European Review for Medical and Pharmacological Sciences 2021; 25: 6908-6919

Effect of Vitamin D supplementation in patients with liver cirrhosis having spontaneous bacterial peritonitis: a randomized controlled study

A.A. MOHAMED[1], A.A. AL-KARMALAWY[2], A.A. EL-KHOLY[3], D.A. EL-DAMAS[4], H.M. ABOSTATE[4], S.M. MOSTAFA[5], M. HAMADA[6], M.A. KHALIK ELKADY[7], Y. HASSAN[8], E. AL-HUSSAIN[8], M.G. KHALIL[9], I. BADAWY[10], D. ELEBEEDY[10], B.A. ALSFOUK[11], M.M. SHAHEENL[2]

[1]Biochemistry, Molecular Biology and Biochemistry Department, National Hepatology and Tropical Medicine Research Institute, Egypt
[2]Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Horus University-Egypt, New Damietta, Egypt
[3]Clinical Pharmacy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
[4]Microbiology, Faculty of Pharmacy, Egyptian Russian University, Cairo, Egypt
[5]Department of Hepatology, National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt
[6]Internal Medicine Department, Faculty of Medicine, Benha University, Cairo, Egypt
[7]Gastroenterology and Hepatology Department, Theodor Bilharz Research Institute, Egypt
[8]Clinical Pathology Department, Faculty of Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt
[9]Toxicology Department, Faculty of Pharmacy, Modern University for Technology and Information (MTI), Cairo, Egypt
[10]Pharmaceutical Biotechnology Department, Faculty of Biotechnology, Misr University for Sciences and Technology, Cairo, Egypt
[11]Department of Pharmaceutical Sciences, College of Pharmacy, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia
[12]Internal Medicine and Nephrology Department, Cairo University, Cairo, Egypt

Amal Ahmed Mohamed and Ahmed A. Al-Karmalawy contributed equally to this work

Abstract. – OBJECTIVE: Spontaneous Bacterial Peritonitis (SBP) is one of the most serious liver cirrhosis with ascites complications. Vitamin D (Vit D) deficiency has been associated with a high risk of infection and mortality in cirrhotic patients. Herein, the assessment of Vit D level as a prognostic marker in SBP patients and the impact of Vit D supplementation on their treatment plan was studied as well.

PATIENTS AND METHODS: Ascetic patients with SBP and Vit D deficiency were divided randomly into treatment and control groups. The control group received standard treatment without Vit D and the treatment group received standard treatment plus Vit D. Clinical monitoring of Vit D was done over 6 months.

RESULTS: At baseline, all patients in both groups revealed an elevated serum and ascetic TLC, AST, ALT, total and direct bilirubin, in addition to elevation in INR and procalcitonin (PCT) level. Univariate regression analysis confirmed that deficiency of Vit D was an independent predictor of infection and mortality ($p < 0.01$; Crude Hazard Ratio: 0.951). Over 6 months, the study revealed significant improvement in serum Vit D level in the treatment group (34.6 ± 9.2 and 18.3 ± 10.0 ng/mL; $p < 0.001$). Moreover, a statistically significant increase in survival rate (64% vs. 42%; $p < 0.05$) and duration (199.5 days vs. 185.5 days; $p < 0.05$) were recorded as well. Univariate and multivariate regression analysis confirmed that Vit D supplementation was positively correlated to survival over 6 months ($p < 0.001$; Adjusted Hazard Ratio: 0.895).

CONCLUSIONS: Vit D deficiency is prevalent in SBP cirrhotic patients and is used as an independent predictor of infection and death. Therefore, Vit D supplementation revealed improvement in their response to treatment.

Key Words:
Ascites, Spontaneous bacterial peritonitis, Vitamin D deficiency, Survival.

Corresponding Author: Ahmed A. Al-Karmalawy, MD; e-mail: akarmalawy@horus.edu.eg

Introduction

Patients with liver cirrhosis had spontaneous bacterial peritonitis (SBP) infection that showed poor prognosis[1-3]. Cirrhosis is the terminal stage of any chronic liver injury which accounts for the fourth major cause of mortality in central Europe[4-6]. Egypt has the highest age-standardized mortality rate of 72.7 deaths for every 100,000 patients. Approximately one-fifth (18.1%) of most deaths in males of age ranged from 45 to 54 years were caused by liver cirrhosis[7-10]. Cirrhosis manifests with the presence of vascular fibrosis and distortion resulting in increased hepatic resistance, which may cause portal hypertension if there was splanchnic hyperperfusion[11-13]. Complications like variceal bleeding and renal dysfunction may be due to portal hypertension[14-16]. Renal dysfunction and splanchnic vasodilation contribute to the development of ascites[17-19]. Ascites are frequently complicated by SBP[20,21], most commonly due to intestinal bacterial translocation, which can trigger renal failure and is associated with increased mortality. New inflammation biomarkers are needed in clinical practice for the early diagnosis of SBP, such as procalcitonin (PCT)[22,23]. Meanwhile, the pathophysiology of SBP is not understood, since translocation of bacteria from the gastrointestinal tract to the ascetic fluid (AF) is thought to be a crucial mechanism behind the development of SBP. Also, SBP is encouraged by impaired defensive mechanisms in cirrhotic patients. It is diagnosed by counting polymorphonuclear (PMN) cell numbers in AF of more than or equal to 250 cells/mm^3, where the infectious organism is isolated in about 70% of cases[24,25].

Procalcitonin (PCT) is a polypeptide precursor for calcitonin synthesized in the thyroid C cells. Microbial toxins and pro-inflammatory mediators, such as IL-1B, IL-6, and TNF-α, triggered PCT release[26-28]. In decompensated cirrhosis, serum and ascetic fluid PCT were examined and we found they were not effective as a marker in the detection of acute bacterial infection (such as SBP) compared to patients without hepatic impairment[29-32]. PCT, however, appears to be a strong marker for bacterial translocation, a well-established mechanism for decompensated cirrhosis infection that can lead to bacterial infection[33,34]. Combining procalcitonin (PCT), the difference in hemoglobin concentration between newly formed and mature red blood cells (dCHC) and mean fluorescence intensity of mature neutrophils (sNFI) in patients with cirrhosis is a valuable predictive composite marker for early detection of ascites infection[35]. The acute stage reactant proteins, such as C-reactive protein (CRP) and procalcitonin (PCT), have been assessed in many clinical situations as indicators for early diagnosis of SBP. CRP and PCT are well known to rise rapidly in response to bacterial infection[36,37]. So, they are used as sensitive predictive and monitoring parameters for bacterial infections and controlling the use of antibiotics. In addition, cirrhotic patients usually have Vit D deficiency[38,39].

The liver is a crucial organ for Vit D biotransformation, where it is the only organ that generates the (25-OH) Vit-D3 from Vit D[40,41]. Low hepatocytes, reduced exposure to sunlight, low adipose tissue, malabsorption of Vit D, and altered hydroxylation of Vit D in the liver are the normal reasons for the decreased levels of Vit D in patients with cirrhosis[42]. It was found that low Vit D in the ascetic fluid is related to the higher risk of AF bacterial infection in cirrhotic patients with ascites[43,44].

