Indian J Gastroenterol
DOI 10.1007/s12664-017-0727-7

ORIGINAL ARTICLE

Vitamin D status in patients with cirrhosis of the liver and their relatives—A case control study from North India

Ravikant Kumar[1] · **Pavan Kumar**[1] · **Kandarp Nath Saxena**[1] · **Manjul Mishra**[1] · **Vivek Kumar Mishra**[1] · **Anju Kumari**[2] · **Manisha Dwivedi**[1] · **Sri Prakash Misra**[1]

Received: 18 May 2016 / Accepted: 4 January 2017

Abstract

Background and Aim Liver diseases interfere with the production of the metabolites of vitamin D required for activation, thus resulting in abnormal calcium and bone metabolism. Previous studies show inconsistent results of vitamin D level in non-cholestatic liver diseases. Our aim was to determine the prevalence of vitamin D insufficiency in cirrhosis as compared to apparently normal relatives and its relationship with etiology and severity.

Methods One hundred and sixty cirrhotic patients attending the Department of Gastroenterology and Hepatology, M L N Medical College, Allahabad, were enrolled, and 25-hydroxy vitamin D [25(OH)D] and calcium levels assessed. Vitamin D status was graded as insufficiency (20–30 ng/mL), deficiency (<20 ng/mL), and severe deficiency (<7 ng/mL). 25(OH)D levels of patients were compared with those of their healthy family members.

Results Forty-six percent of the normal population had 25(OH)D inadequacy, whereas 51.85% of patients with cirrhosis had 25(OH)D deficiency, and 28.12% had insufficiency. Thus, 80% of patients with cirrhosis of the liver had some form of vitamin D inadequacy. 12.5% of cirrhotics had severe vitamin D deficiency. Serum calcium (Ca^{++}) was not significantly different between the patients and control group. The etiology of cirrhosis had no relation with vitamin D levels. Prevalence of deficiency and insufficiency increased with increasing age and mean Child-Turcotte-Pugh and model for end-stage liver disease scores.

Conclusion Vitamin D insufficiency is highly prevalent in patients with cirrhosis irrespective of etiology and significantly more common than their healthy relatives. Measurement of 25(OH) vitamin D and replacement may be considered as part of the overall management of patients with cirrhosis of the liver as well as apparently healthy individuals.

Keywords Child-Turcotte-Pugh score · Cirrhosis · Model for end-stage liver disease score · Vitamin D

Introduction

Vitamin D is a fat-soluble vitamin that plays an integral role in the metabolism of calcium and bone health. There are two forms of vitamin D: ergocalciferol (D2) and cholecalciferol (D3). Vitamin D3 is biosynthesized from 7-dehydrocholesterol through sun-exposed skin, whereas D2 is derived mainly from plants and yeasts and is the main form of oral supplementation. The liver plays an important role in metabolism of vitamin D. Vitamins D2 and D3 are transported to the liver by a vitamin D-binding protein where it is hydroxylated to 25-hydroxy vitamin D [25(OH)D]. 25(OH)D, in turn, is transported to the kidney, undergoes a second hydroxylation by CYP27B1, and is converted into 1, $25(OH)_2$ vitamin D, the active form [1]. The active form mediates its downstream effects via the vitamin D receptor (VDR). Consequently, diseases of the liver interfere with production of the active metabolites of vitamin D, thus resulting in abnormal calcium and bone metabolism. Vitamin D deficiency in adults can precipitate or exacerbate osteopenia and osteoporosis, cause osteomalacia and muscle weakness, and increase the risk of fracture [2, 3]. Vitamin D deficiency has been shown to be associated

✉ Ravikant Kumar
drravi0609@gmail.com

[1] Department of Gastroenterology and Hepatology, M L N Medical College, Allahabad 211 001, India

[2] Department of Physiology, Indira Gandhi Institute of Medical Sciences, Sheikhpura, Patna 800 014, India

Published online: 08 February 2017

Table 1 Demographic profile and serum biochemistry of patients and their relatives

