social Sciences (SPSS). Numerical variables were expressed as mean and standard deviation, whereas categorical variables are count with percentage. Continuous variables were compared using Student's t test; categorical variables were analyzed by Chi-square test. A p-value <0.05 were considered as statistically significant. Association of serum vitamin 25(OH) D with etiology and severity were assessed by Chi-square test. The correlation between the serum 25-hydroxy cholecalciferol and other parameters were evaluated by Pearson's correlation test. Upper gastro-intestinal endoscopy were done using a standard forward viewing endoscope. In order to prevent contamination from another patient, after each procedure endoscope and biopsy forceps were disinfected using glutaraldehyde 2% (CIDEX) solution. Instruments were immersed in solution and kept for 15 minutes. Side channels were also rinsed.

Operational Definition:

Liver failure and the Child-Pugh classification: The final stage of chronic inflammation in the liver is cirrhosis. Liver cirrhosis gives rise to portal hypertension and complications such as bleeding esophageal varices, ascites and encephalopathy. Hepato-cellular failure results in hyperbilirubinemia, hypoalbuminemia and prolonged prothrombin time. The Child-Pugh classification is a scoring system used to assess the prognosis of cirrhosis.

Table : Child Pugh Score (Sherlock & Dooley, 2011)[1]

Assessment criteria	Points scored for abnormality		
	1	2	3
Encephalopathy grade	None	Mild	Marked
Ascites	None	Mild	Marked
Bilirubin (µmol/L)	<34	34-50	>50
Albumin (g/L)	>35	28-35	<28
Prothrombin Time (seconds prolonged)	< 4	4-6	>6
Or INR	< 1.7	1.7-2.4	>2.4
Individual scores should be added. Score <7 = Child's a 7-9 = Child's B >9 = Child's C			

Although it was originally used to predict mortality during surgery, it is now used to determine the prognosis, as well as the required strength of treatment and the necessity of liver transplantation. Scoring is based upon several factors: albumin, ascites, total bilirubin, prothrombin time, and encephalopathy

Survival in cirrhosis (Colledge et al. 2010)[18]

Child-Pugh grade	Survival in cirrhosis (%)			
	1 year	5 years	10 years	Hepatic deaths (%)
A	82	45	25	43
B	62	20	7	72
C	42	20	0	85

Limitations

1. Cross sectional study
2. Sample size was small
3. Study was carried out at a single point and it is not the actual reflection of total population.

RESULTS

This cross sectional study was carried out in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from April 2015 to March 2016 for a period of 1 year. A total of 85 patients with chronic liver disease were included in this study. The results were as follows:

Table-I Shows distribution of patients with CLD according to age. Maximum 36.5% patients were in age group 51-60 years followed by 23(27.1%), 16(18.8%) and 15 (17.6%) in >60, 41-50 and ≤ 40 years age group respectively. Mean age was 53.0 ± 10.7 years within the range of 25-70 years.

Table I: Distribution of patients with CLD according to age (n=85)

Age (years)	Frequency	Percentage
≤ 40	15	17.6
41-50	16	18.8
51-60	31	36.5
>60	23	27.1
Total	85	100.0
Mean ± SD	53.0 ± 10.7	
Range (Min – Max)	25 – 70	

Table-II shows distribution of patients according to gender. Male was predominant in this study. Male female ratio was 2.54:1

Table II : Distribution of patients with CLD according to gender (n=85)

Gender	Frequency	Percentage
Male	61	71.8
Female	24	28.2
Total	85	100.0

Table III show presenting complains of the CLD patients. More than 90.0% patients had ascites and anorexia. Eighty percent patients had weight loss and 71.8% patients had Jaundice. More than 40.0% patients had abdominal pain and melaena.

Table III: Presenting complain of the patients with CLD (n=85)

Presenting complains	Frequency	Percentage
Ascites	79	92.9
Jaundice	61	71.8
Abdominal pain	41	48.2
Haematemesis	33	38.8
Melaena	36	42.4
Anorexia	77	90.6
Weight loss	68	80.0
Fever	11	12.9
Pruritus	2	2.4

Table-IV shows history of past illness. Sixty (77.9%) patients had history of blood transfusion and 73 (94.8%) patients had previous hospitalization.