There are few studies available concerning the impact of Vit D supplementation on people with Vit D deficiencies, particularly those with decompensated cirrhosis[45,46]. The effect of Vit D supplementation on mortality rate in those patients is still unclear. The main purpose of the present work is to assess Vit D and procalcitonin levels as prognostic markers in SBP patients, besides, evaluating the effect of Vit D administration on the response of SBP patients for treatment from liver cirrhosis.

Patients and Methods

Study Design and Setting

This study was a prospective randomized controlled study that included 328 ascetic patients with spontaneous bacterial peritonitis (SBP) who were admitted to the National Hepatology and Tropical Medicine Research Institute from June 2019 to December 2019.

Subject Selection

The inclusion criteria were: patients with liver cirrhosis, ascites with SBP, and aged over 18 years. The excluded patients from the study were: those with non-peritoneal infections (septicemia, skin infection, chest infection, biliary tract infection, urinary tract infection, gastroenteritis,

dental infection, meningitis), administration of non-absorbable antibiotics in the prior 6 weeks, infection with HIV, hepatoma or other forms of malignancy, hepatorenal syndrome on admission, significant cardiac and respiratory disease, pregnancy, patients prepared for transplantation, and signs of gastrointestinal bleeding or bacterial infection in the prior 6 weeks.

Clinical Evaluation

Detailed patient's clinical history and physical examination were performed. Patients were screened for complete blood count (using Phoenix NCC-2310 Hematology Analyzer Device), random blood sugar, liver profile [aspartate aminotransferase (AST), alanine aminotransferase (ALT), direct bilirubin, total serum bilirubin (TSB), alkaline phosphatase (ALP), serum albumin by OLYMPUS biochemistry autoanalyzer (USA), coagulation profile (prothrombin time (PT), and international normalized ratio (INR)] by kcl delta, renal function tests (urea and serum creatinine), viral markers (HBsAg; hepatitis C virus antibodies (HCV Abs) by ELISA, alpha-fetoprotein (AFP) by Eliza according to manufacturing instructions. Values were considered normal if > 10 ng/ml. In addition, abdominal ultrasonography was done for grading of ascites. All studied patients underwent diagnostic paracentesis to obtain AF samples for total leucocyte count (TLC) (polymorph nuclear leucocytes equal or more than 250 cells/mm^3 considered as spontaneous bacterial peritonitis), malignant cells, total protein, albumin, and glucose.

Serum Procalcitonin and Vit D Measurements

Serum procalcitonin (PCT) was measured using the PCT enzyme-linked immunosorbent assay kit for the *in vitro* assessment of serum PCT (Chongqing Biospes Co. Ltd, Chongqing City, China; biopsies). The Vit D level was also measured by ELISA (by direct competitive chemiluminescence immunoassay using commercially available kit Unicel® DxI 800 immunoassay systems (Beckman Coulter, Inc., CA, USA) within 24 hours of admission. Patient with serum Vit D concentration < 25 ng/mL was considered as Vit D deficient or insufficient.

Medication Exposure

The total number of patients who participated in this study with Vit D deficiency was 328 patients, randomly divided into control and treatment groups. The control group included patients who received standard treatment without Vit D (n=168). Whereas the treatment group included patients who received standard treatment plus Vit D (n=160). Patients in the treatment group received a dose of 300000 IU of cholecalciferol as a loading dose by intramuscular injection, followed by a maintenance dose of 800 IU/d orally with oral calcium supplementation in a dose of 1000 mg. Monitoring of Vit D, serum calcium, and phosphorus was measured every month for 6 months or until death, whichever happened first. Administration of Vit D was discontinued if signs of hypercalcemia, renal stone, or Vit D level above 80 nmol/L (> 32 ng/mL) were detected.

Ethical Considerations

Informed consent was received from all participants included in the study. The study protocol complied with the Ethical Guidelines set out in the Declaration of Helsinki (sixth revision, 2008) and Good Clinical Practice guidelines. The National Hepatology and Tropical Medicine Research Institute Committee approved the study (Serial: 5-2020).

Statistical Analysis

The data were analyzed using a statistical package for the social sciences, version 23 (IBM SPSS, Statistics for Windows, Version 23.0, IBM Corp, Armonk, NY, USA). Numerical variables were expressed as mean±SD (standard deviation) and median (range). Comparison between treatment and control groups was conducted as follows: for parametric data, a two-sample *t*-test was used while for the nonparametric data, the Mann-Whitney test was used. For analysis of survival, the log-rank test was used for binary variables while continuous variables were analyzed using the Cox-proportional hazard method. A *p*-value below 0.05 was considered statistically significant.

Results

Demographic Data

Out of 328 patients, 160 patients received standard SBP therapy plus Vit D (treatment group) and 168 patients received only SBP standard therapy (control group). The age was expressed as mean±SD in the treatment group (51.4± 12.1), and in the control group (55.0± 8.5) with a ratio of male to female 85:75 and 81:86, respectively.

Regarding the baseline characteristics, no significant difference (p>0.05) was observed between treatment and control groups, except significantly higher smoking (p<0.05) in the treatment group (Table I). In addition, blood test parameters and ascetic fluid composition were comparable in the control and treatment groups (p> 0.05), except for HBsAg positive and AST levels, which were significantly higher in the control group than in the treatment group (p< 0.05) (Table I). Treatment and control groups showed an elevation in PCT level (1.78± 0.49), (1.84± 0.78) and deficiency of Vit D (22.5± 11.5), (20.2± 11.0), respectively. For both groups, there was no significant difference in those values.

Effect of Vit D Supplementation

After 6 months of treatment and follow-up, most of the blood test parameters and ascetic fluid composition were significantly improved in both groups. However, the level of improvement was significantly higher in the treatment group compared to the control group (p< 0.001) (Table II). The PCT, which was investigated as an early diagnostic marker for ascites infection, showed a highly significant reduction in the treatment group than the control group (p< 0.001) (Figure 1). Furthermore, patients in the control group demonstrated an average 9.4% decrease in the mean serum Vit D level (20.2 ng/mL *vs* 18.3 ng/mL) at the end of 6 months. On the other hand, patients who received Vit D showed a 53.8% increase in mean serum Vit D level (22.5 ng/mL *v*s. 34.6 ng/mL). Also, a highly significant elevation of Vit D in the treatment group in comparison with the control group (p< 0.001) was observed (Table III) (Figure 2).

Survival Rate Analysis

The survival rate was significantly higher in the treatment group (64%) compared to the control group (42%) (p< 0.05). The mean surviv-

Table I. Baseline characteristics, blood tests, and ascetic fluid composition of control and treatment groups.

