RESEARCH ARTICLE

Effect of chronic liver disease continuum upon calcium homeostasis and bone mineral metabolism: An observation from Eastern India

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INTRODUCTION

Chronic liver disease (CLD) involves the progressive decline of various normal functions of the liver such as synthesis of clotting factors and proteins, detoxification of toxic metabolites, and bile excretion persisting over a duration more than 6 months.[1] CLD is a continuous process involving
inflammation, destruction followed by regeneration of the liver parenchyma ultimately culminating to fibrosis and cirrhosis of liver. Cirrhosis, the most complicated sequelae of CLD, has claimed over a million lives in 2010. It is estimated that globally, liver disease causes 1.03 million deaths/year.

CLD plays an important role in causing abnormality of the Calcium-parathyroid hormone (PTH)-vitamin D axis. Prevalence rates of hepatic osteodystrophy range from 13% to 70% in the Western population. A higher prevalence rates between 68% and 95% have been reported from India. Inconsistent data for prevalence rates have been reported from previous studies regarding the serum concentrations of 25(OH)D and its correlation with the progression of liver diseases. Some studies found that 25(OH)D levels decline as the liver disease progresses, whereas others found no significant difference between cirrhotic patients and patients who did not develop cirrhosis or among the Child-Pugh groups and others found it as has no predictive value of the progression of liver disease in etiologies such as hepatitis C.

There is hardly any study in the Eastern part of India to show the effect of CLD on bone mineral homeostasis. In this background, the primary aim of the study was to estimate serum Ca, inorganic phosphate (PO4) along with regulatory hormones of bone metabolism such as iPTH and 25(OH) vitamin D levels in patients suffering from chronic liver disorders of diverse etiology in a tertiary care hospital of Eastern India and to reveal the effects of severity (based on Child-Pugh Turkotte [CTP] score) of CLD on bone mineral metabolism.

MATERIALS AND METHODS

Study Design
An observational, non-interventional, hospital-based cross-sectional study was conducted in the Department of Biochemistry and Department of Medicine, IPGME and R (Kolkata, India) between April 2020 and October 2021.

Sample Size
The sample size considered for the study was 90 with the statistical considerations such as prevalence of CLD of 6%, having a precision of 5%, an alpha error of 0.05, and an anticipated loss to follow-up rate of 5%. Ninety age- and sex-matched volunteers with similar demographic and socioeconomic status were selected as controls.

Inclusion Criteria
The study included patients within age 21–70 years, who gave written consent for participation, having diverse etiologies of CLD, including primary biliary cholangitis, primary sclerosing cholangitis, Wilson’s disease, hepatitis B, hepatitis C, autoimmune hepatitis, NAFLD (non-alcoholic fatty liver disease), and cryptogenic cirrhosis. The Child-Pugh score, which ranges from 5 to 15, was determined by summing the results of five parameters (ascites, bilirubin, albumin, prothrombin time (PT), and encephalopathy).

Exclusion Criteria
The study excluded patients who denied to give consent and who had an acute exacerbation of CLD (bilirubin concentration >5 mg/dL and leukocytosis >10000/mm), recent gastrointestinal bleeding, intestinal resection, acute renal failure, chronic disorders affecting mineral metabolism such as chronic kidney disease, diabetic kidney disease, parathyroid and thyroid gland disorders, hypogonadism, Cushing’s syndrome, and malignancy. The study also excluded patients who have been taking calcium, vitamin D, any hormone replacement therapy, corticosteroids, bisphosphonates, calcitonin, cytoxins, anticoagulants, anticonvulsants, antimetabolites, thyroxin, or interferon. There were no patients in the control group who were known to have metabolic bone disease or were currently on any medications known to influence bone turnover or had a history of recent fracture.

Ethical Approval
The needed ethical approval was collected from the Institutional Ethics Committee.

Consent
All participants signed consent form to participate in the current study.

Biochemical Parameter Analysis
For the biochemical analysis, blood was drawn from the patients and controls, aseptically from the antecubital vein, following an overnight fast of 10 h. The samples were centrifuged to obtain the serum for further analysis. Serum bilirubin, alanine amino transferase, aspartate aminotransferase, serum albumin, serum alkaline phosphate and PT, serum calcium, and serum phosphorous were estimated biochemically using full automated biochemistry analyzer. Parathyroid hormone was estimated by chemiluminescence immunoassay and Vitamin D was estimated by ELISA.

RESULTS
Comparison between cases and controls on the basis of basic demographic characters showed that they were age and sex matched as far as possible. The levels of serum calcium and serum 25(OH) Vit D were significantly lower, whereas serum iPTH levels were significantly higher in patients with CLD compared to controls [Table 1].
The cases were further divided into three groups based on disease severity by increase in Child-Pugh-Turcotte score as mild, moderate, and severe (5–6: Class A; 7–9: Class B and 10 or above: Class C). The analysis of variance test between the investigated parameters revealed significantly lower levels of serum calcium and serum 25(OH) Vitamin D with increase in the severity of CLD. Significantly high serum level of intact parathyroid hormone was found in patients based on the severity of liver disease [Table 2].

