Is Serum-Ascites Vitamin D Gradient a Valid Marker for Diagnosing Spontaneous Bacterial Peritonitis in Patients with Cirrhotic Ascites?

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ABSTRACT

Objective: Spontaneous bacterial peritonitis (SBP) is considered the paradigmatic model of infection in patients with liver cirrhosis. Therefore, there is a need for an accurate and rapid method for SBP diagnosis. The aim of this study was to evaluate the validity of serum-ascites 25-hydroxyvitamin D (25-OH vitamin D) gradient (SADG) as a marker for diagnosing SBP in patients with cirrhotic ascites.

Methods: We conducted a cross-sectional analytic study of 88 patients with portal hypertensive ascites resulting from liver cirrhosis of any etiology. The demographic, clinical, and laboratory characteristics of the patients were recorded. The level of 25-OH vitamin D in serum and ascitic fluid was measured using high-performance liquid chromatography autoanalyzer. The SADG was calculated with the formula: 25-OH vitamin D in serum − 25-OH vitamin D in ascites.

Results: Vitamin D deficiency was detected in 89.8% of the studied patients. The SADG values ranged between 0 and 69.2 ng/mL, with a median value of 5.58 ng/mL. It was significantly lower in patients with SBP than in those without SBP ($P = .004$). The area under the curve for SADG in exclusion of SBP was 0.67 at a cutoff value of ≥5.57 ng/mL.

Conclusion: We found that SADG may be a valid marker of SBP in patients with cirrhotic ascites.

Keywords: vitamin D deficiency, spontaneous bacterial peritonitis, diagnosis, marker, serum-ascites 25-OH vitamin D gradient, cirrhotic ascites

Spontaneous bacterial peritonitis (SBP) is a bacterial infection of ascitic fluid without any intra-abdominal surgically treatable source of infection. It is also the most common infective complication of ascites because of liver cirrhosis and mostly results from gram negative bacteria.[1] When first described, its mortality exceeded 90% but has been reduced to approximately 20% with early diagnosis and treatment. The cornerstone of the diagnosis of SBP is based on a neutrophil count in ascitic fluid of >250/mm^3.[2] However, it has been suggested that the dosage of specific molecules in ascitic fluid, ie, C-reactive protein, lactoferrin, and calprotectin, may be an additional prognostic or diagnostic tool in patients with SBP.[3] With the role of vitamin D increasingly recognized in various body processes, serum-ascites 25-OH vitamin D gradient (SADG) is among these tools that have been recently investigated as a possible diagnostic marker for SBP.[4]

Vitamin D is a steroid hormone involved in several processes in addition to bone and calcium homeostasis.[5] However, vitamin D can increase innate defense and modulate the activation of lymphocytes implicated in the immune response; therefore, it has numerous additional roles such as combating bacteria and preventing both autoimmune diseases and chronic inflammatory states.[6]

Abbreviations:

SBP, spontaneous bacterial peritonitis; 25-OH D3, 25-hydroxyvitamin D3; 25-OH vitamin D, 25-hydroxyvitamin D; SADG, serum-ascites 25-OH vitamin D gradient; PMN, polymorph nuclear leukocyte; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; HPLC, high-performance liquid chromatography; CTP, Child-Turcotte-Pugh; MELD, Model for End-Stage Liver Disease; SD, standard deviation; IQR, interquartile ratio; ROC, receiver operating characteristic; WBC, white blood cell.