Parameters	Control group (N = 168)	Treatment group (N = 160)	*p*-value
Age in years (Mean ± SD)	55.0 ± 8.5	51.4 ± 12.1	< 0.05
Gender (Male: Female)	81:86	85:75	> 0.05
BMI (Kg/m^2) (Mean ± SD)	26.0 ± 2.2	25.9 ± 1.9	> 0.05
Smoking [No. (%)]	62 (37.1%)	65 (40.6%)	< 0.05
Bleeding [No. (%)]	35 (21%)	32 (20%)	> 0.05
Ascites [No.(%)]	167 (100%)	160 (100%)	> 0.05
Encephalopathy [No.(%)]	36 (21.6%)	31 (19.4%)	> 0.05
Fever [No.(%)]	43 (25.7%)	38 (23.8%)	> 0.05
Jaundice [No.(%)]	35 (21%)	31 (19.4%)	> 0.05
HBsAg positive [No.(%)]	24 (14.4%)	19 (11.9%)	< 0.05
HCV Ab positive [No.(%)]	13 (7.8%)	10 (6.3%)	> 0.05
Hb (g/dL) (Mean ± SD)	11.8 ± 2.0	12.0 ± 1.5	> 0.05
Serum glucose (mg/dL) (Mean ± SD)	104.2 ± 8.5	101.6 ± 15.0	> 0.05
Ascetic glucose (mg/dL) (Mean ± SD)	47.3 ± 9.2	45.0 ± 10.2	> 0.05
Platelet (× 109/L) (Mean ± SD)	135.5 ± 27.02	138.0 ± 71.1	> 0.05
Serum Creatinine (mg/dL) (Mean ± SD)	1.61 ± 0.49	1.56 ± 0.42	> 0.05
Total bilirubin (mg/dL) (Mean ± SD)	3.87 ± 0.81	3.18 ± 0.73	> 0.05
Direct bilirubin (mg/dL) (Mean ± SD)	0.93 ± 0.40	0.80 ± 0.49	< 0.05
ALT (IU/L) (Mean ± SD)	89.0 ± 23.1	83.1 ± 22.2	> 0.05
AST(IU/L) (Mean ± SD)	149.0 ± 48.3	138.0 ± 46.9	> 0.05
Serum albumin (g/dL) (Mean ± SD)	1.86 ± 0.30	1.90 ± 0.37	> 0.05
Ascetic albumin (g/dL) (Mean ± SD)	0.73 ± 0.23	0.70 ± 0.29	> 0.05
INR (Mean ± SD)	1.32 ± 0.31	1.29 ± 0.27	> 0.05
AFP (ng/mL) [Median (Range)]	7.9 (3.1-9.2)	8.0 (2.0-8.0)	> 0.05
GGT (IU/L) [Median (Range)]	81.73 (12-187)	75.4 (3.4-176.0)	> 0.05
Blood TLC (× 10^3/μL) [Median (Range)]	11.43 (3.71-23.37)	12.10 (4.71-21.73)	> 0.05
Ascetic TLC (× 10^3/μL) [Median (Range)]	600 (140-7000)	587 (120-4300)	> 0.05
Procalcitonin (ng/mL) (Mean ± SD)	1.84 ± 0.78	1.78 ± 0.49	> 0.05
Sr.Vitamin D level (ng/mL) (Mean ± SD)	20.2 ± 11.0	22.5 ± 11.5	> 0.05

AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; GGT, gamma-glutamyl transferase; Hb, Hemoglobin; HBsAg, Hepatitis B surface antigen; HCV Ab, Hepatitis C virus antibodies; INR, international normalized ratio; SBP, spontaneous bacterial peritonitis; TLC, total leukocytic count.

Table II. Univariate analysis comparing the control and treatment groups regarding blood tests and ascetic fluid composition after 6 months.

Parameters	Control group (N = 168)	Treatment group (N = 160)	*p*-value
Platelet (× 10^9/L) (Mean ± SD)	141.0 ± 66.8	283.1 ± 89.1	< 0.001
Serum Creatinine (mg/dL) (Mean ± SD)	1.30 ± 0.49	0.95 ± 0.16	< 0.001
Total bilirubin (mg/dL) (Mean ± SD)	2.05 ± 0.83	0.76 ± 0.19	< 0.001
Direct bilirubin (mg/dL) (Mean ± SD)	0.62 ± 0.38	0.15 ± 0.07	< 0.001
ALT (IU/L) (Mean ± SD)	65.0 ± 28.8	57.8 ± 22.4	< 0.05
AST(IU/L) (Mean ± SD)	104.3 ± 53.4	66.3 ± 26.3	< 0.001
Serum albumin (g/dL) (Mean ± SD)	2.76 ± 0.44	2.84 ± 0.36	> 0.05
Ascetic albumin (g/dL) (Mean ± SD)	0.68 ± 0.32	0.54 ± 0.32	< 0.05
INR (Mean ± SD)	1.25 ± 0.24	0.98 ± 0.07	< 0.001
AFP (ng/mL) [Median (Range)]	7.1 (2-32)	6 (2.9-9)	< 0.001
GGT (IU/L) [Median (Range)]	51 (2.9-189)	35.5 (25-59)	< 0.001
Blood TLC (× 10^3/µL) [Median (Range)]	8.8 (4.5-17.0)	7.5 (2.5-12.0)	< 0.001
Ascetic TLC (× 10^3/µL) [Median (Range)]	231 (189-291)	124 (90-3400)	< 0.001
Procalcitonin (ng/mL) (Mean ± SD)	1.41 ± 0.86	0.65 ± 0.57	< 0.001
Sr.Vitamin D level (ng/mL) (Mean ± SD)	18.3 ± 10.0	34.6 ± 9.2	< 0.001
Survival at 6 months:			
• Ratio (alive:dead) (%)	42:58	64:36	< 0.05
• Survival (days) [Median (Range)]	185.5 (85-535)	199.5 (85-535)	< 0.05

AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; GGT, gamma-glutamyl transferase; Hb, Hemoglobin; HBsAg, Hepatitis B surface antigen; HCV Ab, Hepatitis C virus antibodies; INR, international normalized ratio; SBP, spontaneous bacterial peritonitis; TLC, total leukocytic count.

al duration was (199.5 days) in the treatment group *vs.* (185.5 days) in the control group ($p< 0.05$) (Table II). For detection of the different factors that were associated with the 6 months survival of the patients, univariate and multivariate analyses were used. The univariate analysis confirmed that higher TLC, higher creatinine, higher bilirubin level, and higher AST level were nonsignificant predictors of death in ascetic SBP patients ($p> 0.05$). However, low Vit D level in those patients was the only significant predictive mortality marker ($p< 0.01$). On the other hand, for assessment of the effect of Vit D supplementation on 6 months survival, both univariate and multivariate analysis revealed a highly significant association ($p< 0.001$) (HR=0.895) (Table IV).

