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Associated vitamin D deficiency is a risk factor for the complication of HCV-related liver cirrhosis including hepatic encephalopathy and spontaneous bacterial peritonitis

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Abstract

The influence of vitamin D, 25-hydroxyvitamin D (25(OH)D), deficiency on hepatitis C virus (HCV)-related cirrhosis had been poorly elucidated especially in patients with hepatic encephalopathy (HE) and spontaneous bacterial peritonitis (SBP). We aimed to investigate the association between vitamin D deficiency and the risk of SBP or HE, including the mortality rate. Serum 25(OH)D levels were prospectively determined in 135 patients. Of them, 45 patients had complications with HE and 45 patients had complications with SBP; 45 cirrhotic patients without complication served as the control group. Vitamin D deficiency was defined as 25(OH)D levels < 20 ng/ml. Receiver operating characteristic (ROC) and Kaplan–Meier method with log-rank test were used in our statistical analysis. Predictors of survival were determined using Cox regression analysis. Serum 25(OH)D levels were significantly ($P < 0.05$) lower in the HE and SBP groups than in the control group (6.81 ± 2.75, 7.15 ± 2.10, 16.28 ± 6.60, respectively). Moreover, serum 25(OH)D levels were significantly lower in the high HE grade than in the low grade ($P < 0.001$). Regarding the SBP group, classic SBP was associated with lower 25(OH)D levels compared to other types ($P < 0.001$). ROC curve revealed that lower 25(OH)D levels less than 7.1 ng/ml and 6.6 ng/ml could predict the mortality in SBP and HE patients, respectively, with high sensitivity and specificity. Serum 25(OH)D levels < 5 ng/ml were associated with significant higher mortality rate (HR = 2.76, $P = 0.001$). Lower 25(OH)D levels were associated with HE and SBP in cirrhotic patients. In addition, it may be considered a prognostic parameter for the severity of liver cirrhosis.

Keywords Vitamin D deficiency · Hepatic encephalopathy · Spontaneous bacterial peritonitis

Introduction

The global prevalence of hepatitis C virus (HCV) infection was estimated to be 71 million viremic infections according to the World Health Organization [1]. Egypt is one of the countries that is most affected by HCV. According to the Egypt demographic and health surveys (EDHS), the prevalence among the adult population aged 15–59 years was 14.7%. [2]. Liver cirrhosis secondary to HCV infection is the main etiology of chronic liver disease (CLD). CLD is a common cause of morbidity and mortality worldwide. It accounts for about two million deaths per year. Of them, over a million deaths was only from liver cirrhosis [3]. Cirrhosis is the most frequent outcome in patients with progressive liver disease of various causes. It is the 12th leading cause of mortality in America and the 7th leading cause of death in people between the ages of 25 and 64 years [4]. Egypt has the highest age-standardized cirrhosis mortality rates of 72.7 deaths per 100,000 patients and almost one-fifth (18.1%) of all deaths in males aged 45–54 years were due to liver cirrhosis [5]. Complications relating to the diseased liver are frequent and severe in patients with cirrhosis. Complications include multiple organ failure that apart from the liver involves the heart, kidneys, lungs, and the immune system. With the progression of cirrhosis, development of

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portal hypertension and hyperdynamic circulation leads to esophageal varices, ascites, infections, cardiomyopathy, hepatorenal syndrome (HRS), hepatic encephalopathy (HE), and hepatopulmonary syndrome [6].

Cirrhotic patients are more susceptible to bacterial infections due to the reduced clearance of bacteria owing to an impaired function of monocytes and macrophages located in the cirrhotic liver, which are critical cells of the innate immune system [7–9]. Furthermore, the overgrowth of gut flora and increased intestinal permeability facilitate the bacterial translocation from the gastrointestinal tract to the porta-hepatis that plays a role in the pathogenesis of the complications of cirrhosis, particularly, bacterial infection and HE [10, 11]. In patients with cirrhosis, infection of the ascitic fluid as in spontaneous bacterial peritonitis (SBP) is a significant risk factor for the development of circulatory dysfunction and HRS [12, 13]. Bacterial translocation is associated with a marked increase in circulating levels of proinflammatory cytokines such as TNF-a, IL-6, and nitric oxide leading to inflammatory response and augmentation of the circulatory dysfunction [14]. Moreover, disturbance in cerebral blood flow is integrated into the pathogenesis of HE and more likely linked to alterations in ammonia and inflammatory status [15]. 1,25-Dihydroxyvitamin D_3 is the active form of vitamin D. The liver is a vital organ for vitamin D biotransformation. It also may be a site of vitamin D storage and is the site of conversion of vitamin D3 to 25-hydroxyvitamin D [25(OH)D] [16]. Recently, studies have found that vitamin D not only regulates calcium and phosphate balance, but also acts as a secosteroid hormone, protects the gut barrier and prevents bacterial translocation as it has an essential role in intestinal epithelial defenses against infectious agents [17]. Furthermore, clinical and epidemiological data have shown that chronic viral hepatitis is associated with lower serum 25(OH)D concentrations [18–21].