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The liver is a vital organ for vitamin D biotransformation, where vitamin D is metabolized, a process carried out by a cytochrome P450 enzyme, into 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 (25-OH D3).[7] 25-OH vitamin D (25-OH D) undergoes a second hydroxylation mainly in the kidney to form 1,25 (OH) D, known as calcitriol, the active form.[8]Research has shown that 25-OH D, the major circulating form of vitamin D, is used to determine a patient's vitamin D status.[8] Recently, 25-OH D levels have been found to be low in patients with liver cirrhosis vs in control patients, and the severity of 25-OH D deficiency in these patients correlated with the severity of liver dysfunction.[1,9,10] The causes of vitamin D deficiency in cirrhosis are the decreased number of hepatocytes, reduced exposure to sunlight, malabsorption of vitamin D, and altered hydroxylation of vitamin D because of liver impairment.[11]

Studies have found that low levels of circulating 25-OH D are associated with infections in patients with cirrhosis.[12,13] Moreover, several recent studies found that vitamin D deficiency was an independent risk factor for infection and even mortality in patients with liver cirrhosis.[14,15]

The diagnosis of SBP is based on neutrophil count in the ascitic fluid > 250/mm^3 as determined by microscopy.[2] The serum-ascites 25-OH vitamin D gradient (SADG) is a novel index in the diagnosis of SBP. The SADG, calculated as the difference between serum and ascites vitamin D, was recently explored by Buonomo et al,[4] aiming to investigate the role of vitamin D levels in ascitic fluid in patients with SBP, and concluded that the SADG was significantly lower in patients with SBP than in those without SBP. However, data in the literature on the SADG as a potential diagnostic biomarker in the diagnosis of SBP in patients with cirrhotic ascites are scarce.

Therefore, this study was conducted to assess the validity of the SADG as a marker for diagnosing SBP in patients with cirrhotic ascites and to identify a cutoff level that can be used for SBP diagnosis.

Patients and Methods

Patient Populations

Between October 2019 and March 2020, patients with portal hypertensive ascites because of liver cirrhosis of any etiology presenting to the Endemic Medicine and Hepatology Department, Faculty of Medicine, Cairo University were consecutively recruited into the cross-sectional analytic study. Patients with cirrhotic ascites aged ≥18 years were recruited. Furthermore, patients who presented with SBP that was diagnosed according to current guidelines by an elevation of ascitic fluid absolute polymorph nuclear leukocyte (PMN) count (≥250 cells/mm^3) were also included in the study.[2] Patients with ascites from causes other than portal hypertension (eg, ascites from peritoneal diseases, malignant ascites, nephrogenic ascites, and pancreatic ascites), infection other than SBP, or patients who received oral vitamin D supplementation in the previous 12 months were excluded. The patients were further subdivided into 2 groups according to the presence or absence of SBP. Group I comprised 44 patients with SBP, and group II comprised 44 patients without SBP; these patients served as a control group.

Patients were subjected to detailed history including demographic data, date of diagnosis of liver cirrhosis, manifestations of hepatic decompensation, etiology of cirrhosis, and date of developing ascites and underwent clinical examination with special emphasis on stigmata of chronic liver disease such as ascites, splenomegaly, spider naevi, palmar erythema, lower limb edema, and gynecomastia in addition to general and local signs of peritonitis and its sequelae (signs of hepatic encephalopathy, reduced urine output or bleeding).

Laboratory evaluation included complete blood count, liver biochemical profile (total serum bilirubin, liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST)], serum albumin, and the international normalized ratio [INR]), renal function tests (serum urea and creatinine), and electrolytes (serum sodium and potassium); all chemistry analysis was performed on a Beckman Coulter AU680 autoanalyzer according to the manufacturer's methods. In addition, serum 25-OH D was measured on the same day that the patient underwent diagnostic paracentesis.

Regarding ascitic fluid analysis, specimens were examined for PMN cell count using manual microscopy, ascitic fluid chemistry including total proteins and albumin on a Beckman Coulter AU680 autoanalyzer, and 25-OH D level in the ascitic fluid using the high-performance liquid chromatography (HPLC) autoanalyzer Agilent 1260.[16] The severity of liver diseases was assessed using the Child-Turcotte-Pugh (CTP) score[17] and the Model for End-Stage Liver Disease (MELD).[18] All patients underwent abdominal ultrasonography to examine liver size and texture, portal vein diameter, spleen size, and splenic vein diameter, to grade the amount of ascites, and to exclude focal hepatic lesions.

