To Compare Serum Vitamin D Levels with Severity of Liver Cirrhosis According to Child-Pugh Score in Amritsar, Punjab

Jaskiran Kaur[1], Gurinder Mohan[2], Manish Chandey[3]

[1, 2, 3] Department of General Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.

ABSTRACT

BACKGROUND

Cirrhosis of liver and its extrahepatic manifestations like hepatic osteodystrophy has been linked to vitamin D deficiency, and has been proposed as a potential therapeutic target. Its metabolism to 25-hydroxylation, makes liver necessary for its activation. In this study sources of vitamin D, functions and its metabolism with a focus on its variation with severity of liver cirrhosis is discussed.

METHODS

This is an observational study conducted on 60 patients with liver cirrhosis. The severity of liver cirrhosis was assessed according to the Modified CPS. By technique of enhanced chemiluminscence values of vitamin D were obtained. The results were statistically analyzed using SPSS Statistics-20.0 version. Association among different variables were calculated using Chi-Square Test and One Way ANOVA. Results were considered significant if p value obtained was below 0.05 and highly significant if it was below 0.001.

RESULTS

Mean age group of study population was 54.83 ± 13.56 years. 27 patients had Alcoholic cirrhosis, 11 patients were HCV positive, 2 patients were HBsAg positive and 20 patients had cirrhosis due to other etiologies. Mean vitamin D level of the study population was 35.34 ± 22.4. 27 patients (45 %) had sufficient vitamin D, 18 patients (30 %) were deficient and 14 patients had insufficient vitamin D levels. 1 patient had vitamin D toxicity. There was no significant correlation between mean vitamin D and etiology of liver cirrhosis (P value = 0.457; Not Significant). There was significant correlation between vitamin D levels with severity of liver disease (p value < 0.001).

CONCLUSIONS

In conclusion, low concentrations of 25 (OH) D are correlated with severity of liver dysfunction irrespective of the etiology.

KEYWORDS

Child-Pugh Score, Liver cirrhosis, Serum Vitamin D

Corresponding Author:
Dr. Manish Chandey,
 # 10, Shastri Nagar,
Near Bhabha School, Majitha Road,
Amritsar - 143001, Punjab, India.
E-mail: chandey96098@yahoo.co.in

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BACKGROUND

Vitamin D is the collective name given to antirachitic substances synthesized in the body and found in foods activated by UV radiations.[1] The major dietary sources of vitamin D are fortified dairy products. Other dietary sources include egg yolks, fish oils, and fortified cereal products. Vitamin D provided by plant sources is in the form of vitamin D2, whereas that provided by animal sources is in the form of vitamin D3. These two forms have equivalent biological potencies and are activated equally efficiently by the hydroxylases in humans.[2]

An UV-B (290 – 315 nm)-dependent, non-enzymatic reaction in human skin converts the cholesterol precursor 7-dehydrocholesterol into previtamin D_3 that further isomerizes into vitamin D_3 (cholecalceferol).[3] 25(OH) D3 is the most stable and abundant metabolite of vitamin D and acts as a biomarker of individual's Vitamin D status.[4] When exposed to UV radiations the cutaneous precursor of vitamin D i.e. 7-dehydrocholestrol, undergoes photochemical cleavage of the carbon bond between carbons 9 and 10 of the steroid ring.

The resultant product, previtamin D, is thermally labile and over a period of 48 hours undergoes a temperature dependent molecular rearrangement that results in the production of vitamin D.[2] Vitamin D is absorbed into the lymphatics and enters the circulation bound primarily to vitamin D-binding protein (VDBP), although a fraction of vitamin D circulates bound to albumin. The human VDBP is a 52-kd alpha globulin that is synthesized in the liver.[2] Incidentally, as VDBP is synthesized in liver, it represents a biomarker for advanced liver injury.[5,6] In the liver, vitamin D undergoes 25-hydroxylation by a cytochrome P450-like enzyme present in the mitochondria and microsomes. The half-life of 25 (OH) D is approximately 2 to 3 weeks.[2] The final step in the production of the active hormone is the renal 1α-hydroxylation of 25 (OH) D to 1, 25 (OH) $_2$D (calcitriol). The half-life of this hormone is approximately 6 to 8 hours.[2]