We hypothesized that 25(OH)D deficiency is associated with increased mortality in cirrhotic patients because of an increase in bacterial infections or aggravating HE. Therefore, we aimed to investigate the association between serum 25(OH)D deficiency and the complications of HCV-related cirrhosis, particularly, SBP and HE. We also aimed to investigate how serum 25(OH)D influences mortality in cirrhotic patients with HE or SBP.

Method

Study design

A hospital-based non-interventional, prospective, observational study was carried out in the ICU Unit of Internal Medicine, and Biochemistry Departments, Faculty of Medicine, Zagazig University Hospitals. The institutional review board approved the study (IRB#:2362-29-9-2015). Written informed consent was obtained from all individual participants included in the study. For patients with altered sensorium, informed consent was obtained from their first-degree relatives.

Patient selection

Eligible patients had a diagnosis of liver cirrhosis secondary to HCV infection, were aged ≥ 18 years and had cirrhosis-related ascites with or without spontaneous bacterial peritonitis or hepatic encephalopathy. We excluded patients with liver cirrhosis secondary to causes other than HCV infection, primary biliary cirrhosis, primary sclerosing cholangitis, history of cancer other than hepatocellular carcinoma within the last 5 years, renal disease, hyperparathyroidism, secondary peritonitis, and ascites due to non-hepatic causes. We also excluded any patients who received 25(OH)D in the previous 2 months. For patients who presented with clinical manifestations of infections as fever and chills or showed elevated C-reactive protein or leukocyte values in the blood tests, urinalysis and chest X-ray were performed. The Child–Pugh classification of each cirrhotic patient was performed [22].

Furthermore, patients also underwent abdominal and pulmonary imaging studies. All cirrhotic patients with ascites underwent diagnostic abdominal paracentesis to obtain an ascetic fluid sample for laboratory investigation. Assessment of severity of patient presenting with hepatic encephalopathy was done according to West Haven criteria [23].

Laboratory assessment

Blood samples were obtained from each patient and left for 30 min for spontaneous clotting. Samples were then centrifuged at 3000 rpm for 5 min and stored at − 20 °C. Serum 25(OH)D level was measured by enzyme-linked immunosorbent assay (ELISA, Immunodiagnostic Systems Ltd, Bolden, UK) at the Central Laboratory of Zagazig University Hospital. The ELISA kit we used cannot cross-react with 1,25(OH) 2D. Vitamin D deficiency was defined as 25(OH)D levels less than 20 ng/ml [24–26].

A combination of the BacT/Alert Microbial Detection System with the VITEK 2 system (bioMérieux, France) was used to achieve rapid bacterial identification and susceptibility testing in the diagnosis of SBP [27, 28]. All patients underwent routine laboratory testing, including liver and renal function tests, total and differential leucocytes counts, and coagulation tests.

Statistical analysis

Statistical analyses were performed using the statistical software program, SPSS, for Windows version 25.0 (SPSS; Chicago, IL, USA) and MedCalc (MedCalc 10 Software bvba, Ostend, Belgium). The Kolmogorov–Smirnov or Shapiro–Wilk tests were used to check for Gaussian distribution of the quantitative variables. Data are presented as a mean ± standard deviation (SD) or as a median and interquartile range (IQR) in case of Gaussian and non-Gaussian distribution, respectively. For quantitative variables, parametric independent *t* test was used for comparison in case of Gaussian distribution, while the Mann–Whitney *U* test was used for non-Gaussian distribution. For comparisons of quantitative variables among the three groups, one-way ANOVA was used. The post hoc analysis was performed using the Tukey test if significant differences were found.

For categorical variables, the Chi-square test (or Fisher's exact test if appropriate) was used. Spearman's rank correlation coefficient (Spearman's rho) was calculated for 25(OH)D levels and various study parameters. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cutoff values of 25(OH)D levels with maximum sensitivity and specificity for predicting mortality in the patients with liver cirrhosis. The best cutoff points were the concentration of serum 25(OH)D at the associated criterion of the highest Youden Index from ROC analysis using MedCalc software.