	Patients	Normal	*p*-value
Age (years)	47.98 ± 12.13	48.26 ± 13.47	NS
M:F	104:56	96:54	NS
Weight (kg)	54.8 ± 11.78	61.8 ± 5.27	NS
Height (m)	1.58 ± 0.08	1.59 ± 0.05	NS
Hb (g/dL)	8.7 ± 0.08	12.98 ± 0.75	<0.001
TLC (per mm^3)	6816 ± 2618	7414 ± 1732	NS
Sugar (mg/dL)	91.6 ± 22.5	81.56 ± 9.8	NS
Urea (mg/dL)	34.2 ± 14.5	24.22 ± 5.6	NS
Creatinine (mg/dL)	0.95 ± 0.23	1.03 ± 0.14	NS
Bilirubin (mg/dL)	2.1 ± 1.1	0.45 ± 0.17	<0.001
SGPT (IU/L)	55.4 ± 23.8	30.1 ± 6.6	<0.001
SGOT (IU/L)	89.9 ± 80.4	27.96 ± 8.2	<0.001
ALP (IU/L)	226.14 ± 104.6	154.25 ± 22.5	NS
Protein (g/dL)	6.13 ± 0.94	7.03 ± 0.31	NS
Albumin (g/dL)	2.98 ± 0.5	3.9 ± 0.25	<0.001
Na^+ (mmol/L)	133.33 ± 6.17	141.32 ± 5.18	NS
K^+ (mmol/L)	4.21 ± 0.69	4.01 ± 0.38	NS
Ca^{++} (mmol/L)	1.04 ± 0.17	1.1 ± 0.14	NS
Ca^{++} (mg/dL)	4.17 ± 0.67	4.4 ± 0.58	NS
25(OH)D (ng/mL)	19.4 ± 9.4	30.6 ± 10.2	<0.001
MELD	14.42 ± 4.32		

Hb hemoglobin, *TLC* total leukocyte count, *NS* not significant, *SGPT* serum glutamic pyruvic transaminase, *SGOT* serum glutamic oxaloacetic transaminase, *ALP* alkaline phosphatase, *MELD* model for end-stage liver disease

with various types of cancer (e.g. colon, prostate, breast), autoimmune, inflammatory, and metabolic disease processes [4]. In humans, the total body vitamin D status is determined by measuring the serum levels of 25(OH)D. Vitamin D is highly fat-soluble, and thus adipose tissue is a depot for vitamin D storage. Currently, there are no commercially available serum assays to evaluate vitamin D stored in adipose tissue. Vitamin D deficiency is defined as serum 25(OH)D levels less than 20 ng/mL, whereas serum levels between 20 and 30 ng/mL constitute insufficiency, and levels between 30 and 100 ng/mL are considered optimal [5, 6]. Based on these definitions, it has been estimated that vitamin D insufficiency and deficiency may be present in as many as one billion people worldwide [7]. Conversely, calcium absorption is deemed to be optimal when 25(OH) vitamin D levels are ≥30 ng/mL.

Vitamin D insufficiency/deficiency in chronic cholestatic liver disease is a well-established fact. But previous studies have reported inconsistent results with respect to serum concentrations of 25(OH)D and its relationship with the severity of non-cholestatic liver disease. Some studies suggested that 25(OH)D levels decrease with progression of liver disease [8]; others did not find a difference between cirrhotic and non-cirrhotic patients or between various Child-Pugh groups [9, 10].

Methods

After the approval of Institutional Ethics Committee, the study was carried out in the Department of Gastroenterology and Hepatology, M L N Medical College, and Swaroop Rani Nehru (SRN) Hospital, Allahabad. One hundred and sixty

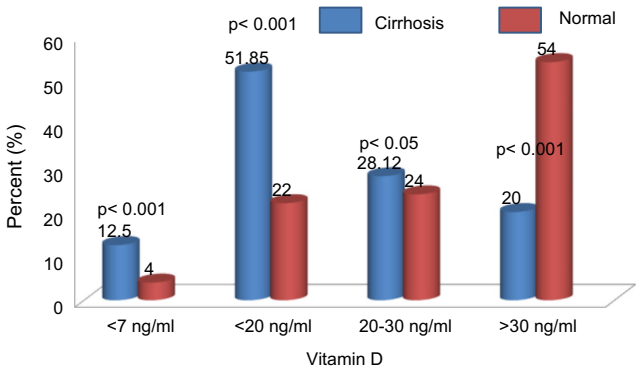

Fig. 1 Vitamin D status in patients with cirrhosis of the liver and normal relatives of patients

