Relation between 25-hydroxy vitamin D serum level and spontaneous bacterial peritonitis in patients with liver cirrhosis

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Background and aim
Besides its role in calcium metabolism, vitamin D has pleiotropic functions, including immunomodulation, cellular proliferation, and differentiation. Low levels of vitamin D increase the risk for bacterial infections in liver cirrhotic patients, including spontaneous bacterial peritonitis (SBP). This work is conducted to study the relation between serum level of the 25-OH vitamin D and SBP in liver cirrhotic patients.

Patients and methods
A total of 90 patients were enrolled in the present study. They were divided into three groups, with 30 patients each: first, patients with compensated liver cirrhosis; second, patients who had decompensated liver cirrhosis without SBP; and third, patients who had decompensated liver cirrhosis with SBP. Different laboratory investigations were carried out, such as liver and kidney functions tests, complete blood count, ascitic neutrophilic count, and serum 25-OH vitamin D level.

Results
In the present study, the lowest serum 25-OH vitamin D level was found in patients with decompensated liver cirrhosis with SBP, with a highly significant difference between the studied groups.

Conclusion
Increased incidence of infections with SBP in cirrhotic patients might be linked to vitamin D deficiency. We recommend vitamin D supplementation to lower the risk of SBP in these cases and thereby mortality rate.

Keywords:
liver cirrhosis, spontaneous bacterial peritonitis, vitamin D

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Introduction

Vitamin D is known to have a major role in calcium metabolism and bone mineralization; hence, its deficiency leads to rickets in children and osteomalacia in adults. Vitamin D has pleiotropic functions, including immunomodulation, cellular proliferation, and differentiation [1]. It is also physiologically crucial for the proper function of other organs such as skeletal muscle, brain, heart, and pancreas [2].

Vitamin D may be involved in innate and acquired immunity. Vitamin D modulates the activation of lymphocytes, leading to a switch toward a T helper 2 response and thereby increases innate defense [3].

Even in sunny countries, vitamin D deficiency has been reported, although it is more frequent at high latitudes, where seasonal variations in 25-OH (25-hydroxy) vitamin D have been described. A low level of 25-OH vitamin D has been associated with increased mortality in the general population [4].

In cirrhotic patients, liver insufficiency decreases the rate of hydroxylation of cholecalciferol, and cholestasis impairs the absorption of fat-soluble vitamins, which leads to lack of vitamin D [5].

A low level of 25-OH vitamin D has also been reported to be associated with increased mortality in patients with alcoholic liver disease and in patients with cirrhosis. In a Belgian cohort study of 324 patients, patients with 25-OH vitamin D severe deficiency (level > 10 ng/ml) showed a significantly higher risk of death compared with those without a deficit [6].

Morbidity and mortality in patients with cirrhosis are mainly caused by bacterial infections. It is hypothesized that the relationship between the increase in mortality and the lack of vitamin D observed in patients with cirrhosis could be owing to an increase in bacterial infections [7].

Spontaneous bacterial peritonitis (SBP), an infection of ascitic fluid without demonstrable intra-abdominal cause, is a complication of cirrhosis, with a reported mortality of 20–40% in adult patients [8].

Patients and methods

Study population

Our study is a cross-section cohort study, which included 90 adult patients from the Internal Medicine Department, Ain Shams University Hospitals, from May 2016 to June 2018. Patients were divided into three groups: group I, compensated liver cirrhosis (30 patients); group II, decompensated liver cirrhosis with ascites (30 patients); and group III, decompensated liver cirrhosis with ascites and SBP (30 patients). Inclusion criteria were patients greater than 18 years old and clinical, laboratory, and ultrasonographic criteria suggestive of chronic liver disease. Exclusion criteria were patients with history of parathyroid diseases or bone diseases, chronic kidney disease, other causes of ascites, and other serous sac effusion. All patients were subjected to the following: first, careful medical history taking; second, thorough complete clinical examination (with special stress on general clinical manifestations of chronic liver disease, hepatomegaly, splenomegaly, ascites, jaundice, palmar erythema, flapping tremors, and spider nevi); and third, laboratory investigations, including serum 25-OH vitamin D. This study was approved by the Ethical Committee of Faculty of Medicine, Ain Shams University.

Laboratory studies

Venous blood samples were withdrawn from all patients (8 ml) and divided into three vacutainer tubes: 2 ml in the first tube with potassium salt of EDTA for complete blood count, 4 ml in the second tube with clot activator and gel for serum separation, and 2 ml in the third tube with sodium citrate for coagulation profile. The following laboratory tests were performed: complete blood count, alanine aminotransferase, aspartate transaminase, serum albumin, serum bilirubin level (direct and indirect), serum ionized calcium, serum urea and creatinine (to exclude chronic kidney disease), prothrombin time, partial thromboplastin time and international normalized ratio, and total neutrophilic count in ascitic fluid (level >250 cell/ml for diagnosis of SBP). Ascitic fluid samples were obtained by paracentesis performed under complete aseptic conditions. A volume of 100 µl of serum from each patient was separated and put in 1.5-ml Eppendorf and stored at −20°C until use for measurement of 25-OH vitamin D serum level by ELISA test.