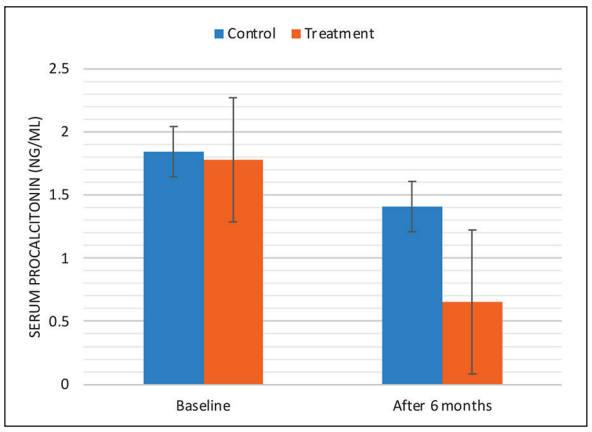

Figure 1. Change in serum Procalcitonin level in both control and treatment groups over 6 months. Results presented as the mean ± SD. ($^{\#}p < 0.001$ for each group after 6 months, $^{*}p < 0.001$ *vs.* the control).

Discussion

Cirrhosis has a noticeable correlation with morbidity and mortality rate in developed countries[47-49]. Mortality is 4 times higher in a patient with cirrhosis and infection with a poor prognosis; 30% of patients die within a month of infection in addition to another 30% within a year[50,51]. Spontaneous bacterial peritonitis (SBP) is one of the most frequently diagnosed infections. The ascetic fluid infection happens when there is no visceral perforation or inflammatory focus in the abdominal cavity. For SBP diagnosis, the polymorphonuclear leukocytic count (PMNLs) from the ascetic fluid obtained through paracentesis must be more than 250 cells/mm[52,54]. In addition, procalcitonin (PCT) appears to be a sensitive diagnostic indicator that can be used to predict diagnosis, monitor bacterial infections, and guide the clinical use of antibiotics. Vit D may enhance

Table III. Comparison of the change in Procalcitonin and Vitamin D levels before and after starting therapy.

Parameters	Baseline	After 6 months	*p*-value
Serum Procalcitonin (ng/mL):			
• Control Group	1.84 ± 0.78	1.41 ± 0.86	< 0.001
• Treatment Group	1.78 ± 0.49	0.65 ± 0.57	< 0.001
Serum vitamin D (ng/mL):			
• Control Group	20.2 ± 11.0	18.3 ± 10.0	> 0.05
• Treatment Group	22.5 ± 11.5	34.6 ± 9.2	< 0.001
p-value	> 0.05	< 0.001	

Results presented as the mean ± SD.

innate defense and modulate the activation of lymphocytes involved in the immune response, while Vit D deficiency or insufficiency might be a predisposal factor to or increase the risk of bacterial infections and SBP in patients with cirrhosis[55,56].

Patients enrolled in the present study were 328; the control group was 168 patients and the treatment group was 160. The mean age in both groups was the fifth decade with a comparable male to female ratio. All patients had ascites with SBP. At baseline, all patients revealed an elevated serum and ascetic TLC, AST, ALT, total and direct bilirubin, in addition to elevation in INR and PCT level. On the other hand, they showed thrombocytopenia and low serum and ascetic albumin level. Regarding elevated TLC, many studies[57-60] reported a significant elevation in TLC in patients with SBP because infection stimulates the immune response that in turn stimulates the release of stored WBCs from bone marrow. As a predictor for the development of SBP, lower platelet count in the current study come in agreement with other studies that confirmed this significant association[61,62]. Due to liver injury, serum AST and ALT levels were elevated agreeing with many other previous studies. Furthermore, the elevated INR and total and direct bilirubin levels in our patients come in agreement with Viallon et al[63], but in disagreement with Magdalena et al[29]. Magdalena et al[29] also revealed that low serum and ascetic albumin concentration is related to the increased rate of SBP and predisposing factor for more infection. Serum PCT has been reported as a sensitive biomarker for monitoring bacterial infection and guides the selection of the accurate antibiotic in many studies[57,58,64]. However, several studies[65-67] show conflicting results regarding the diagnostic value of serum PCT level in patients with SBP. Some studies[68,69] confirmed that significant elevation in serum PCT level could be used as a good predictor for early diagnosis of SBP in decompensated cirrhosis patients, which is compatible with our results.

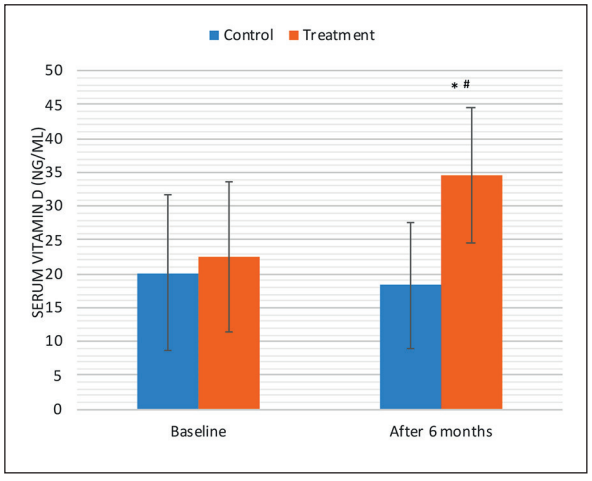

Figure 2. Change in serum vitamin D level in both control and treatment groups over 6 months. Results presented as the mean ± SD. ($^{\#}p < 0.001$ for each group after 6 months, $^{*}p < 0.001$ *vs.* the control).

Regarding Vit D deficiency or insufficiency, the current study revealed a significantly low level of Vit D (< 25 ng/ml) in cirrhotic patients with ascites and SBP. Univariate regression analysis confirmed that Vit D deficiency was an independent predictive marker of infection and mortality in these patients ($p < 0.01$; Crude Hazard Ratio: 0.951). Most previous studies[70,71] showed a very high prevalence of Vit D deficiency or insufficiency in chronic liver disease patients. More studies[72,73] have revealed a relation between deficiency of Vit D and degree of hepatic dysfunction and infection. Therefore, they have suggested using it as a prognostic marker for patients with liver cirrhosis. Malnutrition decreased exposure to sunlight, or malabsorption of Vit D due to intestinal edema as a

Table IV. Cox PH regression model estimates for the risk of clinical recurrence.