Ultrasonographic criteria for the diagnosis of cirrhosis included volume redistribution of the right and left liver lobes, the presence of surface nodularity, the coarseness of texture, the attenuation of hepatic veins, and signs of portal hypertension such as a dilated portal vein (≥14 mm), splenomegaly, ascites, and collaterals.[19]

Measurement of 25-OH D

25-OH D3, after deproteinization with a specific reagent and after purification with Clean-up Columns (extraction with a solid phase extraction column), was injected directly into the isocratic HPLC system (HPLC Agilent 1260). It had sensitivity up to 1.5 ng/mL with a linearity of 2 to 500 ng/mL for detection. Serum 25-OH D3 concentrations <20 ng/mL were defined as severe vitamin D deficiency, levels from 21 to 29 ng/mL were considered as insufficient, and serum levels from 30 to 100 ng/mL were considered normal.[20] The SADG was calculated with the following formula: 25-OH D in serum — 25-OH D in ascites.[4]

The study was designed to respect all ethical guidelines issued by the 1975 Declaration of Helsinki and was approved by the institutional review board of the Faculty of Medicine, Cairo University. Informed consent was obtained before specimen collection.

Statistical Analysis

Descriptive statistics were calculated, categorical variables were presented as frequency and percentages, and numerical variables were presented as mean (standard deviation [SD]) or median (IQR). A comparison between the 2 independent groups was done using the independent-samples *t*-test or the Mann-Whitney *U* test according to the normality of data. For categorical variables, the comparison was done with the χ^2 test. The receiver operating characteristic (ROC) curve was constructed to assess the diagnostic ability of the SADG in detecting SBP. We used STATA 15 for the analysis and deemed *P* values <.05 as significant.

Results

Eighty-eight patients with portal hypertensive ascites resulting from liver cirrhosis of any etiology were included in the present study. The main characteristics of the patients are summarized in **Table 1**. The mean age of patients with SBP was 55.9 years (range, 37–80 years) with male predominance (77.3%), and the mean age of patients without SBP was 58.3 years (range, 42–77 years) with male predominance (59.1%) as well. Regarding the severity of liver diseases, all the patients in both groups were CTP class C or B, and none of them were classified as CTP class A. Specifically, among the 88 patients, 38 (43.2%) belonged to CTP class B and 50 (56.8%) belonged to CTP class C. In addition, there was no statistically significant difference in the CTP or the MELD scores between the 2 groups (*P* = .7 and *P* = .9, respectively).

In comparing the laboratory characteristics in patients with SBP and patients without SBP as shown in **Table 2**, it was observed that the patients with SBP had a significant lower median hemoglobin level (7.45 g/dL [IQR, 4.9–9.3] vs 8.9 g/dL [IQR, 7.15–10.7]; *P* = .03) and a significant higher median blood urea nitrogen level (66 mg/dL [IQR, 42–125.5] vs 49.5 mg/dL [IQR, 29.5–84.5]; *P* = .02). Notably, no difference was observed in relation to analyses on the following laboratory variables: white blood cell (WBC) count, platelets, AST, ALT, total serum bilirubin, serum albumin, INR, and serum creatinine.

Vitamin D deficiency was observed in the majority of the studied patients (n = 79/88, 89.8%). Moreover, it was observed that patients with SBP had nonsignificantly lower

Table 1. Demographic Features of Both Studied Groups

Variables	Patients with SBP (n = 44)	Patients without SBP (n = 44)	*P* Value
Age (y)	55.9 (11.43)	58.3 (7.95)	.3
Sex			
Male	34 (77.3%)	26 (59.1%)	.07
Female	10 (22.7%)	18 (40.9%)	
Severity of liver disease			
A-CTP			
1-score	10.3 (1.7)	10.11 (2.002)	.7
2-class			
B, n (%)	18 (40.9%)	20 (45.5%)	.7
C, n (%)	26 (59.1%)	24 (54.5%)	
B-MELD score	21.6 (6.22)	21.5 (8.1)	.9

CTP, Child-Turcotte-Pugh; MELD, Model for End-Stage Liver Disease; SBP, spontaneous bacterial peritonitis.
Data are expressed as the mean (SD), or number (%).

median serum 25-OH D in relation to patients without SBP (7.3 [IQR, 4.7–14.7] vs 9.4 [5.8–15.3]; *P* = .2), but the median level of 25-OH D in ascitic fluid did not differ between patients with or without SBP (*P* = .08) as shown in **Table 2**.