Vitamin D measurement

The Calbiotech Vitamin D Kit (A Life Science Company, Boston, MA, USA), a solid-phase enzyme-linked immunoassay (ELISA), based on the principle of competitive binding, was used for estimation of vitamin D level in patient × s serum. Before assay procedure, samples, calibrators, and controls were delivered to ambient temperature (20–25°C). Anti-vitamin D antibody-coated wells were incubated with 10 µl of each of vitamin D standards, controls, samples, and vitamin D-Biotin conjugate at room temperature for 90 min. During the incubation, a fixed amount of biotin-labeled vitamin D competes with the endogenous vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti-vitamin D antibody. Following a three-time wash step with 300 µl wash buffer, bound vitamin D-biotin was detected after adding 200 µl of streptavidin-horseradish peroxidase (HRP). Streptavidin-HRP conjugate immunologically bound to the well progressively decreases as the concentration of vitamin D in the specimen increases. Unbound SA-HRP conjugate was then removed, and the wells were washed as before. A solution of TMB reagent (200 µl) was added and incubated at room temperature for 30 min, resulting in the development of blue color. The color development was stopped with the addition of stop solution, and the absorbance was measured spectrophotometrically at 450 nm using Sunrise absorbance microplate reader (Tecan, Port Melbourne VIC 3207, Australia). A standard curve was obtained by plotting the concentration of the standard versus the absorbance. The color intensity is inversely proportional the amount of 25(OH) D in the sample. The vitamin D level was defined as sufficient if between 30 and 100 ng/ml, insufficient between 10 and 30 ng/ml, and deficient if less than 10 ng/ml.

Radiological studies: abdominal ultrasound

Criteria suggestive of chronic liver disease and cirrhosis included increased liver echogenicity (loss of homogeneous texture to be replaced by speckled coarse texture), irregular liver margins, attenuation of intrahepatic portal and hepatic veins, relative enlargement of caudate lobe, and atrophy of right lobe (ratio of caudate/right lobe in cirrhosis > 0.65).

Statistical analysis

Data were collected, revised, coded, and entered to the Statistical Package for the Social Sciences (IBM SPSS, software company in california, United States)

Table 1 Comparison between studied groups regarding laboratory data

	Mean±SD			*F*	*P*	Significance
	Group 1	Group 2	Group 3			
Age	53.5±8.11	54.5±7.06	53.7±6.16	0.088	0.85	NS
Hb	12.17+1.72	11.1+1.81	10.6+1.76	6.207	<0.01	HS
WBC	5.6±1.87	5.6±2.98	9.5±5.82	9.730	<0.01	HS
PLT	144.9±52.10	132±59.62	98.5±48.21	6.023	<0.01	HS
ALT	37.1±19.71	34±14.57	52.1±50.12	2.705	0.07	NS
AST	41.2±27.14	43.7±18.45	86.4±121.12	3.688	0.03	S
Albumin	3.4±0.34	2.66±0.28	2.39±0.38	87.277	<0.01	HS
Total bilirubin	1.3±0.24	3.5±2.35	5.8±6.0	10.823	<0.01	HS
Direst bilirubin	0.3±0.8	1.9±29	3.3±3.25	16.475	< 0.01	HS
INR	1.15±0.13	1.6±0.14	2±0.26	175.78	< 0.01	HS

Data are expressed as the mean±SE. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; highly significant, HS <0.01; INR, international normalized ratio; nonsignificant, NS >0.05; PLT, platelet count; PT, prothrombin time; PTT, partial thromboplastin time; significant, S <0.05; WBC, white blood cell count.

Table 2 Comparison between the group II and III regarding ascitic neutrophils

	Mean±SD		*t*	*P*	Significance
	Group 2	Group 3			
Ascitic neut.	65±38.09	381.2±22.8	−39	<0.01	HS

Highly significant, HS >0.01; nonsignificant, NS >0.05; significant, S <0.05.

version 23. The quantitative data were presented as mean, SD, and ranges when their distribution was found to be parametric. Moreover, qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data was done by using χ^2 test. The comparisons between the two independent groups with quantitative data and parametric distribution were done by using independent *t*-test. The comparisons between more than two independent groups with quantitative data and parametric distribution were done by using one-way analysis of variance test followed by post-hoc analysis using least significant difference test.

Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the *P* value was considered significant as follows: $P > 0.05$: nonsignificant (NS), $P < 0.05$: significant (S), and $P < 0.001$: highly significant (HS).

Results

Comparisons among the three studied groups regarding laboratory data are shown in Table 1. Group III showed lower values concerning hemoglobin, platelets, and albumin (mean: 10.6 ± 1.76, 98.5 ± 48.21, and 2.39 ± 0.38, respectively) than the other two groups but higher results for total and direct bilirubin and international normalized ratio (mean: 5.8 ± 6.0, 3.3 ± 3.25, and 2 ± 0.26, respectively), with a statistically significant difference among all groups.