	Univariate analysis				Multivariate analysis			
	95% CI for HR				95% CI for HR			
	Crude HR	Lower	Upper	*p*-value	Adjusted HR	Lower	Upper	*p*-value
TLCs	1.162	0.982	1.375	> 0.05				
Creatinine	0.872	0.101	7.530	> 0.05				
Total Bilirubin	2.224	0.391	12.666	> 0.05				
Direct Bilirubin	3.584	0.024	537.120	> 0.05				
AST	1.003	0.988	1.019	> 0.05				
Vitamin D	0.951	0.912	0.991	< 0.01				
Vitamin D after treatment	0.895	0.851	0.940	< 0.001	0.895	0.851	0.940	< 0.001

AST, aspartate transaminase; TLC, total leukocytic count.

complication of portal hypertension are the most important mechanisms involved in lowering Vit D levels in cirrhotic patients. Another contributing factor is the low level of Vit D-binding proteins (DBPs) that transport Vit D to the kidney and liver for activation. Therefore, impairment of Vit D hydroxylation in the liver results in low active Vit D levels while the rate of vitamin catabolism is still high[74-76]. Vit D deficiency or insufficiency in cirrhotic patients may predispose or increase the possibility of bacterial infections and SBP[77,78] resulting in the increased SBP incidence in cirrhotic patients with severe Vit D deficiency[79]. SBP was the most frequent infection in cirrhotic patients (62.2%), and Vit D deficiency in such patients was found to be an independent predictive marker of infection. Evidence[80] has shown a direct association between deficiency of Vit D and high-risk mortality in those with the chronic liver disease even if they survive an acute infectious episode. 30% increase in the mortality rate during one year in patients with cirrhosis and infection whatever the stage of liver disease was recorded[81]. This prospective study on cirrhotic patients with other infections also reported that 30-day mortality was observed in 25% of the studied cases. Also, the highest mortality rate was observed among patients with SBP (36%) followed by pneumonia and bloodstream infected patients.

As Vit D deficiency in cirrhotic patients is an independent predictor of infection and death, supplementation with Vit D in these patients may be useful. Vit D regulates the immune system with protection against infections. CYP27B1 enzymes released from dendritic cells, macrophages, and T and B lymphocyte cells, change inactive Vit D to calcitriol[82,83]. This active form then attaches to Vit D receptor (VDR) in a phagocytic cell called antigen-presenting cells and acts as a factor for transcription of beta-defensins and cathelicidin (LL-37) which are the antimicrobial peptides. This mechanism causes an increase in phagocytic and chemotactic effects and protects the epithelial barrier from infection[84,85].

Despite the dosage, regimen, and route of Vit D administration in patients with hepatic cirrhosis are not clear, it is recommended to monitor the level of Vit D periodically in those with Vit D levels < 30 ng/mL[86]. In addition, daily administration of Vit D3 in a dose of 5000 IU or weekly administration of Vit D2 or D3 in a dose of 50000 IU for 3 months, followed by 1000 IU/d indefinitely is recommended as well. Another systemic review suggested the use of Vit D3 for supplementation with single doses of ≥ 300000 IU that are most beneficial in increasing Vit D concentration[87]. Although oral and intramuscular routes are safe and effective, the intramuscular route is preferred as it increases Vit D levels more effectively[88,89]. In the current study, we used cholecalciferol in a dose of 300000 IU intramuscularly as a loading dose followed by a maintenance dose of 800 IU/d orally in addition to supplementation of 1000 mg calcium.

Vit D supplementation is not well established for improving survival in Vit D deficient patients with liver cirrhosis. Few clinical trials have shown that Vit D supplements replenish Vit D levels[90] and enhance the defense against SBP[77] with a low mortality rate in adults[77-91]. It was observed that supplementation of Vit D in patients with decompensated cirrhosis enhances macrophage Vit D receptors in the peritoneal fluid and

increases the expression levels of antimicrobial peptides cathelicidin (LL-37) to prevent SBP[92]. It revealed a significant improvement in functional status using MELD and CTP scoring systems ($p< 0.05$).

Throughout 6 months, our study revealed significant improvement in the level of serum Vit D in the treatment group over the control group ($p< 0.001$). It also demonstrated a statistically significant increase in survival rate (64% *vs.* 42%; $p< 0.05$) and survival duration (199.5 days *vs.* 185.5 days; $p< 0.05$) in the treatment group in comparison with the control group. Univariate and multivariate regression analysis showed a significant association between Vit D supplementation and survival over 6-months ($p< 0.001$; Adjusted Hazard Ratio: 0.895).

Strengths and Limitations

The study showed some limitations; it is a single-centered study, and it lacks a Vit D deficient control group without cirrhosis of the same age range and sex ratio. Due to the time culture sensitivity-testing take, patients usually on admission receive broad-spectrum antibiotics before culture results are obtained, so no culture was performed to identify the causative organism of infection. In addition, we did not study the impact of different Vit D doses, and different routes of administrations in patients with hepatic cirrhosis. Therefore, the results of our study need further confirmation in large multicenter prospective randomized studies.

Conclusions

Collectively, these data suggest that Vit D deficiency in cirrhotic patients with SBP is very common. In addition, Vit D was found to be an independent variable of infection and death in them, so its administration may improve their response to treatment and survival over 6 months.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This research was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University through the Fast-track Research Funding Program.

Institutional Review Board Statement

The National Hepatology and Tropical Medicine Research Institute committee approved this study (Serial: 5-2020).

Informed Consent

Informed consent was obtained from all subjects involved in the study.

Data Availability

Data are available on request due to privacy/ethical restrictions.

ORCID ID

Ahmed A. Al-Karmalawy: 0000-0002-8173-6073.

Authors' Contribution

Amal Ahmed Mohamed and Ahmed A. Al-Karmalawy designed the study. Amal A. El-Kholy, Dalia Ali El-damas, Heba M. Abostate, and Sahar Mohamed Mostafa have performed the experiments. Mohmoud Hamada, Mohamed Abdel Khalik Elkady, Yosra Hassan, Eman Al-Hussain, Mona G.khalil, Ingy badawy, Dalia Elebeedy, and Bshra A. Alsfouk recruited the cases, assessed their clinical stages, and analyzed the data. Mahmoud Maamoun Shaheen revised and analyzed the data. Ahmed A. Al-Karmalawy revised and edited the paper. All the authors wrote the paper and approved the present version for publication.

References

1) Fernández J, Acevedo J, Prado V, Mercado M, Castro M, Pavesi M, Arteaga M, Sastre L, Juanola A, Ginès P, Arroyo V. Clinical course and short-term mortality of cirrhotic patients with infections other than spontaneous bacterial peritonitis. Liver Int 2017; 37: 385-395.
2) Friedrich K, Nüssle S, Rehlen T, Stremmel W, Mischnik A, Eisenbach C. Microbiology and resistance in first episodes of spontaneous bacterial peritonitis: implications for management and prognosis. J Gastroenterol Hepatol 2016; 31: 1191-1195.
3) Cho JH, Park KH, Kim SH, Bang JH, Park WB, Kim HB, Kim NJ, Oh MD, Lee HS, Choe KW. Bacteremia is a prognostic factor for poor outcome in spontaneous bacterial peritonitis. Scand J Infect Dis 2007; 39: 697-702.
4) Loomba R, Wong R, Fraysse J, Shreay S, Li S, Harrison S, Gordon SC. Nonalcoholic fatty liver disease progression rates to cirrhosis and progression of cirrhosis to decompensation and mortality: a real world analysis of Medicare data. Aliment Pharmacol Ther 2020; 51: 1149-1159.
5) Yeverino-Gutiérrez ML, González-González MdR, González-Santiago O. Mortality from alco-

hol-related liver cirrhosis in Mexico (2000-2017). Front Public Health 2020; 8: 524356.

