A Comparative Study of Vitamin D Levels in Non-Cholestatic Chronic Liver Disease and Healthy Controls

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ABSTRACT

BACKGROUND

Chronic Liver Disease (CLD) is defined as the process of long-term progressive destruction and regeneration of the liver, and with advancing disease, hepatic fibrosis (scarring) and cirrhosis frequently occur. Given that liver is involved in bile salt production, absorption of vitamin D, and 25-hydroxylation of vitamin D, it might be expected that vitamin D deficiency would be common in patients with (CLD).

METHODS

The present hospital based observational comparative analysis was conducted in the Department of Medicine, and OPD (Out-Patient Department) and IPD (In Patient Department) of Gastroenterology, of SMS Hospital, Jaipur among a total of 60 participants. The study duration was of one year from 1st May 2016 to 30th April 2017. The minimum sample size required in each group was at 95% confidence interval and 80% power to verify the expected difference of 58.6% in proportion of cases with vitamin D deficiency in non-cholestatic chronic liver disease group with age and sex matched control group (hospital staff and attendants of patients) (76.5% vs. 17.96%) was 30 in each group.

RESULTS

30 study participants were cases and 30 study participants were controls. Out of the total study participants 23 (38.3%) were female and 37 (61.7%) patients were male and the male to female sex ratio was 1.6 : 1. The mean age of 30 cases in our study was 39.1 ± 8.69 years and the mean age of 30 controls was 38.4 ± 8.02 years and no significant difference was observed. Mean serum Vitamin D_3 was lower in CLD cases (23.4 ± 6.44 ng / L) as compared to controls (43.8 ± 5.18 ng / L). This difference was statistically significant with a p value <0.001. In univariate analysis in patients with non-cholestatic CLD, significant (P<0.05) positive correlations were found between serum level of vitamin D and serum bilirubin, serum albumin, platelet count, & haemoglobin. Also, there were significant (P<0.05) negative correlations between vitamin D concentration and serum bilirubin, INR & MELD score. No significant correlation was seen between vitamin D and age, serum level of PTH, calcium, phosphate, ALT, AST, ALP, urea, or creatinine.

CONCLUSIONS

Vitamin D inadequacy is very common in non-cholestatic CLD patients and correlates with the severity of the disease. Therefore, we recommend that clinical guidelines for managing non-cholestatic CLD should include the assessment of vitamin D status in all patients. For vitamin D assessment and replacement in the management of patients with non-cholestatic CLD further studies are required.

KEYWORDS

Chronic Liver Disease, Vitamin D Inadequacy, Non-Cholestatic CLD

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BACKGROUND

Chronic Liver Disease (CLD) is defined as the process of long-term progressive destruction and regeneration of the liver, and with advancing disease, hepatic fibrosis (scarring) and cirrhosis frequently occur.[1] Progression of CLD and deterioration of liver function is associated with various hepatic complications such as chronic liver failure, hepatocellular carcinoma (HCC), and infections. Hepatic osteodystrophy is an important extrahepatic manifestation of advanced liver disease mimicking features of classical osteoporosis with an increased risk for fractures.[2]

Liver plays an important role in vitamin D and bone metabolism. Vitamin D is hydroxylated by liver to 25-hydroxy vitamin D, the main circulating form, and then is converted into the active form 1, 25-dihydroxyvitamin D in kidney.[3] Given that liver is involved in bile salt production, absorption of vitamin D, and 25-hydroxylation of vitamin D, it might be expected that vitamin D deficiency would be common in patients with chronic liver disease (CLD).[4]

Vitamin D is a secosteroid hormone, which is mostly known as a regulator of calcium and bone metabolism. However, vitamin D has pleiotropic effects including cellular proliferation, differentiation and immunomodulation. These extra-skeletal effects have been related to the pathogenesis and treatment of infections, cardiovascular, autoimmune and degenerative diseases and several types of cancer.

Previous studies investigating the correlation between vitamin D, serum PTH level and severity of hepatic dys-function have shown conflicting results. A study demonstrated that about two-third of patients with end stage liver disease and more than 90% of patients who are candidate for liver transplantation do have low vitamin D levels without osteomalacia.[4] There are some other studies, however, which have shown conflicting results.[5]

It is notable that activities such as regulation of synthesis of metalloproteinase and their inhibitors, activation of fibroblasts, and collagen synthesis are also considered as properties of vitamin D.[5] These evidences bring this hypothesis in mind that vitamin D can have a role in progression of hepatic damage and CLD. Considering that there are limited studies about vitamin D status in patients with non-cholestatic CLD, in this study we aimed to evaluate the patterns of vitamin D disturbance and correlate it in a sample of patients with non-cholestatic CLD.