We used CTP class to evaluate the severity of the liver disease and found no significant difference between the CTP class and the status of the serum 25-OH D level in the studied patients (*P* = .4). We also noted that the mean value of serum 25-OH D was lower in CTP class B than in CTP class C with no significant difference between them (11.5 [SD, 8.002] ng/mL vs 12.2 [SD, 12.8] ng/mL; *P* = 7), but the median value of 25-OH D in the ascitic fluid was significantly higher in CTP class B than in CTP class C (2.7 ng/mL [IQR, 1–4.1] vs 1 ng/mL [IQR, 1–1]; *P* = .0007), as shown in **Table 3**.

In the entire sample of patients, the SADG values ranged between 0 and 69.2 ng/mL, with a median value of 5.58 ng/mL (IQR, 3.02–12.2). They were significantly lower in patients with SBP than in those without SBP (3.45 ng/mL [IQR, 2.45–11.95] vs 8.75 ng/mL [IQR, 5–13.3]; *P* = .004), as shown in **Table 2**. However, the median SADG did not differ significantly between patients in CTP class B and in CTP class C (5.2 ng/mL [IQR, 3–10] vs 7 ng/mL [IQR, 3.1–13]; *P* = .5), as shown in **Table 3**.

An ROC curve was designed to evaluate the ability of the SADG in exclusion of SBP; results showed a SADG with a cutoff value of ≥5.57 ng/mL had a sensitivity of 70.5%, a specificity of 68.2%, an overall accuracy of 69.32%, and an area under the curve of 0.67, as shown in **Figure 1**.

Table 2. Analysis of Laboratory Characteristics of Both Studied Groups

Variables	Patients with SBP (n = 44)	Patients without SBP (n = 44)	*P* Value
Laboratory parameters			
White blood cell ($\times 10^3/mm^3$)	8.5 (7.8–10.3)	8.4 (5.85–9.85)	.08
Hemoglobin (g/dL)	7.45 (4.9–9.3)	8.9 (7.15–10.7)	.03[a]
Platelets ($\times 10^3/mm^3$)	77 (56–128)	82.5 (70.5–129.5)	.4
AST (U/L)	51 (37–79.5)	54 (36–76)	.9
ALT (U/L)	24 (13–37.5)	26 (21.5–41)	.5
Total bilirubin (mg/dL)	3.15 (1.2–5.7)	2.2 (1.1–3.4)	.1
Albumin (g/dL)	2.4 (2.1–2.9)	2.15 (1.9–2.8)	.3
INR	1.6 (1.4–1.8)	1.67 (1.39–2)	.4
Blood urea nitrogen level (mg/dL)	66 (42- 125.5)	49.5 (29.5–84.5)	.02[a]
Creatinine (mg/dL)	1.2 (0.98–1.5)	1.1 (0.7- 1.65)	.3
Ascitic fluid analysis			
TLC (mm^3)	410 (360–1360)	110 (60–290)	.09
SAAG (g/dL), mean (SD)	1.87 (0.37)	1.74 (0.33)	.08
Serum 25-OH vitamin D (ng/mL)	7.3 (4.7–14.7)	9.4 (5.8- 15.3)	.2
Ascitic fluid 25-OH vitamin D (ng/mL)	1 (1–4.1)	1 (1–2.2)	.08
SADG (ng/mL)	3.45 (2.45–11.95)	8.75 (5–13.3)	.004[a]

ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; SAAG, serum-ascites albumin gradient; SADG, serum-ascites 25-OH vitamin D gradient; SBP, spontaneous bacterial peritonitis; SD, standard deviation; TLC, total leukocyte count.
Data are expressed either as mean (SD) or median (IQR).
[a]Values were statistically significant.