For all tests, a *P* value of 5% or less at a two-sided test was considered statistically significant. Predictors of survival were determined using a univariate Cox regression hazard model. Kaplan–Meier method with log-rank test was used to compare the overall survival in CLD patients with or without vitamin D deficiency.

Results

The present study included 135 patients with HCV-related cirrhosis aged 58.64 ± 8.07 years. Patients' characteristics are summarized in Table 1. The patients were classified according to their developed complications into three groups: group 1 ($n = 45$) included cirrhotic patients without any significant complications which served as control, group 2 ($n = 45$) included cirrhotic patients with HE, and group 3 ($n = 45$) included cirrhotic patients with SBP. Most of the included patients in HE and control groups were females (71% and 66.7%, respectively). The mean Child–Pugh score in the HE group (11.20 ± 2.07) was significantly higher than in the control group (10.07 ± 1.96). Regarding the patients with SBP, the frequency of males and females was comparable and the mean Child–Pugh score (11.20 ± 2.07) was significantly higher than the control group. In addition, serum K levels were significantly lower in the HE group than in the control group ($P = 0.008$). While in the SBP, compared to the control group, there was no statistically significant difference in serum K level ($P = 0.44$).

The lower the serum 25(OH)D level, the worse was the HE grade. Serum 25(OH)D levels were 10.0 ± 1.52 ng/ml in HE grade 1, 7.01 ± 2.05 ng/ml in HE grade 2, 4.99 ± 0.99 ng/ml in HE grade 3, and 2.33 ± 1.13 ng/ml in HE grade 4, with a statistically significant difference between all HE grades ($P < 0.001$). Post hoc analysis revealed a statistically significant difference between each of two HE grades ($P < 0.05$, Fig. 1a).

On the other hand, we reported 12 patients with classic SBP, 5 patients with mononuclear bacterascites (MNB), and 28 patients with culture-negative neutrocytic ascites (CNNA). Serum 25(OH)D level was significantly different between the three SBP groups ($P < 0.001$). Post hoc analysis showed that serum 25(OH)D level was significantly lower in patients with classic SBP (4.67 ± 1.39 ng/ml) compared to patients with CNNA (8.47 ± 1.41 ng/ml, $P < 0.001$), but not when compared to patients with MNB (5.71 ± 0.73 ng/ml, $P = 033$) as seen in Fig. 1b. There was a significant negative correlation between 25(OH)D and Child–Pugh score, albumin, and potassium level in cirrhotic patients with HE. Furthermore, there was a significant negative correlation between the MELD score and the serum 25(OH)D level in patients with SBP ($r = -0.29$, $P = 0.04$); Table 2.

A total of 23 patients in the SBP group and 30 patients in the HE group improved, while 22 patients in the SBP group and 15 patients in the HE group died. Serum 25(OH)D level was significantly higher in the improved patients than in patients who died in both groups, as shown in Table 3.

ROC curve analysis revealed that serum 25(OH)D level of less than 7.2 ng/ml could predict the mortality in cirrhotic patients with 80.9% sensitivity and 58.8% specificity; Fig. 2a. Furthermore, the cutoff level of vitamin D less than 7.1 ng/ml can predict the mortality in SBP patients with a sensitivity of 79.1% and specificity of 77.2%; Fig. 2b. In patients with HE, 25(OH)D level of less than 6.6 ng/ml can predict the mortality with 70.1% sensitivity and 75% specificity; Fig. 2c. The areas under the curve were 0.680, 0.739, and 0.797, respectively.

In the HE group, 25(OH)D levels < 5 ng/ml were associated with high mortality rate (HR: 2.76; 95% CI 1.69–4.15; $P = 0.001$), while in patients with SBP, the mortality rate with serum 25(OH)D level < 5 ng/ml did not differ significantly from patients with high 25(OH)D levels (> 5 ng/ml). The 25(OH)D levels < 10 ng/ml was not associated with high mortality rate in patients with HE or SBP compared to high 25(OH)D levels (> 10 ng/ml); Fig. 3. In addition, Cox regression analysis was performed to identify the risk factors associated with high mortality in cirrhotic patients. MELD score, Child–Pugh score, severe 25(OH)D deficiency,