1,25 $(OH)_2D$ is secreted into the circulation bound to VDBP, and once it has entered its target cells, it binds to specific member of the nuclear receptor superfamily, the vitamin D receptor (VDR), which forms a heterodimer with retinoid X receptor (RXR).[7] Subsequently, this heterodimer associates with the vitamin D response element (VDRE) in the promoter of target genes and induces or represses gene transcription.[8] VDR binding is associated with the differential expression of a plethora of genes, and through this vitamin exerts specific effects, particularly the regulation of immune functions.[9] The physiological role of vitamin D is the regulation of calcium homeostasis for maintaining bone mineralization[10] as well as the modulation of innate and adaptive immunity[11] for improving the response to infections by microbes, such as *Mycobacterium tuberculosis*, and preventing autoimmune diseases, such as multiple sclerosis.[12]

Anatomically Cirrhosis is defined as a process with diffuse fibrosis and nodule formation. It is the end result of the fibrogenesis that occurs with chronic liver injury.[13] Cirrhosis progression and worsening liver function are linked to a variety of hepatic complications, including chronic liver disease, infections and hepatocellular carcinoma (HCC). Hepatic osteodystrophy is an important extra hepatic manifestation of advanced liver disease mimicking features of classic osteoporosis with an increased risk for fractures.[14] Recently the role of vitamin D in cirrhosis has received much attention, given its inherent activation process by the liver and the high prevalence of vitamin D deficiency in this patient group.[15]

Vitamin D plays a role in the inhibition of zinc-dependent endoproteinases, specifically matrix metalloproteinases (MMP), which degrade extracellular matrix components. Hence, a reduced concentration of vitamin D is associated with an increased circulation of MMPs, and liver fibrosis is associated with the over-accumulation of extracellular components.[16]

In a landmark article published 52 years ago, Child and Turcotte, two surgeons who performed portocaval shunt surgery, classified patients with cirrhosis in three "functional hepatic reserve" categories: A, B, and C, corresponding to minimally, moderately and severely altered hepatic functional reserve, respectively. His classification was based on 5 variables, three clinical (ascites, encephalopathy and nutritional status) and two biochemical (serum albumin and bilirubin).[17]

The Child-Turcotte classification was modified by Pugh et al in 1973 by substituting the normalized ratio (INR) for nutritional status, and by arbitrarily adjusting limits for serum albumin and better defining the encephalopathy grading system. Pugh et al also assigned 1, 2 or 3 points to each of the five variables enabling the calculation of a score, the Child-Turcotte-Pugh (CTP) score, ranging from 5–15, with scores of 5 and 6 corresponding to CTP class A, scores of 7–9 corresponding to CTP class B and scores of 10–15 corresponding to CTP class C.[18]

Measure	1 Point	2 Points	3 Points
Total bilirubin, µmol/L m g/dL	<34 <2.0	34-50 2.0-3.0	>50 >3.0
Serum albumin, g/dl g/L	>3.5 >35	3.0-3.5 30-35	<3.0 <30
Prothrombin time, seconds prolonged INR	<4.0 <1.7	4.0-6.0 1.7-2.3	> 6.0 >2.3
Ascites	NONE	Easily controlled	Poorly controlled
Hepatic encephalopathy	NONE	Minimal	Advanced

Table 1. Modified Child-Pugh Score[19]

Hepatic osteodystrophy is bone disease and metabolic disorders caused by Chronic Liver Disease (CLD), with osteopenia and osteoporosis being typical extrahepatic manifestations of CLD. Likewise hepatic osteodystrophy can pursue long after liver transplantation and is therefore considered a long term complication of CLD.[20]

While chronic liver disease is linked to a variety of bone diseases, osteoporosis and osteomalacia are the most common types of hepatic osteodystrophy. If bone resorption outpaces bone formation, or if reduced bone formation occurs alongside normal bone loss, bone mass will deteriorate and the risk of fracture will rise in advanced cases. Hepatic ostedystrophy is caused by an increase in cytokine levels in chronic liver disease and liver cirrhosis. Osteoblastic function disorder is believed to be caused by decrease in growth factors, such as insulin, or an excess of growth inhibitors, such as bilirubin, in patients with cirrhosis.

Serum osteocalcin levels have been shown to decrease as osteoblast function declines. A decrease in serum osteocalcin levels and an increase in deoxypyridinoline (DPD) levels can be explained by lower bone turnover.[21] The central role of vitamin D deficiency in the pathogenesis of osteomalacia associated with chronic liver disease is shown, first by the presence of low serum 25OH levels in osteomalacic patients and secondly, by the therapeutic response to vitamin D.