Ascitic neutrophils showed a high statistically significant differences between groups II and III ($P > 0.01$), being higher in group III. Table 2 and Fig. 1 show the correlation between vitamin D level and ascitic fluid WBC count. There was a highly significant negative correlation between the two parameters. Moreover, in our cirrhotic patients, a highly significant positive correlation between albumin and vitamin D concentration is reported (Fig. 2).

Ionized calcium showed no significant differences among the studied groups ($P < 0.05$). As for vitamin D, it showed a statistically highly significant difference between the studied groups ($P > 0.01$), with reduction in group III than the other two groups (Table 3).

Discussion

A low level of vitamin D in patients with chronic liver disease can be attributed to multiple mechanisms, such as low sunlight exposure, malnutrition, intestinal edema complicating portal hypertension leading to low intestinal absorption of vitamin D, or bile salt disruption caused by cholestasis. Another contributing factor is the low levels of vitamin D-binding proteins and albumin, which transfer vitamin D to the liver and kidney for subsequent activation, besides the low production of the active form of vitamin D caused by impaired hydroxylation by the liver [9].

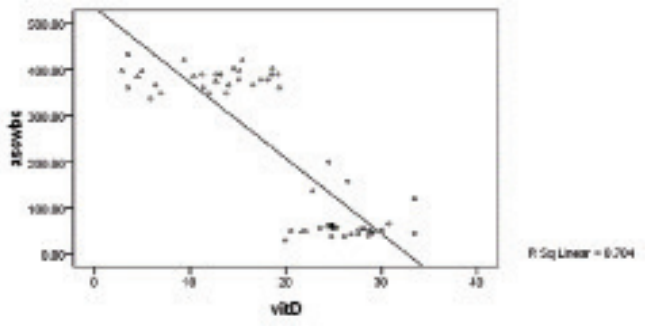
Gentile *et al.*[10] studied the effect of infection in cirrhotic patients after one-year follow-up and reported 30% mortality. Another study revealed that the 30-day mortality in cirrhotic patients with infection was 25%. SBP in 36% of patients and pneumonia in 31% were associated with the highest mortality rates, followed by primary blood stream infection (29%) [11].

Table 3 Comparison between the three groups regarding vitamin D level

	Groups (mean±SD)			ANOVA test		
	Group 1	Group 2	Group 3	*F*	*P*	Significance
Ionized Ca	4.8±0.18	4.8±0.15	4.9±0.22	2.613	0.079	NS
Vitamin D	38.61±13.12	26.1±3.34	12±5.14	0.75.995	<0.01	HS

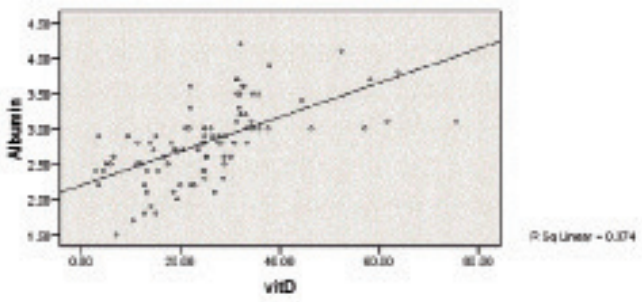
ANOVA, analysis of variance; highly significant (HS) <0.01; significant (S) <0.05; nonsignificant (NS) >0.05.

Figure 1



A negative correlation between vitamin D level and ascitic fluid white blood cells count.

Figure 2



A positive correlation between vitamin D level and serum albumin.

In our study, vitamin D showed a statistically highly significant difference between the studied groups ($P > 0.01$) with reduction in group three than the other two groups In Egypt, Ramadan *et al.*[12] studied the relation between vitamin D level among cirrhotic patients and the risk of infections. They also reported SBP as the most common infection among cirrhotic patients (62.2%). Respiratory tract infection was the second most common (22.2%), followed by skin and soft tissue infection, and urinary tract infection as the least common.

Low 25-OH-vitamin D levels reflect hepatic dysfunction and are associated with mortality in patients with liver cirrhosis [13]. We found in our cirrhotic patients a negative correlation between markers of severe cirrhosis and vitamin D concentrations. Similarly, Kubesch *et al.*[14] and Thiele *et al.*[15] reported a significant negative correlation between vitamin D level and markers of severe cirrhosis.

Regarding platelet count, in our study, we found a highly significant difference between the studied groups, being lowest in group III. Correspondingly, Wehmeyer *et al.*[16] stated that platelet count may be an important predictor of degree of cirrhosis in patients with decompensated liver diseases.

In the present work, we report a highly significant difference among the groups concerning ascitic neutrophils. Moreover, there was a significant negative correlation between vitamin D level and ascitic neutrophil count. This comes in concordance with a study done by Reginato *et al.*[17] revealing increased ascitic neutrophils in cases of SBP and Buonomo *et al.*[18] showing a significant negative correlation between vitamin D level and ascitic neutrophil count.

Jha *et al.*[19] conducted a study on 101 patients using vitamin D for treatment of patients with liver cirrhosis, compared with the control group. They stated that vitamin D supplementation in patients with liver cirrhosis was significantly associated with survival over 6 months.

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Conflicts of interest
There are no conflicts of interest.

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