METHODS

The present hospital based observational comparative analysis was conducted in the Department of Medicine, and OPD (Out-Patient Department) and IPD (In Patient Department) of Gastroenterology, of SMS Hospital, Jaipur. The study duration was of one year from 1st May 2016 - 30th April 2017. The minimum sample size required was 14 in each group at 95% confidence interval and 80% power to verify the expected difference of 58.6% in proportion of cases with vitamin D deficiency in Non cholestatic chronic liver disease group with age and sex matched control group (hospital staff and attendants of patients) (76.5% vs 17.96%). We enhanced the sample size to 30 in each group. Venous blood samples were obtained after an overnight 10–12 h of fasting. All patients with non-cholestatic CLD (Hepatitis B, Hepatitis C, Autoimmune Hepatitis and Cryptogenic cause) attending department of Medicine and Gastroenterology OPD and indoor were included in the study. Clearance from Institutional Ethics Committee was taken before start of study. Patient on medication with vitamin D or calcium supplement, bisphosphonates, calcitonin, hormone replacement therapy, corticosteroids or anti-viral drugs were excluded from the study.

The diagnosis of CLD was based on consistent clinical findings, serologic markers of hepatitis B and C, hepatitis B virus DNA, and hepatitis C virus RNA measurements by polymerase chain reaction method, auto antibodies (anti-nuclear antibody, anti-smooth muscle antibody), bio-chemical features (including iron studies, ceruloplasmin, and urinary copper), endoscopic and imaging (including abdominal ultrasonography) evidence, and histological examinations (liver biopsy examination). The diagnosis of cirrhosis was established by liver biopsy or definitive clinical or bio- chemical evidence of hepatocellular failure and / or portal hypertension. Liver biopsy was performed for patients who did not have contraindication and samples were studied for fibrosis and inflammation. The Model for End-Stage Liver Disease (MELD) score was also calculated. The resulting study groups were subjected to detailed history and examination followed by vitamin D and PTH and other relevant tests.

Data were analyzed using SPSS software (version 16.0; SPSS Inc., Chicago, USA). Variables were expressed as percentage or mean ± standard deviation (SD). Patients with and without cirrhosis were compared using Chi-square analysis for categorical variables and independent sample t test for continuous variables. Comparison of variables between categories of vitamin D was performed using analysis of variance (ANOVA). Pearson correlation coefficients were calculated between vitamin-D and other variables. p value <0.05 was considered significant.

RESULTS

In the present study, a total of 60 study participants were enrolled. Out of them 30 study participants were cases and 30 study participants were controls. Out of the total study participants, 23 (38.3%) were female and 37 (61.7%) patients were male and the male to female sex ratio was 1.6: 1. The mean age of 30 cases in our study was 39.1 ± 8.69 years and the mean age of 30 controls was 38.4 ± 8.02 years and no significant difference was observed. As evident from the table 1 case and control groups were contain 19 males and 11 females & 18 males and 12 females respectively. No significance difference was observed in sex composition of case and control groups. (Table 1)

Parameters	Case	Control	P Value
Mean age (years)	39.1 ± 8.69	38.4 ± 8.02	0.724
Female	11 (36.7%)	12 (40%)	0.791
Male	19 (63.3%)	18 (60%)	

Table 1. Distribution of Patients According to Age and Gender

Group	N	Mean	Std. Deviation
Case	30	23.4	6.44
Control	30	43.8	5.18

Table 2. Comparison of Mean Serum Vitamin D_3 (ng / dL) in the Study Groups

t= -13.592; at 58 degree of freedom; p<0.001 (S)

Table 2 indicates that mean serum Vitamin D_3 was lower in CLD cases (23.4 ± 6.44 ng / L) as compared to controls (43.8 ± 5.18 ng / L). This difference was statistically significant with p value <0.001.