Further indirect evidence for the pathogenetic role of vitamin D deficiency comes from the demonstration of secondary hyperparathyroidism in osteomolacic patients. Calcium malabsorption has been reported in both parenchymatous and biliary liver disease. There is evidence that this is at least partly caused by vitamin D deficiency thus some improvement has been reported after parenteral or oral vitamin D supplementation.[22]

Low levels of vitamin D are not only associated with degree of liver dysfunction, but also with a less favorable outcome. Reports showed their association with higher mortality in alcoholic liver disease as well as cirrhosis of other origins. Other results that 25 (OH) D3 levels are inversely correlated with the MELD score are in agreement with data showing that serum 25 (OH) D3 levels decrease with increasing degrees of liver insufficiency and decreased levels of 25 (OH) D3 levels are associated with unfavorable outcomes.[23]

It is important to further investigate whether its deficiency can be an independent outcome indicator and whether vitamin D should be regularly supplemented in all patients requiring ICU admission. Thus low vitamin D levels not only relate to the clinical sequelae, but also a prognostic marker in chronic liver disease.

METHODS

This was a cross sectional observational study conducted in 60 diagnosed patients of liver cirrhosis visiting OPD/Indoor of SGRDIMSR, Vallah, Sri Amritsar**.** It was conducted from 1st January 2019 to 31st December 2020. This study was carried out after approval from hospital ethical committee and obtaining informed consent from patients or their relatives.

Cirrhosis was labeled on the basis of:

- Clinical examination and history taking.
- Radiological (increased and coarse liver echo pattern, portal vein diameter > 1.3 cm and spleen size > 13 cm)
- Biochemical (prolonged prothrombin time and reduced level of serum albumin)

The severity of liver cirrhosis was assessed and according to the Modified Child-Pugh score. Patients were grouped into:

- Group 1:- patients of liver cirrhosis with Child-Pugh class-A (Score 5 - 6)
- Group 2:- patients of Liver cirrhosis with Child-Pugh class-B (Score 7 - 9)
- Group 3:- patients of Liver cirrhosis with Child-Pugh class-C (Score 10 - 15)

Inclusion Criteria

- Patients diagnosed to have liver cirrhosis (ultrasound/CT abdomen/MRI evidence of chronic liver disease) of any etiology.

Exclusion Criteria

- Patients on hepatotoxic drugs
- Hepatocellular carcinoma
- Pregnancy and Lactating mothers
- Malabsorption Syndromes
- Patients of nephrotic syndrome or chronic kidney disease
- Patients who were receiving calcium or vitamin D supplements

Assessment of Vitamin D

5 ml of whole blood sample was taken in plain (red) vial. It was centrifuged and processed. By technique of enhanced chemiluminscence values of vitamin D was obtained.[24] A review of the most resent literature suggests the recommendation for 25-OH Vitamin D levels are:

Level	Range (ng/ml)
Deficient	<20
Insufficient	20-<30
Sufficient	30-100
Potential toxicity	>100

Table 2. Expected Values

From above parameters, we studied the variation of serum vitamin D levels with severity of liver cirrhosis.

Statistical Analysis

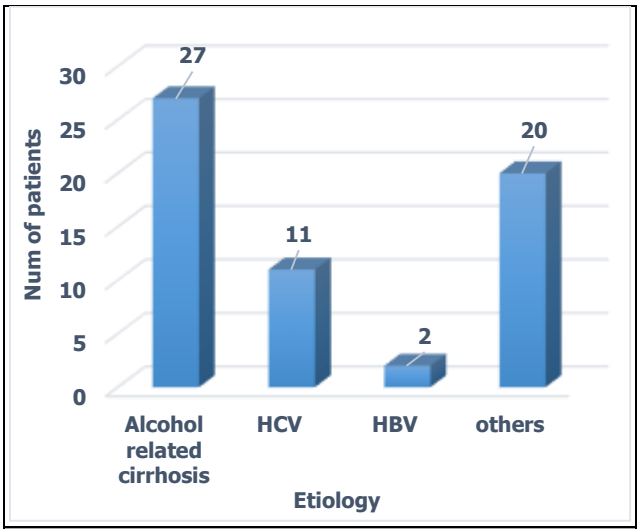
The results obtained from the study were statistically analyzed using SPSS -20.0 version. The observations were tabulated as frequency, percentage and mean ± SD. Association among different categorical variables were calculated using Chi-Square Test. One Way ANOVA test was used to compare mean values among subgroups**.** Results were considered significant if p value was below 0.05 and highly significant if it was below 0.001 and non-significant if more than 0.05.

