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Is Vitamin D Deficiency a Risk Factor for Spontaneous Bacterial Peritonitis?

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ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is a severe worldwide liver condition.

Objective: The aim of this study was investigating the association between serum 25(OH)D deficiency and the complications of hepatitis c virus (HCV) related cirrhosis, notably, SBP.

Patients and Methods: This prospective case control study was carried out on 100 patients, with cirrhosis and ascites. The patients were divided into two groups: Group I cirrhotic patients with ascites and SBP, which were divided into two equal subgroups according to addition of 25(OH)D to treatment of SBP: group Ia did not receive vitamin D and group Ib received vitamin D. Group II: cirrhotic patients with simple ascites and without SBP. Group Ib of patients were receiving a dose of 2.000 I. U of 25 hydroxycholecalciferol per day for at least 1 week.

Results: There was a significant correlation between serum vitamin D and end-stage liver disease (MELD) score (r = -0.51, P= 0.012; r = -0.37, P= 0.016, respectively) in subgroup (GI a) not receiving vitamin D and control group. Serum level of vitamin D was also significantly correlated with ascitic polymorphonuclear neutrophils (PMN) count (cell/µL) in both SBP subgroups (GI a and b) (r = -0.61, P= 0.002; r = -0.61, P= 0.002, respectively). The receiver operating characteristic (ROC) curve analysis estimated a sensitivity of 78.3%, a specificity of 69.0%, a positive predictive value (PPV) of 58%, a negative predictive value (NPV) of 85.3% and an accuracy of 73.7% at the best cutoff value of ≤13.96 (ng/mL). The AUC was 0.76 with a highly significant P-value < 0.001. A positive highly significant correlation between serum vitamin D and serum albumin was observed in both subgroups and control (r = 0.67, P = 0.001 and r =0.50, P=0.014, r = 0.44, P=0.004, respectively).

Conclusions: MELD scores >15 were related with an increased risk of SBP. It was also shown that Escherichia coli and Staph aureus were the most frequent bacteria among SBP patients.

Keywords: Vitamin D Deficiency, SBP, MELD score, Cirrhosis.

INTRODUCTION

As a monomicrobial infection of ascitic fluid without a communicable source of infection, SBP is a major morbidity worldwide hepatic condition. SBP is related with a higher risk of infection and mortality and can arise from a variety of aetiologies due to immune system abnormalities, which are frequent in patients with MELD [1].

Low ascitic fluid protein content and advanced liver disease have been found to be risk factors for SBP. Research indicates that the most potent predictor of SBP is an ascitic fluid total protein (TP) level ≤ 1 gm /dL, which indicates a reduced opsonization capability and low complement concentration [2].

Polymorphonuclear (PMN) cell count in ascitic fluid (AF) more than or equal to 250 cells/mm^3, where 60–70% of cases show the isolation of the infectious organism, is the diagnostic tool for SBP [3].

Up to 92% of individuals with chronic liver disease have some form of insufficiency or deficit in Vit-D, and the severity of the shortage is correlated with the severity of liver dysfunction. This correlation between the degree of liver malfunction and Vit-D insufficiency was thought to be caused by deterioration of the synthetic liver [4-6].

Patients with cirrhosis and alcoholic liver disease have been linked to higher death rates when deficient in Vit-D; however, the source of this correlation and its causal linkage remain unclear. It is possible that a Vit-D shortage raises infection rates, which in turn raises death rates in cirrhosis patients. It is true that Vit-D controls the immune system and that bacterial infections shorten the longevity of cirrhosis patients [7].

The aim of this study was investigating the association between serum 25(OH)D deficiency and the complications of HCV-related cirrhosis, notably, SBP and impact of addition of 25(OH)D to treatment regimen on outcomes of patients with SBP and Vit-D deficiency.

PATIENTS AND METHODS

This prospective case control study was carried out on 100 patients aged up to 59 years old, of both genders, with cirrhosis and ascites.

Exclusion criteria were patients with malignant or tuberculous ascites; patients with alcoholic liver cirrhosis; patients with Wilson disease; patients with hemochromatosis and glycogen storage disease; patients with renal disease; patients with DM; patients with collagen diseases; patients with sepsis; patients with infection other than SBP; patients who received vit-D supplementation within the last six months; and patients who are not available for follow-up

Patients were grouped based on the presence of ascites as follows: Group I included cirrhotic patients with ascites and SBP, patients were diagnosed to have

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SBP according to **Rimola *et al.* [8],** which were divided into two equal subgroups according to addition of 25(OH)D to treatment of SBP: group Ia did not receive Vit-D and group Ib received Vit-D as part of their treatment regimens. Group II (Control group) included cirrhotic patients with simple ascites and without SBP, those had ascites attributable to decompensated liver disease and had no clinical symptoms or indicators of SBP.

Group Ib of patients were receiving a dose of 2.000 I.U. of 25 hydroxycholecalciferol per day for at least 1 week during patient stay at National Liver Institute.

All patients were subjected to history taking, clinical examination, the model for ESLD (MELD) and Child–Pugh scoring systems for cirrhotic patients was calculated, laboratory investigations [CBC, RFT, liver profile [ALT, AST, TSB, ALP, prothrombin activity (PA)], serum level of 25(OH) Vit-D was assessed by ELISA, and abdominal ultrasonography was used to grade the ascites.

Every patient in the study who had ascites and cirrhosis and underwent diagnostic paracentesis had an ascitic fluid (AF) sample taken for the purpose of measuring Vit-D using chemiluminescence, PMN cell count, culture, and sensitivity. In order to diagnose SBP, the AF had to have more than or equal to 250 PMN cells/mm^3, and the AF culture had to be positive without an intra-abdominal infection source.