Table 1 Characteristics of enrolled patients at the baseline

	Control group (*N* = 45) Mean ± SD	HE group (*N* = 45) Mean ± SD	*P* value	SBP group (N = 45) Mean ± SD	*P* value
Age (year)	58.07 ± 8.26	59.51 ± 8.03	0.472	58.33 ± 7.91	0.87
Gender					
Female *N* (%)	30 (66.7%)	32 (71%)	0.158	22 (48.9%)	0.13
Male *N* (%)	15 (33.3%)	13 (29%)		23 (51.1%)	
WBC ($\times 10^3$/mm^3)	8.48 ± 2.87	10.5 ± 4.81	0.001	9.21 ± 6.37	0.625
Hemoglobin (g/dl)	10.68 ± 1.89	9.66 ± 1.88	0.012	10.15 ± 1.62	0.1505
Platelet count ($\times 10^3$/mm^3)	82.04 ± 30.5	92.42 ± 51.78	0.250	79.20 ± 35.34	0.6837
ALT (U/l)	37.98 ± 24.54	46.38 ± 46.50	0.274	43.98 ± 30.8	0.3112
AST (U/l)	58.88 ± 29.10	87.04 ± 68.60	0.011	74.85 ± 52.01	0.1475
Total bilirubin (mg/dl)	3.20 ± 3.05	7.36 ± 8.57	0.003	5.03 ± 4.57	0.007
Direct bilirubin (mg/dl)	2.01 ± 2.36	5.44 ± 6.92	0.002	3.40 ± 3.65	0.0083
Total protein (mg/dl)	6.31 ± 0.80	5.78 ± 0.89	0.004	6.09 ± 0.93	0.2451
Albumin (mg/dl)	2.16 ± 0.52	2.16 ± 0.41	0.966	2.02 ± 0.39	0.1869
INR	1.64 ± 0.32	1.78 ± 0.41	0.085	1.95 ± 0.87	0.1222
Serum creatinine (mg/dl)	1.03 ± 0.26	1.03 ± 0.60	0.966	1.13 ± 0.26	0.0614
Serum urea (mg/dl)	28.08 ± 14.48	33.7 ± 21.92	0.151	28.54 ± 17.49	0.89
Random blood sugar (mg/dl)	135.28 ± 20.87	158.8 ± 27.73	0.030	141.64 ± 25.70	0.0790
Serum K level (mEq/l)	4.20 ± 0.61	3.84 ± 0.66	0.008	4.11 ± 0.52	0.44
Vitamin D level (ng/ml)	16.28 ± 6.60	6.81 ± 2.75	0.001	7.15 ± 2.10	0.0001
Vitamin D class *N* (%)					
Deficient ≤ 20 ng/ml	35 (77.8%)	45 (100%)	–	45 (100%)	–
Non deficient > 20 ng/ml	10 (22.2%)	0		0	
Child–Pugh Class *N* (%)					
Class B	21 (46.7%)	11 (24.4%)	0.02	10 (22.2%)	0.03
Class C	24 (53.3%)	34 (75.6%)		35 (77.8%)	
Child–Pugh score	10.07 ± 1.96	11.20 ± 2.07	0.002	11.20 ± 2.07	0.0075
MELD score	16.2 ± 3.85	19.5 ± 5.88	0.005	19.69 ± 6.19	0.001

ALT alanine transaminase, *AST* aspartate transaminase, *HE* hepatic encephalopathy, *INR* international normalized ratio, *MELD* model for end-stage liver disease, *SBP* spontaneous bacterial peritonitis, *WBC* white blood cell

creatinine level, and urea level were associated with high mortality in patients with HE, while in patients with SPB, only a severe 25(OH)D deficiency was associated with high mortality, as seen in Supplementary file 1.

Discussion

Vitamin D deficiency is prevalent in chronic liver disease patients. Even patients with mild liver disease are affected, although patients with liver cirrhosis more commonly have severe deficiency [29]. Our study demonstrates that low serum levels of 25(OH)D are associated with severe liver damage, leading to significant complications such as HE and SBP. Furthermore, a low threshold (< 7.2 ng/ml) was significantly linked to high mortality rates.

Most of the included patients in this study were females (62%). Women commonly present with autoimmune hepatitis, benign liver lesions, primary biliary cirrhosis, and toxin-mediated hepatotoxicity, but less commonly have malignant liver tumors and viral hepatitis. There is a decreased rate of decompensated cirrhosis in women with hepatitis C virus infection [30].