RESULTS

Graph 1 Showed that 27 patients out of 60 had Alcohol related cirrhosis, 11 patients were HCV positive, 2 patients were positive for HBsAg and 20 patients had cirrhosis due to other etiologies.

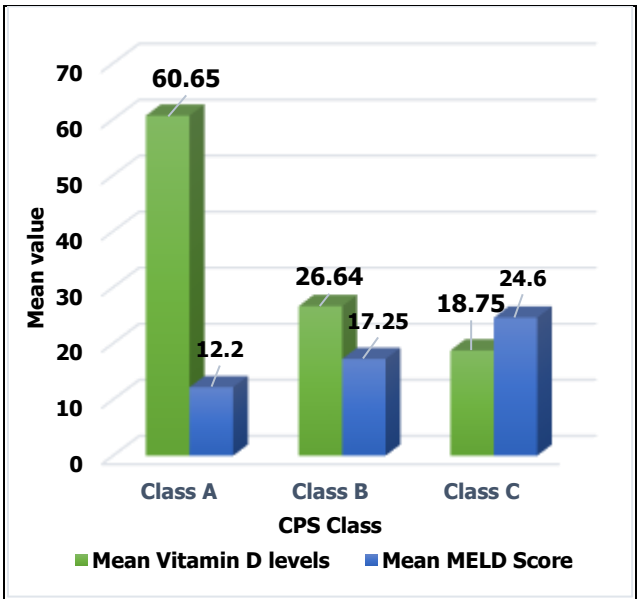
Vitamin D (ng/ml)	Number of Patient	Percent (%)
Deficient (<20)	18	30.0
Insufficient (20 - <30)	14	23.3
Sufficient (30 – 100)	27	45.0
Toxicity (>100)	1	1.7
Total	**60**	**100.0**
Mean ± SD	35.34±22.4	

Table 3. Mean Vitamin D Levels ± SD of the Study Population



Graph 1. Etiology of Liver Cirrhosis

Table 3 depicted that Mean Vitamin D levels ± SD of the study population was 35.34 ± 22.4. Out of total 60 patients 27 patients (45 %) had sufficient Vitamin D levels followed by 18 patients (30 %) with Vitamin D deficiency and 14 patients with insufficient Vitamin D levels. 1 patient had Vitamin D levels in toxicity range.



Graph 2. Mean Vitamin D Levels

Graph 2 showed Mean Vitamin D levels and mean MELD Score in different classes according to Child-Pugh Score. Patients in Class A had mean Vitamin D levels of 60.65±18.34 and mean MELD Score of 12.20 ± 4.26, patients in Class B had mean Vitamin D levels of 26.64±9.08 and mean MELD Score is 17.25 ± 6.87 and patients in Class C had mean Vitamin D levels of 18.75 ± 9.98 and mean MELD Score of 24.60 ± 7.39. P value of < 0.001 showed highly significant correlation between Child-Pugh Score, mean Vitamin D value and mean MELD Score.

Table 4 depicted that in Child-Pugh Class A out of 20 patients, 1 patient had insufficient Vitamin D, 18 patients had sufficient Vitamin D and 1 patient had toxic levels. In Child-Pugh Class B out of 20 patients 5 were deficient in Vitamin D, 8 had insufficient and 7 patients had sufficient Vitamin D levels. In Child-Pugh Class C out of 20 patients 13 were deficient in Vitamin D levels, 5 had insufficient and 2 patients had sufficient Vitamin D. P value of < 0.001 showed highly significant correlation between CPS Score and Vitamin D levels.