6) Mezzano G, Juanola A, Cardenas A, Mezey E, Hamilton JP, Pose E, Graupera I, Ginès P, Solà E, Hernaez R. Global burden of disease: acute-on-chronic liver failure, a systematic review and meta-analysis. Gut 2020 gutjnl-2020-322161. doi: 10.1136/gutjnl-2020-322161. Online ahead of print.
7) Medhat MA, Gushken F, Khaled T, Hassan E, Abd El-Rehim A, Kamel S. Acute-on-chronic liver failure in Egypt: an underestimated complication of liver cirrhosis. Eur J Gastroenterol Hepatol 2021; 30: 00.
8) Abo-Mandil ME, Alshahat SAE-R, El-Badry A, El-Sheety AG, El-Faramawy MS, Ismael NF. Genotypic Prevalence of Cryptosporidium in Egyptian Patients with Liver Cirrhosis. Al-Azhar International Medical Journal 2020; 1: 225-231.
9) Abd-Elsalam S, Habba E, Elkhalawany W, Tawfeek S, Elbatea H, El-Kalla F, Soliman H, Soliman S, Yousef M, Kobtan A, El Nawasany S, Awny S, Amer I, Mansour L, Rizk F. Correlation of platelets count with endoscopic findings in a cohort of Egyptian patients with liver cirrhosis. Medicine (Baltimore) 2016; 95.
10) Hassanein M, Abdelhamid M, Ibrahim B, Sobhy M, Nasr G, Aboleineen MW, Reda A, Farag N, Elshazly A, Abdeldayem TK, Elmesseiry F, Boshra H, Sobhy H, Elbahry A, Youssef A, Ashmawy M, Abdelmoneim A, Saleh A, Elrakshy Y, Ebeid H. Gender differences in Egyptian patients hospitalized with heart failure: insights from the European Society of Cardiology Heart Failure Long-Term Registry. ESC Heart Fail 2018; 5: 1159-1164.
11) Grinspan LT, Verna EC. Chronic Liver Failure and Hepatic Cirrhosis. Liver Anesthesiology and Critical Care Medicine 2018: 21-39.
12) Pinzani M, Rosselli M, Zuckermann M. Liver cirrhosis. Best Pract Res Clin Gastroenterol 2011; 25: 281-290.
13) Suk KT, Kim DJ. Staging of liver fibrosis or cirrhosis: The role of hepatic venous pressure gradient measurement. World J Hepatol 2015; 7: 607.
14) Simonetto DA, Liu M, Kamath PS, editors. Portal hypertension and related complications: diagnosis and management. Mayo Clin Proc 2019: Elsevier.
15) Thabut D, Bernard-Chabert B. Management of acute bleeding from portal hypertension. Best Pract Res Clin Gastroenterol 2007; 21: 19-29.
16) Blei AT. Portal hypertension and its complications. Curr Opin Gastroenterol. 2007; 23: 275-282.
17) Martell M, Coll M, Ezkurdia N, Raurell I, Genescà J. Physiopathology of splanchnic vasodilation in portal hypertension. World J Hepatol 2010; 2: 208-220.
18) Jeffers L. Aquaretics in the treatment of ascites. Gastroenterol Hepatol (N Y) 2010; 6: 559-564.
19) Fortune B, Cardenas A. Ascites, refractory ascites and hyponatremia in cirrhosis. Gastroenterol Rep (Oxf) 2017; 5: 104-112.
20) Sheer TA, Runyon BA. Spontaneous Bacterial Peritonitis. Dig Dis 2005; 23: 39-46.
21) Zafar MU, Marjara J, Tarar ZI, Ghous G, Patel P. Spontaneous Bacterial Peritonitis in Cardiogenic Ascites. Cureus 2020; 12: e10995-e.
22) Bunchorntavakul C, Chamroonkul N, Chavalitdhamrong D. Bacterial infections in cirrhosis: A critical review and practical guidance. World J Hepatol 2016; 8: 307-321.
23) Koutsounas I, Kaltsa G, Siakavellas SI, Bamias G. Markers of bacterial translocation in end-stage liver disease. World J Hepatol 2015; 7: 2264-2273.
24) Alexopoulou A, Agiasotelli D, Vasilieva LE, Dourakis SP. Bacterial translocation markers in liver cirrhosis. Ann Gastroenterol 2017; 30: 486-497.
25) Ponziani FR, Zocco MA, Cerrito L, Gasbarrini A, Pompili M. Bacterial translocation in patients with liver cirrhosis: physiology, clinical consequences, and practical implications. Expert Rev Gastroenterol Hepatol 2018; 12: 641-656.
26) Becker KL, Nylén ES, White JC, Müller B, Snider RH, Jr. Procalcitonin and the Calcitonin Gene Family of Peptides in Inflammation, Infection, and Sepsis: A Journey from Calcitonin Back to Its Precursors. J Clin Endocrinol Metab 2004; 89: 1512-1525.
27) Davies J. Procalcitonin. J Clin Pathol 2015; 68: 675.
28) Mussap M, Degrandi R, Cataldi L, Fanos V, Plebani M. Biochemical Markers for the Early Assessment of Neonatal Sepsis: the Role of Procalcitonin. J Chemother 2007; 19: 35-38.
29) Lesińska M, Hartleb M, Gutkowski K, Nowakowska-Duława E. Procalcitonin and macrophage inflammatory protein-1 beta (MIP-1β) in serum and peritoneal fluid of patients with decompensated cirrhosis and spontaneous bacterial peritonitis. Adv Med Sci 2014; 59: 52-56.
30) Cai ZH, Fan CL, Zheng JF, Zhang X, Zhao WM, Li B, Li L, Dong PL, Ding HG. Measurement of serum procalcitonin levels for the early diagnosis of spontaneous bacterial peritonitis in patients with decompensated liver cirrhosis. BMC Infect Dis 2015; 15: 55.
31) Choi JJ, McCarthy MW. Novel applications for serum procalcitonin testing in clinical practice. Expert Rev Mol Diagn 2018; 18: 27-34.
32) Elsadek HM, Elhawari SA, Mokhtar A. A novel serum index for accurate diagnosis of spontaneous bacterial peritonitis in cirrhotic patients without other infections. Egyptian Liver Journal 2020; 10: 10.
33) Engelmann C, Berg T. Management of Infectious Complications Associated with Acute-on-Chronic Liver Failure. Visc Med 2018; 34: 261-268.
34) Salzedas Netto Alcides, Silva Rm, Martins Jl, Menchaca Diaz Jl, Bugni Gm, Watanabe Andrea,

Silva Fjp, Ulysses Fagundes, Morais Mb, Koh Ivan. Can bacterial translocation be a beneficial event? Transplant Proc 2006; 9: P60.

35) Han Wang, Yan Li, Fangfang Zhang, Ning Yang, Na Xie, Yuanli Mao, Boan Li. Combination of PCT, sNFI and dCHC for the diagnosis of ascites infection in cirrhotic patients. BMC Infect Dis 2018; 18: 389.