Low serum levels of 25(OH)D have long been observed in chronic liver diseases, especially with liver cirrhosis due to chronic HCV infection [31, 32]. Our study included 135 patient with HCV-related cirrhosis and revealed a statistically significant correlation between the severity of cirrhosis (Child–Pugh score) and low serum levels of 25(OH)D in patients with complications of HE ($P = 0.02$) and SBP ($P = 0.04$). In line with our results, Trépo et al. reported that 25(OH)D level below 10 ng/ml was significantly associated with complications of liver disease, including the presence of ascites ($P = 0.0001$) and encephalopathy ($P = 0.005$). Moreover, SBP was more prevalent with low vitamin D concentration [33]. Similarly, Anty et al. reported that 25(OH)

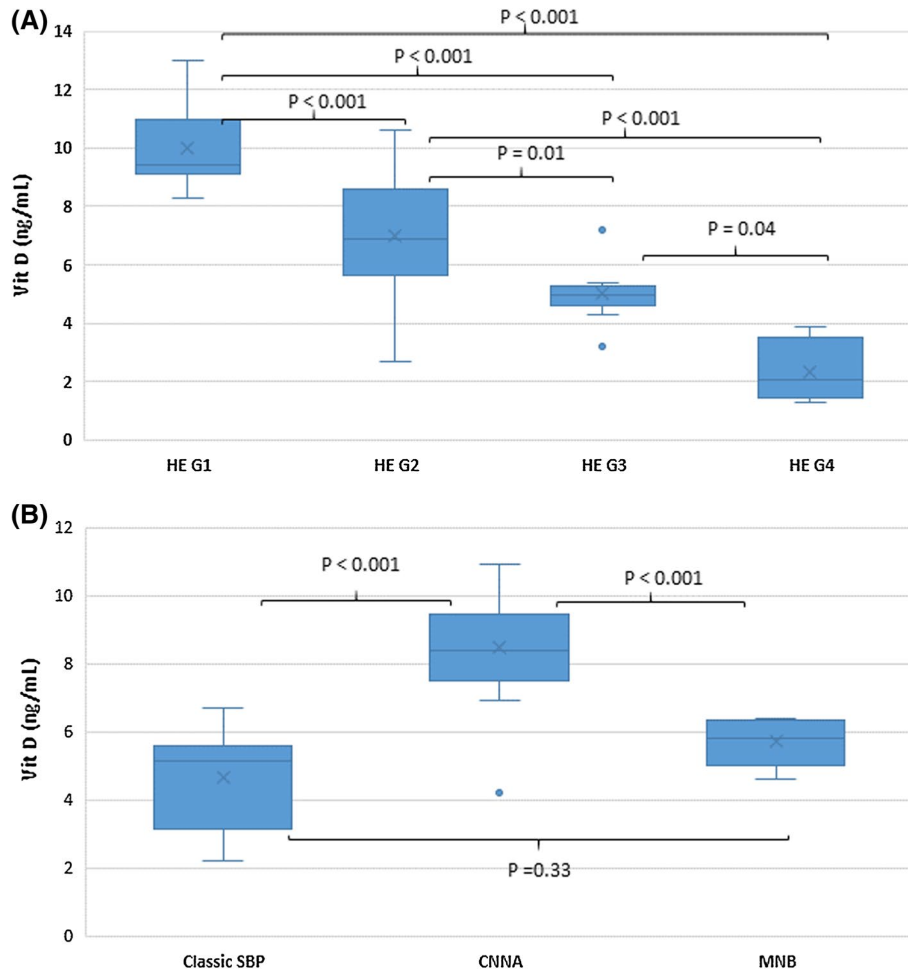

Fig. 1 **a** Comparison between vitamin D level and different hepatic encephalopathy grades with post hoc analysis. **b** Comparison between vitamin D level and subtypes of spontaneous bacterial peritonitis with post hoc analysis

D level was inversely correlated with the Child–Pugh score ($r=0.28$, $P=0.009$) [34].

We compared the cirrhotic patients with complications (HE and SBP group) with the patients without complications (control group). Our results revealed that serum vitamin D level was significantly lower in the patients with complications compared to the control group. Although 25(OH)D deficiency is common among patients with CLD, it is more severe in cirrhotic patients with HE or SBP. Vidot et al. reported that mild 25(OH)D deficiency was not associated with an increase in HE. However, moderate and severe 25(OH)D deficiency was significantly associated with HE. Previously, patients with SBP reported a significantly lower 25(OH)D levels compared with non-infected patients [34, 35]. We have confirmed that more severe liver cirrhosis is associated with high vitamin D deficiency. Also, previous studies have shown that the higher incidence of infectious complications and worse prognosis, the more severe is the liver cirrhosis [35, 36].