Table 2 Etiological association of 25(OH)D deficiency in cirrhosis of the liver

Etiology (*n*)	Vitamin D	Vitamin D deficiency *N* (%)	Vitamin D insufficiency *N* (%)	Vitamin D <7 ng/mL *N* (%)
Alcohol (52)	22.79±13.95	27 (51.9%)	13 (25%)	5
Cryptogenic (39)	18.10±11.48	22 (56.4%)	12 (30.7%)	9
Hepatitis B (38)	21.15±15.02	21 (55.2%)	12 (31.5%)	6
Hepatitis C (12)	25.28±8.90	6 (50%)	1 (8.33%)	
NAFLD (9)	24.01±11.82	4 (44.4%)	3 (33.3%)	
AIH (7)	25.92±6.16	2 (28.57%)	3 (42.8%)	
Wilson (3)	35.33±7.07	1 (33.3%)	1 (33.3%)	
Total (*n*)=160	18.9±9.4	83 (51.85%)	45 (28.12%)	20 (12.5%)

NAFLD non-alcoholic fatty liver disease, *AIH* autoimmune hepatitis

patients with cirrhosis of the liver attending S R N Hospital between November 2013 and April 2015 were included in the study. Patients with chronic kidney disease, celiac disease, inflammatory bowel disease, cholestatic liver disease, gastric bypass, and those taking steroid (even for AIH), vitamin D and/or calcium supplementation were excluded from the study. Celiac disease was diagnosed on the basis of serology and histology.

Baseline investigations were done. Child-Turcotte-Pugh (CTP) and model for end-stage liver disease (MELD) scores were calculated in all patients. Vitamin D and calcium status of 150 apparently normal relatives of patients were estimated for comparison.

Diagnosis and etiology of cirrhosis of the liver

This was based on history, clinical examination, biochemistry, ultrasonography (USG) of the abdomen, esophagogastroduodenoscopy (OGD), and liver biopsy if required. HBsAg, anti-HCV, serology for autoimmune markers, serum ceruloplasmin, and serum ferritin levels were estimated to determine etiology of cirrhosis of the liver. Baseline investigations including complete hemogram, liver function tests (LFTs), OGD, USG of the abdomen, and ascitic fluid analysis were done. Duodenal and colonic/ileal biopsy in suspected cases of celiac or inflammatory bowel disease, respectively, were taken.

Table 3 Univariate analysis of variables associated with severe 25(OH) vitamin D deficiency (<7 ng/mL) in patients with cirrhosis of the liver

25(OH) vitamin D	<7 ng/mL	>7 ng/mL	*p*-value
Age >55 years	5 (9.6%)	47 (90.38%)	0.001
Age <55 years	15 (13.8%)	93 (86.11%)	
Male	12 (11.5%)	92 (88.4%)	0.001
Female	8 (14.2%)	48 (85.71%)	
Bilirubin (mg/dL)	2.92±1.6	2.38±1.63	NS
ALT (IU/L)	82.6±40.4	56.13±40.43	NS
Albumin (g/dL)	2.67±0.5	3.02±0.5	NS
INR	1.68±0.59	1.45±0.59	0.001
Creatinine (mg/dL)	1.07±0.23	0.94;±0.23	NS
Ca^{++} (mmol/L)	0.99±0.16	1.05±0.18	NS

ALT alanine transaminase, *INR* international normalized ratio, *NS* not significant

Vitamin D and calcium estimation

Vitamin D status was assessed by measuring serum concentration of 25(OH)D. The test was performed using automated chemiluminescence immunoassay (DiaSorin LIAISON) with a laboratory reference range of 30–100 ng/mL (commercial assay multiply by 2.496 for nmol/L). According to the assay, the normal 25(OH)D level was taken as >30 ng/mL. Levels between 20 and 30 ng/mL were categorized as vitamin D insufficiency. Levels <20 ng/mL were labeled as vitamin D deficiency. Severe vitamin D deficiency was defined as a serum level of 25(OH)D <7 ng/mL.