Table IV: History of past illness of the patients with CLD (n=85)

History of past illness	Frequency	Percentage
Blood transfusion	60	77.9
Previous hospitalization	73	94.8
Total	85	100.0

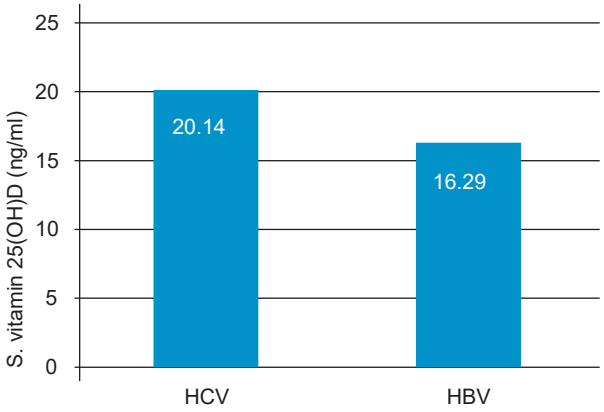

Figure-1: *Bar chart showing mean S. vitamin 25(OH)D in different aetiology stages of CLD.*

Figure 1 shows the s. vitamin 25(OH)D level in HCV and HBV. Mean vitamin 25(OH) D was 16.29±7.96 in 69 HBV and 20.14±9.76 in 16 HCV patients.

Table V shows the clinical features of the patients with CLD. Common clinical features anaemia, splenomegaly, leukonychia, jaundice, bone pain were found in 83 (97.6%), 79(92.9%), 52(61.2%), 52(61.2%) and 45(52.9%) of patients respectively. Others hepatomegaly, flapping tremor, ankle oedema, abdominal lump and lymphadenopathy were found in 32(97.6%), 23(27.1%), 8(9.4%), 5(5.9%) and 1(1.2%) of patients respectively.

Table V: Clinical features of the patients with CLD (n=85)

Clinical examination findings	Frequency	Percentage
Anaemia	83	97.6
Jaundice	52	61.2
Lymphadenopathy	1	1.2
Ankle oedema	8	9.4
Leukonychia	52	61.2
Flapping tremor	23	27.1
Bone pain	45	52.9
Splenomegaly	79	92.9
Hepatomegaly	32	37.6
Abdominal lump	5	5.9

Table VI shows level of s. vitamin 25(OH)D in different stages of CLD. Mean s. vitamin 25(OH)D was gradually decreased as the changes of stage from lower to higher. There was statistical significant difference in s. vitamin 25(OH)D among the different stages of CLD.

Table VI: S. vitamin 25(OH)D in different stages of CLD (n=85)

Stage	Mean ± SD	Range (Min – Max)	P value
Child-pugh A(n=25)	27.12 ± 5.98	19.60 – 43.50	
Child-pugh B(n=32)	15.97 ± 5.31	10.60 – 27.30	
Child-pugh C(n=28)	9.57 ± 1.15	8.20 – 13.00	
Analysis			
A vs B vs C			<0.001
A vs B			<0.001
A vs C			<0.001
B vs C			<0.001