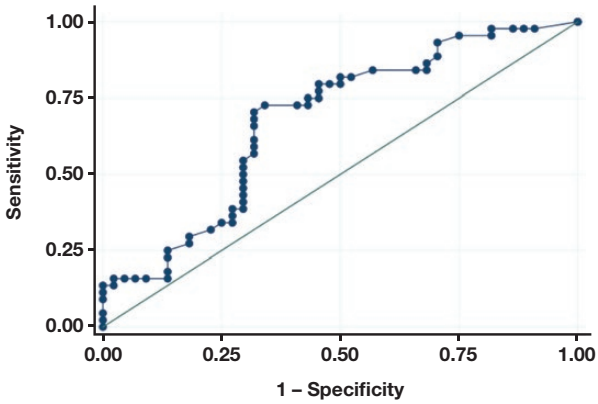
Discussion

SBP is considered the paradigmatic model of infection in patients with liver cirrhosis and is peculiar to those with the decompensated disease, being associated with significant morbidity and mortality. In addition to the diagnostic tools available, multiple efforts have been developed for the rapid diagnosis of SBP. The SADG is among these tools that have been recently investigated

Table 3. Vitamin D Assessment in Relation to Liver Disease Severity

Variables	CTP Class B (n = 38)	CTP Class C (n = 50)	*P* Value
Vitamin D status n (%)			
Sufficient	3 (7.9%)	1 (2%)	.4
Insufficient	2 (5.3%)	3 (6%)	
Deficient	33 (86.8%)	46 (92%)	
Serum 25-OH D (ng/mL), mean (SD)	11.5 (8.002)	12.2 (12.8)	.7
Ascitic fluid 25-OH D (ng/mL)	2.7 (1–4.1)	1 (1–1)	.0007[a]
SADG (ng/mL)	5.2 (3–10)	7 (3.1–13)	.5

CTP, Child-Turcotte-Pugh; IQR, interquartile ratio; SADG, serum-ascites 25-OH vitamin D gradient; SD, standard deviation.
Unless otherwise stated, numerical data are expressed as mean (SD) or median (IQR).
[a]Statistically significant value.



Variable	Cutoff Point	AUC	Sensitivity	Specificity	Overall Accuracy
SADG (ng/mL)	≥5.57	0.67	70.5%	68.2%	69.32%

Figure 1

ROC curve analysis of validity of SADG in exclusion of SBP. AUC, area under the curve; ROC, receiver operating characteristic; SADG, serum-ascites 25-OH vitamin D gradient; SBP, spontaneous bacterial peritonitis.

as a possible diagnostic marker for SBP.[4] This study was conducted to assess the validity of the SADG as a marker for diagnosing SBP in patients with cirrhotic ascites.

In reviewing the laboratory characteristics, we noted that there were no significant differences concerning WBC count, serum albumin, total serum bilirubin, or INR between patients with and without SBP. Similar findings were noted by the Evans et al. study,[21] which assessed 427 patients with ascites and observed that 3.5% had SBP. At the same time, we noticed that there was an increase in WBC count in the SBP group when compared to the non-SBP group; this finding could help predict the appearance of SBP in patients with ascites with no clinical signs or symptoms of infection, owing to the immunocompromised state observed in liver cirrhosis.[22]

In addition, we observed that patients in the SBP group showed a nonsignificant increase in serum creatinine compared with those in the non-SBP group (P = .3), whereas the blood urea nitrogen level was significantly higher in those in the SBP group compared with the level in those in the non-SBP group (P = .02). Patients with cirrhosis are susceptible to renal dysfunction as a result of a variety of etiologies, and measures of renal dysfunction (serum creatinine, blood urea nitrogen/azotemia) are strong predictors of mortality in decompensated cirrhosis.[23] Infection, particularly SBP, is a common precipitating factor of renal dysfunction in cirrhosis and may be associated with septic shock in up to 10% of patients.[2,24] Traditionally, the diagnosis of renal dysfunction in liver disease is still based on serum creatinine, but it is not a reliable marker of renal function in cirrhosis because it still has several well-known limitations.[25]