Fasting blood sample was collected from the antecubital vein without tourniquet in a-3 mL plain conical centrifuge capped tube filled till the brim. Ionized (free) calcium was estimated by automated analyzer (9180 Electrolyte Analyzer, Roche Diagnostics). The normal reference range for ionized calcium was taken as 1.13–1.26 mmol/L (4.52–5.28 mg/dL).

Statistical analysis

Univariate analysis was performed to identify whether age, gender, severity of liver disease, or etiology was associated with vitamin D deficiency and calcium level. Categorical variables were compared using chi-square test, and continuous variables were analyzed by Student's *t* test; a *p*-value of <0.05 was considered statistically significant.

Table 4 Relation between vitamin D status and severity of cirrhosis of the liver

CTP class	No.	Vitamin D mean±SD	<20 ng/mL	<7 ng/mL	*p*-value
A	16	34.67±13.88	3 (18.75%)	0	A–B = 0.95
B	73	25.71±12.55	23 (31.5%)	4 (5.4%)	B–C = 0.0021
C	71	15.64±9.66	57 (80.28%)	16 (22.5%)	A–C = 0.00034

CTP Child-Turcotte-Pugh

Results

Out of 160 patients, 104 (65%) were male with mean age of 47.98±12.13 years (Table 1). The mean hemoglobin and albumin levels were significantly lower in patients with cirrhosis. Serum bilirubin and transaminases were significantly higher in cirrhotics as compared to those of normal controls. The mean 25(OH)D level in cirrhotics was 19.4±9.4 ng/mL which was significantly lower than that of controls where it was 30.6 ±10.2 [$p < 0.001$]. Mean serum Ca^{++} in cirrhotics and normal controls were 4.17±0.67 and 4.4±0.58 mg/dL, respectively [p = ns].

The most common etiology of cirrhosis of the liver was alcoholic liver disease, found in 53 (33%) patients. In 39 (24%) patients, a diagnosis of cryptogenic cirrhosis was made. Alcohol was followed by hepatitis B, hepatitis C, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis (AIH), and Wilson's disease.

Vitamin D status in cirrhosis of the liver and normal population

Vitamin D deficiency, defined as 25(OH)D <20 ng/mL was found in 83 (51.85%) patients with cirrhosis of the liver and 33 (22%) normal controls [$p<0.001$] (Fig. 1). Optimal 25(OH)D level was found in 32 (20%) cirrhotics and 81 (54%) normal controls. Severe 25(OH)D deficiency, 25(OH)D deficiency, and 25(OH)D insufficiency were significantly more prevalent in cirrhotics than in the normal population.

Etiological association of 25(OH)D deficiency

25(OH)D deficiency/insufficiency was seen irrespective of etiology of liver cirrhosis (Table 2). Mann-Whitney *U* test showed no significant association between etiology of disease and 25(OH)D deficiency/insufficiency. Out of 52 patients with alcoholic cirrhosis, 40 (77%) had 25(OH)D deficiency/insufficiency, and 86 (81.42%) non-alcoholic cirrhotic patients had 25(OH)D deficiency/insufficiency [p = 0.729]. Similar was the situation for cryptogenic vs. non-cryptogenic cirrhosis of the liver.

Variables associated with severe 25(OH) D deficiency (<7 ng/mL)

Severe 25(OH)D deficiency (<7 ng/mL) was more common in older (>55 years) than younger patients (<55 years) [13.8 vs. 9.8% (p = 0.001)], female cirrhotics compared with their male counterparts [14.2 vs. 11.5% (p = 0.001)]. Liver function tests such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and bilirubin as well as serum creatinine and calcium were not significantly different statistically in those with severe deficiency and those without (Table 3). INR was significantly high in patients with severe vitamin D deficiency (1.68±0.59 vs. 1.45±0.59, p<0.05).

Relation between vitamin D status and severity of cirrhosis of the liver

Out of 160 patients with liver cirrhosis, 16 belonged to CTP-A with a mean 25(OH)D level of 34.67±13.88 ng/mL, with none of them having severe 25(OH)D deficiency (Table 4). Mean vitamin D levels differ significantly in different CTP groups as shown in Table 4. Four (5.4%) and 16 (22.5%) patients in CTP-B and CTP-C, respectively, had severe 25(OH)D deficiency. There was a statistically significant difference in prevalence of vitamin D deficiency between CTP-A to CTP-C and CTP-B to CTP-C.