Child Pugh Score	Vitamin D								Total
	Deficiency		Insufficient		Sufficient		Toxicity		
	n	%	n	%	n	%	n	%	
Class A	0	0	1	5	18	90	1	5	20
Class B	5	25	8	40	7	35	0	0	20
Class C	13	65	5	25	2	10	0	0	20

Table 4. Child-Pugh Score and Vitamin D Levels

x^2=36.508; df=6; p=< 0.001; Highly significant

	Etiology	Mean ± SD	P – Value	Remarks
Vitamin D	Alcohol	30.83±21.25	0.457	Not significant
	HBV	48.35±18.87		
	HCV	41.42±21.88		
	Other	36.80±24.56		
CPS Score	Alcohol	9.93±2.98	0.127	Not significant
	HBV	6.50±2.12		
	HCV	7.91±2.58		
	Other	8.55±3.18		
MELD Score	Alcohol	21.29±7.43	0.029	Significant
	HBV	11.50±4.94		
	HCV	14.90±8.81		
	Other	15.95±7.37		

Table 5. Comparison among Different Aetiologies of Liver Cirrhosis

Table 5 showed mean value of Vitamin D, mean Child-Pugh Score and mean MELD Score in patients with different etiologies of liver cirrhosis in the study population. It revealed no significant differences between Vitamin D status and severity of liver cirrhosis due to different etiologies. It also revealed significant relationship between mean MELD Score and etiology of liver cirrhosis.

In the study population, out of 60 patients 11 were females and 49 were males. Mean age of the study population was 54.83 ± 13.56 years. 27 (45 %) patients out of 60 patients had cirrhosis attributable to chronic alcohol intake. 13 patients had their disease due to viral etiology out of which 11 (18.3 %) and 2 (3.3 %) were due to HCV and HBV respectively. 20 (33.3 %) out of 60 patients had their disease due other causes. Mean vitamin D in the study population was 35.34 ± 22.4 ng / ml. Females and males had mean vitamin D level of 39.19 ± 23.62 ng/ml and 34.48 ± 22.32 ng/ml respectively. 1.7 % patients (1 out of 60) had vitamin D toxicity, 23.3 % (14 out of 60) had insufficient, 30 % (18 out of 60) had deficiency, 45 % (27 out of 60) had sufficient vitamin D. Patients with HCV and HBV as cause of liver cirrhosis had 41.42 ± 21.8 and 48.35 ± 18.8 mean vitamin D levels respectively.

In patients with Alcoholic cirrhosis mean vitamin D was 30.83 ± 21.2 and in patients with other causes of cirrhosis mean vitamin D was 36.80 ± 24.5. There was no significant correlation between mean vitamin D levels and etiology of liver cirrhosis. (P value = 0.457; Not Significant). Mean CPS and MELD Scores were 8.98 ±3.05 and 18.01 ± 8.06 respectively. Class A had mean vitamin D levels of 60.65 ± 18.34 with mean MELD Score was 12.20 ± 4.26. Class B had mean vitamin D levels of 26.64 ± 9.08 with mean MELD Score of 17.25 ± 6.87. Class C had mean vitamin D levels of 18.75 ± 9.98 and mean MELD Score was 24.60 ± 7.39. There was significant correlation between vitamin D levels

with severity of liver disease according to Child-Pugh Score and MELD Score (p value < 0.001).

DISCUSSION

The present observational study was conducted with the aim of assessing vitamin D levels in patients of cirrhosis and to study the relation between vitamin D levels and severity of liver dysfunction. In the study population, 11 were females and 49 were males. Mean age of the study population was 54.83 ± 13.56 years. In comparison a study conducted by Fisher L et al[25] in 100 patients (63 men, 37 women) the mean age of the study population was 49.0 ± 12.1 years.

Mean vitamin D in the study population was 35.34 ± 22.4 ng / ml. 1 patient out of 60 had vitamin D toxicity (> 100 ng / ml). 23.3 % (14 out of 60) of the study population had insufficient vitamin D (20 - < 30 ng / ml), 30 % (18 out of 60) had vitamin D deficiency (<20ng/ml), 45 % (27 out of 60) had sufficient (30 – 100 ng / ml) vitamin D. In comparison to study conducted by Fisher L et al[25] out of 100 patients 91 had inadequate vitamin D. 23 % patients had insufficient vitamin D levels, 68 % patients had vitamin D deficiency and 9 % patients had sufficient vitamin D levels.

In this study the patients with HCV as cause of liver cirrhosis had 41.42 ± 21.8 mean vitamin D levels, patients with HBV as cause of cirrhosis had 48.35 ± 18.8 mean vitamin D levels. In patients with Alcohol as etiology of cirrhosis mean vitamin D was 30.83 ± 21.2 and in patients with other causes of cirrhosis mean vitamin D was 36.80 ± 24.5. (P value = 0.457; Not Significant).