The classic function of vitamin D is to regulate calcium homeostasis and bone formation and resorption. However, vitamin D could increase innate defense and modulate the activation of lymphocytes implicated in the immune response,[26] so vitamin D insufficiency may predispose to or increase the risk of bacterial infections and SBP in patients with cirrhosis.[7] We observed a high prevalence of vitamin D deficiency in our patients (n = 79/88, 89.8%), and patients with SBP had lower serum 25-OH D concentrations than patients without SBP. These findings were consistent with the observations of Zhang, Zhao, and Ma,[11] who showed a high prevalence of vitamin D insufficiency in patients with cirrhosis and ascites. Moreover, the authors found that serum vitamin D deficiency was observed in patients with SBP. A low level of vitamin D in patients with chronic liver disease (CLD) may be attributed to several mechanisms such as insufficient exposure to sunlight or an inadequate dietary intake of vitamin D.[11] In addition, patients affected by chronic liver disease may have impeded intestinal luminal absorption of the dietary sources of vitamin D because of intestinal edema, which complicates portal hypertension, or to cholestasis-induced bile salt disruption.[27]

Furthermore, we used CTP class to evaluate the severity of liver disease in relation to the status of vitamin D. We found that 92% of patients with CTP class C had vitamin D

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deficiency, indicating that vitamin D status may be determined in part by chronic liver disease severity. Our finding was in agreement with the results of Arteh et al[9] and Fisher and Fisher,[26] who showed that patients with cirrhosis have much lower vitamin D concentrations than patients without cirrhosis.

In the current study, however, the SADG was found to be significantly lower in patients with SBP than in those without SBP. This finding agrees with the results of Buonomo et al,[4] who investigated 54 patients with liver cirrhosis and ascites for the possible role of vitamin D in ascites in patients with SBP. The available evidence indicates that there seems to be a compartmentalization of vitamin D from serum to ascites in patients with SBP that exists to increase local, peritoneal levels of vitamin D, which acts as a chemotactic and antibacterial agent that exerts its effects locally in ascitic fluid. This compartmentalization could explain our finding of a low SADG in patients with SBP. Our results are also potentially in accordance with those obtained by Trépo et al,[27] who reported a tendency to an increased incidence of SBP (15.7% vs 6.9%) in patients with cirrhosis with vitamin D deficiency.

Another important finding of this study based on the ROC curve was that the SADG with a cutoff value of ≥5.57 ng/mL had a sensitivity of 70.5%, a specificity of 68.2%, and an area under the curve of 0.67 in exclusion of SBP.

Vitamin D deficiency is associated with an increased incidence of bacterial infections and increased all-cause mortality risk in patients with liver cirrhosis.[13,14] Indeed, it can modulate the host's innate and adaptive immune defense mechanisms through diverse immunomodulatory functions mediated by the vitamin D receptor.[28] On the level of ascites, this immunological role seems to be carried out through vitamin D–mediated enhancement of peritoneal leukocytic, phagocytic, chemotactic, and antimicrobial effects, including in particular the antimicrobial peptide cathelicidin.[29,30]

Conclusion

We found that a lower SADG was associated with SBP in patients with cirrhotic ascites and that it may be a valid marker for diagnosing SBP in such patients. Vitamin D deficiency is universal in patients with cirrhotic ascites regardless of SBP diagnosis. Adequate vitamin D is vital for the body to improve antimicrobial immune response.

This study has some limitations. We assessed the diagnostic significance of the SADG in SBP diagnosis in patients with cirrhotic ascites and excluded those who received vitamin D supplementation from our study. Therefore, more powered studies are needed to determine whether SADG has any prognostic significance in patients with SBP and to establish a reliable cutoff value for diagnosing SBP. The response of the SADG and the recurrence of SBP after vitamin D supplementation need further evaluation. **LM**

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