Vitamin D has been identified as an anti-infective agent. The association between 25(OH)D deficiency and liver cirrhosis has been identified in HCV and alcoholic liver disease [33, 37, 38]. The mechanisms of increased risk of infection in cirrhotic patients with a low 25(OH)D level are still unclear, but can be explained by many theories. Hypovitaminosis D is commonly associated with hypocalcemia. In chronic liver disease patients, hypocalcemia is a well-known abnormality in critically ill patients in the course of sepsis [39, 40]. It was reported that the 25(OH)D levels inversely correlate with the expression of toll-like receptors (TLRs) in monocytes, leading to a negative correlation between 25(OH)D levels and infection [31]. It is mediated by antigen-presenting cells such as macrophages. The 25(OH)D is activated in these cells by 1, 25 hydroxylase to generate 1, 25-(OH)D, which increase the synthesis of antibacterial protein as cathelicidin [41, 42].

To date, the association between 25(OH)D deficiency and HE has not been explained. We reported that the lower 25(OH)D level, the higher is the HE grade. This association may be explained by deficient anti-inflammatory properties of 25(OH)D. Systemic inflammation and changes in hepatic metabolism as increased ammonia levels are considered precipitants of HE and worsen the existing HE [37, 38]. The present European and American guidelines

Table 2 Correlation between vitamin D level and other parameters

	All Patients ($n=135$)		HE group ($n=45$)		SBP group ($n=45$)		Control group ($n=45$)	
	r^a	P value	r^a	P value	r^a	P value	r^a	P value
Age (year)	−0.21	0.02	−0.20	0.18	−0.13	0.38	−0.29	0.04
WBC ($\times 10^3$/mm^3)	−0.16	0.05	−0.16	0.27	−0.09	0.55	0.01	0.91
Hemoglobin (g/dl)	0.13	0.12	0.02	0.89	0.04	0.75	−0.06	0.57
Platelet count ($\times 10^3$/mm^3)	0.02	0.79	0.05	0.70	0.15	0.31	0.13	0.37
ALT (U/L)	−0.13	0.11	−0.11	0.44	−0.16	0.29	−0.11	0.46
AST (U/L)	−0.22	0.01	−0.21	0.04	−0.18	0.22	−0.14	0.34
Total bilirubin (mg/dl)	−0.21	0.02	−0.15	0.30	−0.04	0.76	0.01	0.91
Direct bilirubin (mg/dl)	−0.23	0.01	−0.14	0.33	0.09	0.97	−0.02	0.86
Total protein (mg/dl)	0.09	0.29	−0.11	0.43	0.02	0.98	−0.11	0.46
Albumin (mg/dl)	0.09	0.47	−0.29	0.03	0.001	0.91	0.10	0.50
INR	−0.11	0.21	−0.15	0.30	0.14	0.32	0.29	0.04
Serum urea(mg/dl)	−0.08	0.34	−0.04	0.71	−0.13	0.37	−0.01	0.93
Serum K level (mEq/l)	0.14	0.09	−0.21	0.05	0.02	0.87	−0.11	0.46
MELD score	−0.22	0.01	−0.16	0.26	−0.29	0.04	0.18	0.23
Child–Pugh score	−0.24	0.006	−0.36	0.01	−0.25	0.05	0.13	0.39

ALT alanine transaminase, *AST* aspartate transaminase, *HE* hepatic encephalopathy, *INR* international normalized ratio, *MELD* model for end-stage liver disease, *SBP* spontaneous bacterial peritonitis, *WBC* white blood cell

[a]Spearman's rank correlation coefficient

Table 3 Comparing the vitamin D levels between the improved patients and deceased patients

Groups	Improved patients		Deceased patients in ICU		MD. 95% CI	P value
	N (%)	Mean ± SD	N (%)	Mean ± SD		
SBP group ($N=45$)	23 (51.2%)	8.36 ± 1.72	22 (48.8%)	5.9 ± 1.96	0.93 (3.58, 1.33)	< 0.0001
HE group ($N=45$)	30 (66.7%)	7.93 ± 3.21	15 (33.3%)	5.42 ± 2.60	2.51 (0.58, 4.43)	0.01

HE hepatic encephalopathy, *SBP* spontaneous bacterial peritonitis, *MD* mean difference, *CI* confidence interval