36) Lin KH, Wang FL, Wu MS, Jiang BY, Kao WL, Chao HY, Wu JY, Lee CC. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection in patients with liver cirrhosis: a systematic review and meta-analysis. Diagn Microbiol Infect Dis 2014; 80: 72-78.

37) Lippi G, Danese E, Cervellin G, Montagnana M. Laboratory diagnostics of spontaneous bacterial peritonitis. Clin Chim Acta 2014; 430: 164-170.

38) Schaalan MF, Mohamed WA, Amin HH. Vitamin D deficiency: correlation to interleukin-17, interleukin-23 and PIIINP in hepatitis C virus genotype 4. World J Gastroenterol 2012; 18: 3738-3744.

39) Reda R, Abbas AA, Mohammed M, El Fedawy SF, Ghareeb H, El Kabarity RH, Abo-Shady RA, Zakaria D. The Interplay between Zinc, Vitamin D and, IL-17 in Patients with Chronic Hepatitis C Liver Disease. J Immunol Res 2015; 2015: 846348.

40) Chen SC, Ke CY, Subeq YM, Yang WT, Huang SG, Shiao AS, Lee RP. Protective Effect of Calcitriol on Organ Damage Induced by 5-Fluorouracil Treatment. Nutr Cancer 2020: 1-10.

41) Hajialilo M, Noorabadi P, Tahsini Tekantapeh S, Malek Mahdavi A. Endothelin-1, α-Klotho, 25(OH) Vit D levels and severity of disease in scleroderma patients. Rheumatol Int 2017; 37: 1651-1657.

42) Saumoy M, Ando Y, Jesudian A, Aden B, Jacobson I, Gambarin-Gelwan M. Vitamin D Deficiency is Not Associated with Increased Risk of Hepatocellular Carcinoma in Patients with Cirrhosis: 362. Am J Gastroenterol ACG 2013; 108.

43) El Sayed Zeid A, El Sayed Salem P, El Hadidi AS, Ibrahim TG. Vitamin D and LL-37 in cirrhotic patients with culture-positive spontaneous bacterial peritonitis. Egypt J Intern Med 2019; 31: 247-253.

44) Abstracts. Journal of Investigative Medicine 2011; 59: 372.

45) Abu-Mouch S, Fireman Z, Jarchovsky J, Zeina A-R, Assy N. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. World J Gastroenterol 2011; 17: 5184-5190.

46) Antico A, Tampoia M, Tozzoli R, Bizzaro N. Can supplementation with vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. Autoimmun Rev 2012; 12: 127-136.

47) Lim Y-S, Kim WR. The Global Impact of Hepatic Fibrosis and End-Stage Liver Disease. Clin Liver Dis 2008; 1.46-733: 2.

48) Hou F, Wang R, Han B, Bai Z, Li Y, Qi X. Liver Cirrhosis. Liver Diseases: Springer 2020. p. 239-245.

49) Berumen J, Baglieri J, Kisseleva T, Mekeel K. Liver fibrosis: Pathophysiology and clinical implications. Wiley Interdiscip Rev Syst Biol Med 2021; 13; e1499.

50) Grgurević Ivica, Trkulja Vladimir, Božin Tonči, Madir Anita, Miletić Maja, Marušić Srećko, Škrlin, Jasenka, Šestan Crnek Sandra, Dobrović Karolina. Infection as a predictor of mortality in decompensated liver cirrhosis: exploring the relationship to severity of liver failure. Eur J Gastroenterol Hepatol 2020; 32: 1458-1465.

51) Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014; 383: 1749-1761.

52) Căruntu FA, Benea L. Spontaneous bacterial peritonitis: pathogenesis, diagnosis, treatment. J Gastrointestin Liver Dis 2006; 15: 51-56.

53) Veeramani C. A Clinical Study of Risk Factors and Clinical Features in patients of SBP with Cirrhosis and Ascites in a Tertiary Care Hospital Salem: Government Mohan Kumaramangalam Medical College, Salem 2020.

54) Pazhanivel M. Prevalence and risk factors for spontaneous ascitic fluid infection in outpatient cirrhotics undergoing therapeutic paracentesis: Stanley Medical College, Chennai 2010.

55) Dong R, Wan B, Lin S, Wang M, Huang J, Wu Y, Wu Y, Zhang N, Zhu Y. Procalcitonin and liver disease: a literature review. J Clin Transl Hepatol 2019; 7: 51.

56) Wu H, Chen L, Sun Y, Meng C, Hou W. The role of serum procalcitonin and C-reactive protein levels in predicting spontaneous bacterial peritonitis in patients with advanced liver cirrhosis. Pak J Med Sci 2016; 32: 1484.

57) AHMED AA, MAHMOUD HA, SALMAN A. The Value of Serum Pro-Calcitonin in Diagnosis of Spontaneous Bacterial Peritonitis (SBP) and Predicting Response to Treatment. Med J Cairo Univ 2020; 88: 381-388.

58) Parthiban P. A study of prevalence of bacterial infection in patients with liver cirrhosis: A cross sectional study: Kilpauk Medical College, Chennai 2018.

59) Wilson V, Velayudhan KK, Rao H, Sukumaran SV. Low Absolute Eosinophil Count Predicts In-Hospital Mortality in Cirrhosis With Systemic Inflammatory Response Syndrome. Cureus 2021; 13.

60) Abu Rahma MZ, Mahran ZG, Shafik EA, Mohareb DA, Abd El-Rady NM, Mustafa MA, Khalil M, Abo-Amer YE, Abd-Elsalam S. The Role of Serum Procalcitonin Level as an Early Marker of Ascitic Fluid Infection in Post Hepatitic Cirrhotic Patients. Antiinflamm Antiallergy Agents Med Chem 2021; 2.7-61.

61) Schwabl P, Bucsics T, Soucek K, Mandorfer M, Bota S, Blacky A, Hirschl AM, Ferlitsch A, Trauner M, Peck-Radosavljevic M, Reiberger T. Risk factors for development of spontaneous bacterial peritonitis and subsequent mortality in cirrhotic patients with ascites. Liver Int 2015; 35: 2121-2128.

62) Metwally K, Fouad T, Assem M, Abdelsameea E, Yousery M. Predictors of spontaneous bacterial peritonitis in patients with cirrhotic ascites. J Clin Transl Hepatol 2018; 6: 372.

63) Viallon A, Zeni F, Pouzet V, Lambert C, Quenet S, Aubert G, Guyomarch S, Tardy B, Bertrand JC. Serum and ascitic procalcitonin levels in cirrhotic patients with spontaneous bacterial peritonitis: diagnostic value and relationship to pro-inflammatory cytokines. Intensive Care Med 2000; 26: 1082-1088.

64) Chinese Society of Hepatology, Chinese Medical Association, Xu X, Duan Z, Ding H, Li W, Jia J, Wei L, Linghu E, Zhuang H. Chinese guidelines on the management of ascites and its related complications in cirrhosis. Hepatol Int 2019; 13: 1-21.

65) Harbarth S, Holeckova K, Froidevaux C, Pittet D, Ricou B, Grau GE, Vadas L, Pugin J. Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Respir Crit Care Med 2001; 164: 396-402.