This is in comparison with the observation of study conducted by Malham et al.[26] The median 25- (OH) D blood concentration in ALC patients was 24 nmol/L, and the median serum level of 25- (OH) D was 45 nmol/L in Primary Biliary Cirrhosis (PBC) patients. Where as in contrast a study conducted by Putz - Bankuti et al[27] mean vitamin D in patients with Alcoholic cirrhosis was 17.2 ± 9.3 and in patients with cirrhosis of non-alcoholic origin mean vitamin D was 15.4 ± 9.4 ng/ml (p= 0.480).

Mean CPS Score of the study population was 8.98 ± 3.05. In Child-Pugh Class A out of 20 patients, 1 (5 %) patient had insufficient vitamin D, 18 (90 %) patients had sufficient vitamin D and 1 patient had toxic levels. In Child-Pugh Class B out of 20 patients 5 (25 %) were deficient in vitamin D, 8 (40 %) had insufficient and 7 (35 %) patients had sufficient vitamin D levels.

In Child-Pugh Class C out of 20 patients 13 (65 %) were deficient in vitamin D levels, 5 (25 %) had insufficient and 2 (10 %) patients had sufficient vitamin D. Patients in Class A had mean vitamin D levels of 60.65 ± 18.34, mean MELD Score was 12.20 ± 4.26, patients in Class B had mean vitamin D levels of 26.64 ± 9.08 with mean MELD Score of 17.25 ± 6.87 and patients in Class C had mean vitamin D levels of 18.75 ± 9.98 and mean MELD Score was 24.60 ± 7.39 (p value < 0.001).

From these results it was observed that hypovitaminosis is related to the increasing severity of the liver dysfunction according to CPS Score and inversely related to MELD Score.

This was in comparison to study conducted by Paternostro et al.[28] The prevalence of vitamin D (< 10 ng/ml) deficiency was 40 percent i.e. (79 / 199), with 14 percent in class A of Child-Pugh, 39 percent in Child-Pugh class B and 47 percent in Child-Pugh class C (p = 0.001). Moreover, a significantly (P < 0.0001) higher prevalence of vitamin D deficiency in patients with cirrhosis was observed in a study conducted by Stokes et al,[29] as evidenced by a negative relationship between vitamin D status and disease severity (assessed by CPS).

Patients in Child-Pugh Class C had lower (P < 0.001) mean 25 (OH) D concentrations than those in class A (9.0 ± 4.0 vs 18.3 ± 6.7 ng/ml) respectively. These results are backed up by Miroliaee et al[30] and Rode et al[31] who found that 75 percent of cirrhotic patients have 25 (OH) D levels below 20 ng/ml. In a cohort of 75 patients with cirrhosis, Putz-Bankuti et al[27] found an inverse relationship between serum 25 (OH) D levels and liver disease severity. The data reflect the subjects in Child-Pugh class C have approximately half the 25 (OH) D concentrations of class A and in most cases, the difference is statistically significant (P < 0.001).

Similarly, the median of plasma 25 (OH) D level was 48 nmol/L (p25th: 32.5; p75th: 56.1) and 27 (15.5 %) had 25 (OH) D deficiency (< 25 nmol / L) in a study conducted by Guzmán-Fulgencio.[32]

A prospective cohort study was conducted by Finkelmeier et al,[23] and it showed that the levels of 25 (OH) D3 varied considerably between the Child-Pugh scores and showed a negative association with the end-stage liver disease (MELD) score model. There were significant differences among Child Pugh scores with the highest levels in Child A and the lowest levels in Child C patients ($P <$ 0.001). Furthermore, the relationship between the levels of 25 (OH) D3 and the MELD score was assessed. A strong inverse association between 25 (OH) D3 concentrations and the MELD score (r = -0.277, P < 0.001) was observed, suggesting a relationship between cirrhosis severity and low levels of 25 (OH) D3. Vitamin D levels were found to be negatively associated with both MELD and CPS in a study conducted by Jamia et al,[33] implying that vitamin D levels become deficient as the disease progress (p < 0.05).

The findings are consistent with those of several other studies that have shown that vitamin D levels are inversely linked to CPS and MELD. In comparison to patients with lower CPS and MELD scores, patients with higher CPS and MELD score have significantly lower vitamin D levels.

CONCLUSIONS

In conclusion, the low concentration of 25 (OH) D is correlated with severity of liver dysfunction irrespective of the etiology of the disease.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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