Mean CTP and MELD scores in patients with severe vitamin D deficiency were 10.65±1.53 and 16.9±3.82, respectively (Table 5). There was significant difference in CTP and MELD scores in different categories of 25(OH)D. Mean 25(OH)D level progressively decreased as CTP and MELD scores increased.

Table 5 Relation of 25(OH) vitamin D level with CTP and MELD scores

25(OH) vitamin D	<7 ng/mL	<20 ng/mL	20–30 ng/mL	≥30 ng/mL	*p*-value
CTP (mean±SD)	10.65±1.53	10.09±1.65	8.88±2.0	7.55±1.69	<0.05
MELD (mean±SD)	16.9±3.82	15.98±4.61	13.59±3.57	11.60±2.33	<0.05

CTP Child-Turcotte-Pugh, *MELD* model for end-stage liver disease

Discussion

Vitamin D is now widely recognized to have multiple extra-skeletal health functions in addition to maintenance of skeletal health. The liver is one of the major organs involved in its metabolism. Among the 150 normal relatives of patients with cirrhosis of the liver, 25(OH)D deficiency/insufficiency was observed in 69 (46%). In spite of the tropical situation (25° 28′ N, 81° 54′ E) of Allahabad (place of study) with adequate sunlight, vitamin D deficiency/insufficiency is prevalent even in normal population. Arya et al. from Lucknow reported prevalence of vitamin D deficiency in 78.3% of hospital staff [11]. In another study from Varanasi (125 km from Allahabad), Agrawal and Sharma reported that 58% of adult males aged greater than 50 years had vitamin D deficiency [12]. The possible reasons include inadequate sunlight exposure and skin pigmentation in Indians.

Out of 160 patients with cirrhosis of the liver, 128 (80%) had 25(OH)D deficiency/insufficiency. In a study of 118 patients of non-cholestatic liver disease, Arteh et al. found some form of vitamin D deficiency in 92% of patients [13]. Fisher and Fisher reported that 68% of patients of non-cholestatic liver disease had vitamin D deficiency [14].

25(OH)D deficiency/insufficiency was significantly more common in patients with liver cirrhosis than normal relatives [80% vs. 46%, $p < 0.05$]. As the dietary pattern (available food), socioeconomic status, and sunlight were the same in both groups, a high prevalence of vitamin D deficiency/insufficiency may be attributed to underlying liver disease leading to restricted diet and sun exposure in addition to altered liver metabolism.

Alcohol was the most common etiology of liver cirrhosis (33%), followed by hepatitis B and hepatitis C. Choudhary et al. reported alcohol as the leading cause of cirrhosis (58.2%) [15]. In our study, there was no association between etiology of cirrhosis and vitamin D status similar to the findings of Arteh et al. [13]. On univariate analysis, only age greater than 55 years, female gender, and INR were associated with severe vitamin D deficiency (<7 ng/mL). Liver function tests such as AST, ALT, albumin, and bilirubin as well as serum creatinine and ionized calcium were not statistically different significantly between those with severe deficiency and those without.

Mean vitamin D level decreased with increase in the severity of liver disease as assessed by CTP. In the CTP-A class, none had severe vitamin D deficiency but the CTP-C class 16/71 (22.5%) had severe vitamin D deficiency. One mechanism for the higher prevalence of vitamin D deficiency in cirrhotics is impairment in the 25-hydroxylation of vitamin D leading to low serum levels of 25(OH)D in relation to the degree of liver dysfunction [16]. In one study, deficient hepatic hydroxylation was observed only in patients with advanced CLD, whereas others found no impairment to the production of vitamin D metabolites even in advanced stages of liver disease [17, 18]. High prevalence of vitamin D deficiency/insufficiency in cirrhosis of the liver in our study raises the question of whether impaired synthetic function of liver was responsible for low levels of vitamin D in some of our patients. However, the biochemical tests for liver dysfunction such as serum albumin, bilirubin, and transaminases did not correlate with severe deficiency. Several other factors could contribute to the development of vitamin D deficiency, including inadequate exposure to the sun, insufficient dietary intake, and impaired cutaneous synthesis of vitamin D in the presence of jaundice.