Variable	Correlation Co-efficient	P Value
Age	-0.118	0.535
Bilirubin	-0.507	0.004 (S)
ALP	-0.161	0.396
SGOT	-0.129	0.496
SGPT	-0.098	0.608
Albumin	0.384	0.036 (S)
INR	-0.472	0.009 (S)
Platelet	0.373	0.043 (S)
Haemoglobin	0.418	0.002 (S)
Calcium	0.303	0.104
Phosphorus	-0.076	0.689
PTH	0.015	0.937
Urea	0.110	0.561
Creatinine	0.006	0.976
MELD score	-0.671	0.002 (S)

Table 3. Correlation of Vitamin D_3 with Different Parameters among CLD Cases

In univariate analysis in patients with non-cholestatic CLD, significant (P<0.05) positive correlations were found between serum level of vitamin D and serum bilirubin, serum albumin, platelet count & haemoglobin. Also, there were significant (P<0.05) negative correlations between vitamin D concentration and serum bilirubin, INR &MELD score. No significant correlation was seen between vitamin D and age, serum level of PTH, calcium, phosphate, ALT, AST, ALP, urea, or creatinine. (Table 3)

DISCUSSION

In this case control analytic study conducted at SMS Hospital, Jaipur, thirty cases of non-cholestatic CLD diagnosed by symptoms, biochemical features, serological marker, endoscopy, abdominal sonography & histological examination (liver biopsy) and thirty age & sex matched controls were studied.

In this study, we evaluated the association between parameters of calcium–phosphate metabolism (vitamin D, PTH, calcium and phosphate) and the parameters that are clinically valuable in cirrhotic and noncirrhotic patients with non-cholestatic CLD (ALT, AST, ALP, bilirubin, albumin, INR, Child–Pugh score and MELD score).Mean age of CLD cases in present study was 39.1 ± 8.69 years and the mean age of controls was 38.4 ± 8.02 years and there no significant difference was observed. Mean age of case and control were approximately similar to study done by Miroliaee et al[6] where mean age of case and control were 42.39 ± 13.02 years and 40.98 ± 9.29 years respectively. There was no significant difference in age and sex between healthy controls and patients.

The causes of CLD were viral hepatitis C (n=11), viral hepatitis B (n=6), autoimmune hepatitis (n=3), and cryptogenic (n=10). Cirrhosis was evident in 19 patients. The main causative factor for cirrhosis was viral hepatitis C (36.8%), whereas in the non-cirrhotic group cryptogenic (36.4%) and viral hepatitis C (36.4%) were more prevalent. Similar study was done by Miroliaee[6] et al enrolled 90 consecutive patients with evidence of non-cholestatic CLD due to hepatitis C (n=28), hepatitis B (n=26), autoimmune hepatitis (n=19), and cryptogenic causes (n=17). Cirrhosis was evident in 51 patients. The main causative factor for cirrhosis was viral hepatitis C (31.4%), whereas in the noncirrhotic group viral hepatitis B (33.3%) and C (30.8%) were more prevalent.

We demonstrated that the majority of non-cholestatic CLD patients (86.6%) had insufficient serum vitamin D concentrations. In present study mean serum Vitamin D_3 of 30 cases was 23.4 ng / mL and mean serum Vitamin D_3 of 30 controls was 43.8 ng / mL. Standard deviation was 6.44 in case group and 5.18 in control groups. Mean serum Vitamin D_3 was lower in cases (23.4 ± 6.44 ng / mL) as compared to controls (43.8 ± 5.18 ng / mL). This difference was significant as p value was <0.001. Although we found a strong association between serum 25 (OH) D concentration and liver injury, this does not establish the relationship as causal. One would expect older patients to have lowered 25 (OH) D levels. However, there was no age difference in our series. Other possible factors contributing to vitamin D insufficiency in CLD may include the following: (1) reduced exposure to sunlight (patients with CLD and greater liver function abnormalities possibly spend less time outdoors), (2) dietary insufficiency, (3) malabsorption, (4) low levels of serum proteins that bind with vitamin D, (5) impaired cutaneous synthesis of vitamin D in jaundiced patients, (6) decrease hepatic hydroxylation of vitamin D to 25 (OH)D, (7) increase catabolism and removal of 25 (OH)D. One could speculate that in individual CLD patients, inadequacy in vitamin D status is determined by different pathogenic factors.

Similar results obtained in study done by Miroliaee[6] et al in which the mean value of serum Vitamin D_3 (nmol / L) in controls and cases were found to be 95.28 ± 29.41 and 40.721 ± 22.43 nmol / L respectively (p< 0.001). Zhao et al[7] found that serum 25 (OH)D3 levels in chronic hepatitis B patients (7.83 ± 3.47 ng / mL) were significantly lower than that in healthy controls (9.76 ± 4.36 ng / mL, P<0.001). Similar study done by Fisher et al.[8] obtained that serum 25 (OH) D levels were inadequate in 91 patients: vitamin D deficiency (<50 nmol / L) was found in 68 patients and vitamin D insufficiency (50-80 nmol / L) was found in 23 patients. (P<0.05).