66) Cai ZH, Fan CL, Zheng JF, Zhang X, Zhao WM, Li B, Li L, Dong PL, Ding HG. Measurement of serum procalcitonin levels for the early diagnosis of spontaneous bacterial peritonitis in patients with decompensated liver cirrhosis. BMC Infect Dis 2015; 15: 1-7.

67) Watkins RR, Lemonovich TL. Serum procalcitonin in the diagnosis and management of intra-abdominal infections. Expert Rev Anti Infect Ther 2012; 10: 197-205.

68) Shi kun Yang, Li Xiao, Hao Zhang, Xiao xuan Xu, Pan ai Song, Fu you Liu, Lin Sun.Significance of serum procalcitonin as biomarker for detection of bacterial peritonitis: a systematic review and meta-analysis. BMC Infect Dis 2014; 14: 1-13.

69) Zuwala-Jagiello J, Simon K, Kukla M, Murawska-Cialowicz E, Gorka-Dynysiewicz J, Grzebyk E, Pazgan-Simon M. Increased circulating endocan in patients with cirrhosis: relation to bacterial infection and severity of disease. J Physiol Pharmacol 2017; 68: 273-282.

70) Zhou Q, Li L, Chen Y, Zhang J, Zhong L, Peng Z, Xing T. Vitamin D supplementation could reduce the risk of acute cellular rejection and infection in vitamin D deficient liver allograft recipients. Int Immunopharmacol 2019; 75: 105811.

71) del Castillo MCM, Rodriguez AS, Abad JJHB, Garcia-Buen-Abad R, Atkinson N, Rodriguez MEM. ACCEPTED: LIVER. Am J Gastroenterol 2017 ;112: S490-S576.

72) Osman W, Al Fahdi F, Al Salmi I, Al Khalili H, Gokhale A, Khamis F. Serum Calcium and Vitamin D levels: Correlation with severity of COVID-19 in hospitalized patients in Royal Hospital, Oman. Int J Infect Dis 2021; 107: 153-163.

73) Nakchbandi IA, Van Der Merwe SW. Current understanding of osteoporosis associated with liver disease. Nat Rev Gastroenterol Hepatol 2009; 6: 660.

74) Ambika B. Estimation of serum vitamin 25 (oh) d level in chronic liver disease patients and its clinical correlation a cross sectional study at a tertiary care government royapettah hospital/government kilpauk medical college in chennai: Kilpauk Medical College, Chennai 2018.

75) Ashraf A, McCormick K. 9 Vitamin D deficiency in concert with functional hypoparathyroidism as an etiologic factor of neonatal seizures. BMJ 2005.

76) Tharu B, Bishal K, Basnet S, Adhikari N, Tharu S. MON-LB63 Hypocalcemia-A Clue to Digeorge Syndrome; A Very Rare Case Diagnosed in a 41 Year Male. J Endocr Soc 2020; 4: MON-LB63.

77) Siddiqui M, Manansala JS, Abdulrahman HA, Nasrallah GK, Smatti MK, Younes N, Althani AA, Yassine HM. Immune modulatory effects of vitamin D on viral infections. Nutrients 2020; 12: 2879.

78) Quiel L, Alderuccio J, Ntalageorgos T, Popescu A, Bergasa N. Assessment of Lymphoma Risk in Patients with Chronic Hepatitis Virus Infection. Am J Gastroenterol 2013; 108: S106-S61.

79) Basu PP, Aloysius MM, Shah NJ, Brown Jr RS, Center N. Topic: 2 Alcoholic Liver Disease.

80) Zeid AES, Salem PES, El Hadidi AS, Ibrahim TG. Vitamin D and LL-37 in cirrhotic patients with culture-positive spontaneous bacterial peritonitis. Egypt J Intern Med 2019; 31: 247-253.

81) Bartoletti M, Giannella M, Lewis R, Caraceni P, Tedeschi S, Paul M, Schramm C, Bruns T, Merli M, Cobos-Trigueros N, Seminari E, Retamar P, Muñoz P, Tumbarello M, Burra P, Torrani Cerenzia M, Barsic B, Calbo E, Maraolo AE, Petrosillo N, Galan-Ladero MA, D'Offizi G, Bar Sinai N, Rodríguez-Baño J, Verucchi G, Bernardi M, Viale P. A prospective multicentre study of the epidemiology and outcomes of bloodstream infection in cirrhotic patients. Clin Microbiol Infect 2018; 24: 546. e1-. e8.

82) Medrano M, Carrillo-Cruz E, Montero I, Perez-Simon JA. Vitamin D: effect on haematopoiesis and immune system and clinical applications. Int J Mol Sci 2018; 19: 2663.

83) Goncalves-Mendes N, Talvas J, Dualé C, Guttmann A, Corbin V, Marceau G, Sapin V, Brachet P, Evrard B, Laurichesse H, Vasson MP. Impact of vitamin D supplementation on influenza vaccine response and immune functions in deficient elderly persons: a randomized placebo-controlled trial. Front Immunol 2019; 10: 65.

84) Vitamin D, Cholangitis PB. Liver associations with vitamin D and genes–Overview April 2018.

85) Owens S. The use of a once oral monthly ergocalciferol supplement to improve serum 25 (OH) D levels in bariatric surgery subjects 2013.

86) Lim LY, Chalasani N. Vitamin d deficiency in patients with chronic liver disease and cirrhosis. Curr Gastroenterol Rep 2012; 14: 67-73.

87) Kearns MD, Alvarez JA, Tangpricha V. Large, single-dose, oral vitamin D supplementation in adult populations: a systematic review. Endocr Pract 2014; 20: 341-351.

88) Masood MQ, Khan A, Awan S, Dar F, Naz S, Naureen G, Saghir S, Jabbar A. Comparison of vitamin D replacement strategies with high-dose intramuscular or oral cholecalciferol: a prospective intervention study. Endocr Pract 2015; 21: 1125-1133.

89) Tellioglu A, Basaran S, Guzel R, Seydaoglu G. Efficacy and safety of high dose intramuscular or oral cholecalciferol in vitamin D deficient/insufficient elderly. j.maturitas 2012; 72: 332-338.

90) Stojanovic OI, Lazovic M, Lazovic M, Vuceljic M. Association between atherosclerosis and osteoporosis, the role of vitamin D. Arch Med Sci: AMS 2011; 7: 179.

91) Zhang C, Zhao L, Ding Y, Sheng Q, Bai H, An Z, Xia T, Wang J, Dou X. Enhanced LL-37 expression following vitamin D supplementation in patients with cirrhosis and spontaneous bacterial peritonitis. Liver Int 2016; 36: 68-75.

92) Fernández NF, Torres PL, Matias DJ, Plaza FJ, Goñi JLO. Vitamin D deficiency in chronic liver disease, clinical-epidemiological analysis and report after vitamin d supplementation. Gastroenterol Hepatol (English Edition) 2016; 39: 305-310.