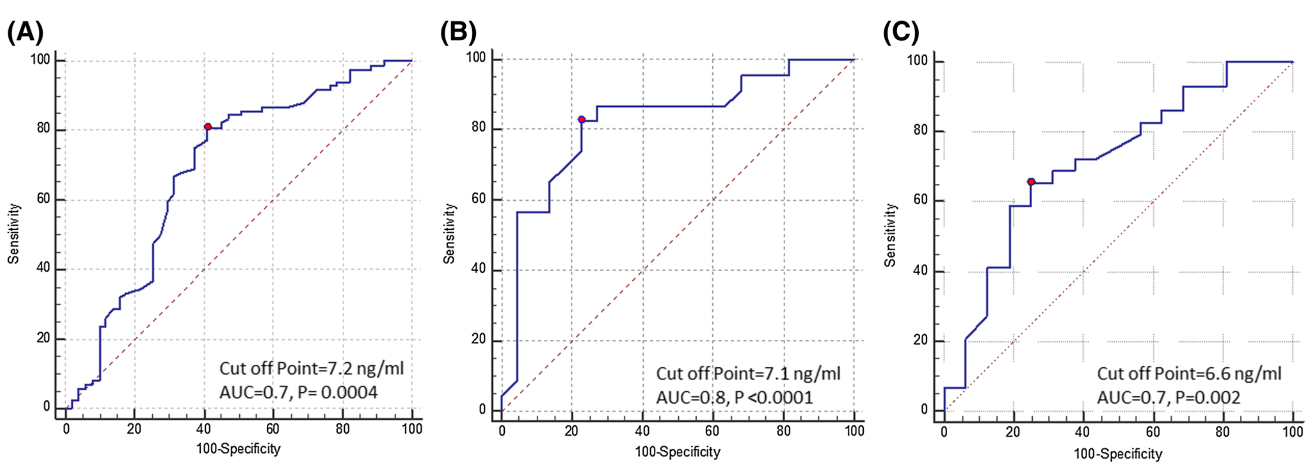

Fig. 2 **a** ROC curve analysis for vitamin D level and the overall mortality in complicated cirrhosis. **b** ROC curve analysis for vitamin D level and the mortality in spontaneous bacterial peritonitis patients. **c** ROC curve analysis for vitamin D level and the mortality in hepatic encephalopathy patients