Correlation analysis between the parameters yielded mixed results. Strong positive correlation was found when the levels of serum calcium, phosphate, and 25(OH) Vitamin D were compared against each other. Contrary to this, serum iPTH showed a negative correlation with serum calcium, phosphate, and 25(OH) Vitamin D [Table 3].

**DISCUSSION**

The results of the study revealed a high prevalence of hypocalcaemia and Vitamin D deficiency in the CLD patients. Further analysis of our study results showed that the severity of hypovitaminosis D and hypocalcaemia was strongly correlated with higher CTP scores. This study further revealed significantly higher levels of serum iPTH in patients with hypovitaminosis D, thus indicating toward a significant dysregulation of the Calcium-PTH-Vitamin D axis. Furthermore, hypovitaminosis D is considered an independent risk factor for the low serum calcium level.[9-11]

There is a defect in alpha hydroxylation of Vitamin D by the kidney in CLD. This is due to decline in the primary substrate for this enzyme, 25-OH Vitamin D, produced by the liver. Liver plays an essential role in the synthesis of Vitamin D carrier proteins and has a fundamental action in Vitamin D metabolism. The carrier proteins, Vitamin D-binding protein (DBP) and albumin, are bound to a significant portion of Vitamin D. A study done by Bikle et al. suggests that low levels of total vitamin D do not affect the biological activity when normal levels of unbound Vitamin D are maintained. Moreover, it has been found that DBP has an excess capacity to bind with Vitamin D. Even a 50% decrement in DBP does not result in significant reduction of Vitamin D levels.[16] This might explain why decreasing serum 25(OH)D levels are more often seen only in advanced liver disease, where this capacity is fully depleted.

The study results were such as a study conducted by Narayanasamy et al. recently, which reported hypocalcaemia in 85.59% of CLD patients and a prevalence of 69.3% in Vitamin D deficient patients.[12] The results were quite similar...
to a study performed by Jamil et al. which revealed that vitamin D levels were negatively correlated to CPT scores.\textsuperscript[17]

This study further revealed significantly higher levels of serum iPTH in CLD patients. The study conducted by Dibble JB had shown similar results.\textsuperscript[18,19] It has been found that the concentration of PTH in CLD increases even with adequate replacement of Vitamin D.\textsuperscript[20] Conflicting results have been yielded by some interventional studies where patient with CLD received Vitamin D/Calcium supplements. Several studies reviewed by Collier et al. had focused on patients with PBC.\textsuperscript[21] There was no delay in the progression of osteoporosis, nor increase bone mineral density (BMD) in some studies where Vitamin D supplementation was done.\textsuperscript[22] However, Shiomi et al. observed an increase in BMD after 1 year of Vitamin D supplements (1 µg calcitriol/day) in cirrhosis patients.\textsuperscript[23]

Along with several musculoskeletal manifestations such as hepatic osteodystrophy, Vitamin D deficiency is responsible for early decompensation and increase in mortality rates in CLD patients.\textsuperscript[24] Many studies indicate that the proper replacement of Vitamin D can ameliorate functional status, prognosis, and long-term morbidity of patients with liver disease. Thus, this deficiency should be addressed right away so that patients with CLD can be benefited.\textsuperscript[25]

In CLD population, Vitamin D deficiency and hypocalcaemia are highly prevalent. Moreover, hypovitaminosis D is correlated to higher CTP score.

In the backdrop of CLD of various grades, this study findings emphasize the correlations between hypovitaminosis D, hypocalcaemia, hypophosphatemia, and secondary hyperparathyroidism, indicating toward a significant dysregulation of the Calcium- PTH-vitamin D axis.

**Limitations of the Study**

The sample size for the study was relatively less. This study included CLD patients of diverse etiologies; the outcome of result and correlations of the analytes based on particular etiologies remain to be established. The study subjects were selected from the patients attending this hospital, so the trend in the general population of the community might not be evenly reflected in this study. It was a cross-sectional, observational study. For determining any potential therapeutic advantages of the analytes, further longitudinal and interventional studies are needed.

**CONCLUSION**

The majority of CLD patients showed hypovitaminosis D, and as the disease advances, the levels decrease. We live in a Vitamin D deficient endemic zone. In CLD patients, a lack of calcium and Vitamin D lead to the development of secondary hyperparathyroidism which often results in the development of osteoporosis. Results of this study suggest that serum 25(OH)D and calcium levels should be measured as a part of the routine tests performed in the patients of CLD so that adequate replacement by Vitamin D supplements can be initiated as a therapeutic adjunct in managing such patients.

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