In univariate analysis in patients with non-cholestatic CLD, significant (P<0.05) positive correlations were found between serum level of vitamin D and serum bilirubin, serum albumin, platelet count & haemoglobin. Also, there were

significant (P<0.05) negative correlations between vitamin D concentration and serum bilirubin, INR &MELD score. No significant correlation was seen between vitamin D and age, serum level of PTH, calcium, phosphate, ALT, AST, ALP, urea, or creatinine. Fisher et al.[8] showed that serum vitamin D levels less than 25 nmol / l would be a reliable predictor of higher INR and serum bilirubin as well as lower serum albumin and platelet count. Hen et al.[9] reported a positive correlation of serum 25 (OH) D concentrations with albumin levels ($r = 0.655$, $P < 0.0001$).Christos Konstantakis et al[10] showed that there is evidence of a significant relation of 25(OH) D levels with the degree of liver dysfunction, considering that an inverse correlation of 25 (OH) D levels with both Child-Pugh score and Model for End-Stage Liver Disease has been reported. Our results, demonstrating the high rate of vitamin D deficiency in CLD patients, could possibly suggest that screening and treatment of vitamin D deficiency should be considered in the management of patients with CLD.

It should be noted that vitamin D insufficiency is not only a causative factor for bone diseases in the general population but also a risk factor for a wide range of chronic inflammatory and autoimmune diseases (inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis and diabetes mellitus), cancers (colon, prostate and breast), and metabolic disorders (metabolic syndrome and hypertension)[9]. Vitamin D can influence hepatic injury, fibrosis, and tissue remodelling by different mechanisms. Vitamin D and its derivates are potent regulators of cell proliferation, differentiation, and immunomodulation. These effects include inhibition of certain matrix metalloproteinases (MMPs) and induction of their inhibitors, suppression of proliferation of fibroblasts, and increased collagen production. Vitamin D insufficiency is associated with increased circulating MMP-2 and -9, which is correctable by supplementation. Hepatocytes produce the major MMPs and tissue inhibitors involved in liver extracellular matrix remodelling. MMP-2 and -9 are of particular relevance to the liver because they are critically involved in the degradation of components of the basement membrane such as collagen IV and fibronectin, 2 main components of the space of Disse. Inhibition of MMPs protects from hepatic ischemic injury.[10]

Therefore, determination and treatment of vitamin D deficiency may represent an important therapeutic target in the follow-up of CLD patients. Vitamin D deficiency may present with bone pain, proximal muscle weakness, and bone fracture, but many patients are asymptomatic. After liver transplantation and corticosteroid therapy many patients will be symptomatic. Our results, demonstrating the high rate of vitamin D deficiency in CLD patients, could possibly suggest that screening and treatment of vitamin D deficiency should be considered in the management of patients with CLD.

The study demonstrated that the majority of non-cholestatic CLD patients had significant insufficient serum vitamin D concentrations. Serum Parathyroid Hormone was significantly higher in CLD cases and serum levels of calcium and phosphate were normal in many patients with vitamin D deficiency. This can be explained by reabsorption of minerals from bones. So, osteopenia and osteomalacia could be expected in these patients. Our study also showed a significant correlation between low serum vitamin D level and markers of liver function insufficiency including coagulopathy, hypoalbuminemia, hyperbilirubinemia, and thrombocytopenia.

In our study univariate analysis in patients with non-cholestatic CLD, significant positive correlations were found between serum level of vitamin D and serum bilirubin, serum albumin, platelet count & haemoglobin. Also, there were significant negative correlations between vitamin D concentration and serum bilirubin, INR & MELD score. No significant correlation was seen between vitamin D and age, serum level of PTH, calcium, phosphate, ALT, AST, ALP, urea, or creatinine.

CONCLUSIONS

In conclusion, vitamin D inadequacy is very common in non-cholestatic CLD patients and correlates with the severity of the disease. Therefore, we recommend that clinical guidelines for managing non-cholestatic CLD should include the assessment of vitamin D status in all patients. For vitamin D replacement in management of patients with non-cholestatic CLD further studies are required.

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