DISCUSSION

In this study, males were predominant. Male female ratio was 2.54:1. Males were predominant and male female ratio was 2.70:1.[20] The male female ratio was in the range of 2.3:1 to 2.6:1 among the patients with cirrhosis and HCC.[21] All these results are similar to this study result. Male female ratio of patients with CLD in Malayasia was 4.4:1.[22] Maximum 36.5% patients were in age group 51-60 years followed by 23 (27.1%), 16 (18.8%) and 15 (17.6%) in >60, 41-50 and <40 years age group respectively. Mean age was 53.0 ± 10.7 years within the range of 25-70 years. Mean age of patients with CLD in Malayasia was 52 years which is almost similar to this study.[22] Regarding presenting complains, more than 90.0% patients had ascites and anorexia. Eighty percent patients had weight loss and 71.8% patients had Jaundice. Almost fifty patients had abdominal pain (48.2%) and melaena (42.4%). Haematemesis was present in 38.8% patients and fever was present in 12.9% patients. Dhole et al.[23] found jaundice in (73.0%), abdominal distension in (51.0%) and Ascites in (41.8%) Patients.[23] Ascites is more in this study compare to Dhole et al.[23] but other presenting complains are similar to Dhole et al. Regarding clinical examination findings of the CLD patients, most of the patients had anaemia (97.6%) and Splenomegaly (92.9%). More than 50.0% patients had jaundice (61.2%), Leukonychia (61.2%) and bone pain (52.9%). Hepatomegaly, flapping tremor, ankle oedema, abdominal lump and lymphadenopathy were present in 37.6%, 27.1%, 9.4%, 5.9% and 1.2% patients. Hepatomegaly was seen in 63% patients, splenomegaly was seen in 60% patients and anaemia in 56.0% patients.[23] In this study, anaemia and splenomegaly is more comparing Dhole et al. hepatomegaly was less comparing Dhole et al.[23] In this study mean s. vitamin 25(OH) D was 17.03 ± 8.41. Putz-Bankuti et al.[24] reveled mean 25(OH)D was 16.0 ± 9.2 ng/ml and Lange et al. (2011) revealed mean 25(OH)D was 17 ng/ml (range: 3-80).[24] Mean s. vitamin 25(OH) D was 16.29 ± 7.96 in 69 HBV patients and 20.14 ± 9.76 in 16 HCV patients. Mean s. vitamin 25(OH) D found in this study is almost similar to the above studies. Mean s. vitamin 25(OH) D was 7.65 ±4.19 in HBV patients which is less comparing these study.[15] Chronic hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease; it is estimated to affect 130 to 150 million people worldwide, a significant number of whom also develop cirrhosis and hepatic cancer.[16] A high percentage of these patients (46% to 92%) have low vitamin D levels, and more than 25% suffer from severe deficiency.[7] It has been hypothesized that the high incidence of vitamin D deficiency in these patients may be caused by HCV's effect on direct or indirect 25-hydroxylation through cytokine induction or oxidativc stress,[28] and that the virus may suppress 25(OH)D levels due to a disruption in lipid metabolism; as shown a recent study where HCV decreases the production of 7-dehydrocholesterol, the endogenous precursor of vitamin D.[27] In this study, 28.2% patients were in child Pugh class A, 36.4% in child Pugh class B and 32.9% in child Pugh class C group. Majority of CLD patients 63.0% fall in child Pugh class A group, followed by 32.0% fall in child Pugh class B & 5.0% fall in child Pugh class C.[20] Child Pugh class B is similar in both studies but child pugh class A in higher and child pugh class C is lower in this study comparing.[20] Mean s. vitamin 25(OH) D were 27.12 ± 6.11, 15.97 ± 5.40 and 9.57 ± 1.15 in Child-pugh A, Child-pugh B and Child-pugh C stages respectively. Mean s. vitamin 25(OH) D was gradually decreased as the changes of stage from lower to higher. There were significant differences in s. vitamin 25(OH)D among Child Pugh scores with the highest levels in Child A and the lowest levels in Child C patients (Finkelmeier, 2015). The studies by Crawford et al.[26], Fisher et al.[19], Chen et al.[25] and Putz-Bankuti et al.[24] show that patients with severe cirrhosis (Child-Pugh class C) have approximately half the amount of serum 25-hydroxyvitamin D concentrations compared with patients with less severe cirrhosis (Child-Pugh class A).

CONCLUSIONS

In this study, 85 patients were enrolled and the mean S. 25(OH) D was 17.03 which was in the lower limit. The patients were also categorized according to severity with Child-Pugh classification and showed the level of vitamin D is inversely related to the severity of the disease.

RECOMMENDATION

A multicenter, population based with control and having a larger sample size study should be done. So that, it can be properly evaluated and will be more correct.

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