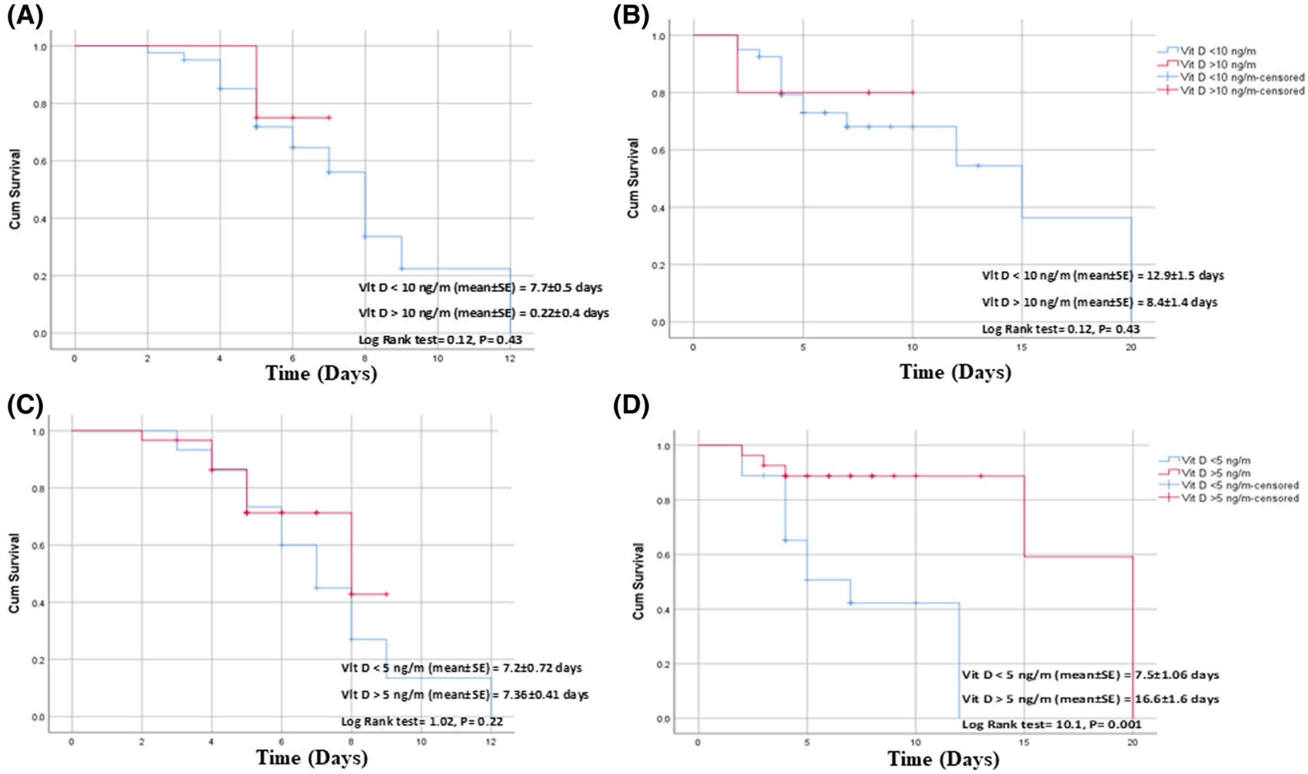

Fig. 3 **a** Survival analysis in spontaneous bacterial peritonitis patients with vitamin D level <10ng/ml. **b** Survival analysis in hepatic encephalopathy patients with vitamin D level <10 ng/ml. **c** Survival analysis in spontaneous bacterial peritonitis patients with vitamin D level <5 ng/ml. **d** Survival analysis in hepatic encephalopathy patients with vitamin D level <5 ng/ml

for the management of liver disease do not clearly propose vitamin D supplementation in CLD with or without complications. The European Association for the Study of the Liver recommended calcium and vitamin D supplementation for the management of patients with cholestatic liver diseases. However, few clinical trials are available to support this suggestion [43]. Well-designed clinical trials are recommended to investigate the effect of vitamin D supplementation on patients with complicated liver cirrhosis.

We reported a significant negative correlation, only in the HE group, between the albumin level and serum 25(OH)D ($r = -0.29$, $P = 0.03$). Patients with HE or SBP were associated with lower serum albumin level, especially those with 25(OH)D deficiency [37].

High mortality rate was associated with serum 25(OH)D deficiency. A total of 38 (42.2%) patients with 25(OH)D < 10 ng/ml died. Serum 25(OH)D < 5 ng/ml was associated with 80% mortality in the SBP group and 55.6% mortality in the HE group. Because patients with cirrhosis had lower serum 25(OH)D levels, we suggested that 25(OH)D levels might be of prognostic value in these patients [33, 44, 45]. The ROC analysis showed that the cutoff level of vitamin D < 7.1 ng/ml can predict the mortality in SBP, while, in patients with HE, 25(OH)D level of < 6.6 ng/ml can predict the mortality with high sensitivity and specificity.

A low level of 25-OH vitamin D has been associated with increased mortality in the general population [46, 47]. A prospective trial revealed that vitamin D with calcium supplementation is associated with reducing the mortality rate in older adults [48]. The previous experimental study conducted by Trépo and colleagues revealed that 25(OH)D concentration below 10 ng/ml was significantly associated with complications of liver cirrhosis, including the presence of ascites ($P = 0.00012$) and HE ($P = 0.0052$).

HE can be even more worrisome when associated with bacterial infections. Survival of cirrhotic patients with HE has been investigated, confirming bacterial infections as the leading cause of death [49]. Vitamin D levels less than 5 ng/ml were associated with a significantly higher mortality rate in patients with HE (HR: 2.76, $P = 0.001$). These results were consistent with the study of Paternostro et al., that reported a high mortality rate in cirrhotic patients with serum 25(OH)D levels less than 6 ng/ml (HR 1.723, $P = 0.013$) [50].

Our study has some limitations and strengths. This study was the first to conduct a comprehensive analysis including cirrhotic patients with complications in a prospective study design. Nevertheless, this study is monocentric that limits the generalizability of the results. The reported CIs of the ROC curves in the present study were relatively wide. It is evident that the sample size effect can be witnessed regarding the width of the confidence interval, notifying that the true estimate is independent of the effect of sample size and its corresponding confidence interval.

Conclusion

Low levels of 25(OH)D levels were associated with HE and SBP in cirrhotic patients. In addition, it may be considered as a prognostic parameter for severity of liver cirrhosis. Finally, we recommend a randomized clinical trial to assess the impact of 25(OH)D supplementation on the infection rate, the severity of HE, and the mortality rate in cirrhotic patients.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no conflict of interest.

Statement of human and animal rights All human rights are preserved according to the declaration of Helsinki guidelines.

Informed consent Written informed consent was obtained from all individual participants included in the study. For patients with altered sensorium, informed consent was obtained from their first-degree relatives.

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