One of the limitations of our study was that we did not measure parathyroid hormone (PTH) levels, and hence we could not further explore the interaction between PTH, vitamin D, and calcium levels in liver disease, especially in those with advanced stages. Moreover, the clinical significance of vitamin D deficiency/insufficiency on skeletal as well as extra-skeletal health has not been evaluated in this study. Bansal et al. [19] reported high prevalence (66%) of hepatic osteodystrophy (HOD) among Indian patients with non-cholestatic disease. But he did not find a significant correlation between vitamin D and HOD and concluded that HOD has a multifactorial pathogenesis beyond vitamin D metabolism.

We conclude that vitamin D deficiency is highly prevalent in apparently healthy subjects as well as in patients with cirrhosis of the liver irrespective of etiology of cirrhosis in spite of adequate availability of sunlight in North India. High CTP and MELD scores, increasing age (>55 years), and female gender and INR were associated with severe vitamin D deficiency. Measurement of 25(OH)D and replacement may be considered as part of the overall management of patients with cirrhosis of the liver.

Compliance with ethical standards

Conflict of interest RK, PK, KNS, MM, VKM, AK, MD, and SPM declare that they have no conflict of interest.

Ethics statement The authors declare that the study was performed in a manner to conform with the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning human and animal rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

After the approval of the Institutional Ethics Committee, the study was carried out in the Department of Gastroenterology and Hepatology, M L N Medical College, and Swaroop Rani Nehru (SRN) Hospital, Allahabad.

References

1. De Luca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80 Suppl :1689S–96S.
2. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–81.

3. Looker AC, Mussolino ME. Serum 25-hydroxyvitamin D and hip fracture risk in older US white adults. J Bone Miner Res. 2008;23:143–50.
4. Peterlik M, Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. Eur J Clin Investig. 2005;35:290–304.
5. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008;87:1080S–6S.
6. WHO. WHO Scientific Group on the Prevention and Management of Osteoporosis. Prevention and management of osteoporosis: Report of a WHO Scientific Group. Geneva: World Health Organization; 2003.
7. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006;81:353–73.
8. Chen CC, Wang SS, Jeng FS, et al. Metabolic bone disease of liver cirrhosis: is it parallel to the clinical severity of cirrhosis? J Gastroenterol Hepatol. 1996;11:417–21.
9. Duarte MP, Farias ML, Coelho HS, et al. Calcium-parathyroid hormone-vitamin D axis and metabolic bone disease in chronic viral liver disease. J Gastroenterol Hepatol. 2001;16:1022–7.
10. Monegal A, Navasa M, Guanabens N, et al. Osteoporosis and bone mineral metabolism disorders in cirrhotic patients referred for orthotopic liver transplantation. Calcif Tissue Int. 1997;60:148–54.
11. Arya V, Bhambri R, Godbole MM, Mithal A. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. Osteoporos Int. 2004;15:56–61.
12. Agrawal NK, Sharma B. Prevalence of osteoporosis in otherwise healthy Indian males aged 50 years and above. Arch Osteoporos. 2013;8:116.
13. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. Dig Dis Sci. 2010;55:2624–8.
14. Fisher L, Fisher A. Vitamin D and parathyroid hormone in outpatients with noncholestatic chronic liver disease. Clin Gastroenterol Hepatol. 2007;5:513–20.
15. Choudhary NS, Tomar M, Chawla YK, et al. Hepatic osteodystrophy is common in patients with noncholestatic liver disease. Dig Dis Sci. 2011;56:3323–7.
16. Hepner GW, Roginsky M, Moo HF. Abnormal vitamin D metabolism in patients with cirrhosis. Am J Dig Dis. 1976;21:527–32.
17. Skinner RK, Sherlock S, Long RG, et al. 25-Hydroxylation of vitamin D in primary biliary cirrhosis. Lancet. 1977;1:720–1.
18. Compston JE. Hepatic osteodystrophy: vitamin D metabolism in patients with liver disease. Gut. 1986;27:1073–90.
19. Bansal R, Kumar M, Ranjan P, Sachdeva M, Kumar A. Prospective study of profile of hepatic osteodystrophy in patients with noncholestatic liver cirrhosis and impact of bisphosphonate supplementation. United European Gastroenterol. 2016;4:77–83.