Assessment of serum level of 25 (OH Vit-D): Epitope Diagnostics, Inc., (Catalog Number KT-715/V9/CE/2020-03, Germany).

Ethical approval:

The Ethical Committee of National Liver Institute, Faculty of Medicine, Menoufia University, Menoufia, Egypt, gave its clearance before the study could be carried out. The patient provided written, informed consent. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

SPSS V. 27.0 was used for the statistical analysis. The normality of the data distribution was assessed using the Shapiro-Wilks test and histograms. T-test and ANOVA (F) test with post hoc test (Tukey) were used to analyse quantitative parametric data, which were reported as mean±SD.

In order to compare each group, quantitative non-parametric data were given as the median and interquartile range (IQR) and were examined using the Mann Whitney test and the Kruskal-Wallis test. The X^2 test was used to analyse the qualitative variables, which were given as frequency and percentage (%). The Pearson correlation coefficient (r) was computed to show the direction and degree of connection between two continuous, at least regularly distributed numerical variables. The optimal cutoff value and AUC were among the test features that were evaluated using the ROC curve. A statistically significant result was defined as a two-tailed P value < 0.05.

RESULTS

There was no significant difference regarding age and gender between all studied groups **(Table 1)**.

Table (1): Demographic data in SBP cases and non-SBP control group and SBP subgroups received and did not receive Vit-D and non-SBP control group

		GI SBP (n= 46)	GII Non-SBP (n= 42)	P-value
Age (years)		59.30 ± 7.09	61.26 ± 6.18	0.173
Gender	**Male**	26 (56.5%)	21 (50.0%)	0.540
	Female	20 (43.5%)	21 (50.0%)	

		GI a SBP not receiving vit. D (n=23)	GI b SBP receiving vit. D (n= 23)	GII Non SBP (n= 42)	
Age (years)		60.09 ± 5.65	58.52 ± 8.34	61.26 ± 6.18	0.290
Gender	**Male**	11(47.8%)	15 (65.2%)	21 (50.0%)	0.412
	Female	12 (52.2%)	8 (34.8%)	21 (50.0%)	

There was insignificant difference between two groups regarding Vit D deficiency, ALT, AST, GGT, Total bilirubin, direct bilirubin, ALB, INR, creatinine, Na+, K+, and hemoglobin. While there was highly significant increase in group I than group II regarding Vit-D, ALP, WBCs and Ascitic PMN count. There was highly significant increase in group II than group I regarding urea and platelets. The comparison between clinicopathological parameters in SBP and non-SBP groups showed non-significant difference regarding liver and spleen sizes and MELD score. However, there was a significant difference regarding MELD score categories. Similarly, there was significant statistical difference regarding Child-Pugh classification, ascites, and ascetic culture results **(Table 2)**.

Table (2): Laboratory investigations and clinicopathological parameters in SBP cases and non-SBP control group

		GI SBP (n= 46)	GII Non SBP (n= 42)	P-value
Vit-D (ng/mL)		18.82 (12.74)	17.58 (10.14)	**<0.001** *
Vit D deficiency		27 (58.7%)	25 (59.5%)	0.937
ALT (U/L)		31.50 (17.50)	41.00 (21.00)	0.071
AST (U/L)		41.00 (21.00)	37.00 (41.50)	0.655
ALP (U/L)		171.15 ± 32.40	134.26 ± 33.43	**<0.001** *
GGT (U/L)		52.48 ± 12.98	52.48 ± 13.00	1.000
Total bilirubin (mg/dL)		3.15 (2.25)	2.80 (1.63)	0.216
Direct bilirubin (mg/dL)		1.30 (1.15)	1.23 (0.73)	0.095
ALB (g/dL)		2.15 (0.63)	2.55 (0.80)	0.065
INR		1.90 (0.45)	1.80 (0.62)	0.277
Urea (mg/dL)		47.00 (27.00)	48.00 (30.25)	**0.031***
Creatinine (mg/dL)		1.20 (0.42)	1.20 (0.40)	0.819
Na+ (mmol/L)		123.00 (7.25)	127.00 (7.25)	0.156
K+ (mmol/L)		4.56 ± 0.48	4.41 ± 0.50	0.145
HB (g/dL)		8.68 ± 1.54	9.25 ± 1.68	0.099
Platelets (10^3 cell/µL)		78.50 (27.00)	106.00 (41.75)	**0.001***
WBCs (10^3 cell/µL)		15.20 (4.45)	7.25 (1.75)	**<0.001***
Ascitic PMN count (cell/µL)		370.50 (221.00)	175.00 (51.25)	**<0.001***
Clinicopathological parameters				
Liver size (cm)		12.50 (1.50)	12.50 (1.10)	0.337
Spleen size (cm)		19.00 (3.50)	19.00 (2.70)	0.369
MELD score		19.33 ± 3.94	17.93 ± 4.42	0.120
MELD ≤ 15		5 (10.9%)	13 (31.0%)	**0.033***
MELD > 15		41 (89.1%)	29 (69.0%)	
Child Pugh score	**B**	6 (13.0%)	17 (40.5%)	**0.033***
	C	40 (87.0%)	25 (59.5%)	
Ascites	**Mild**	1 (2.2%)	9 (21.4%)	**0.003***
	Moderate	22 (47.8%)	23 (54.8%)	**0.003***
	Marked	23 (50.0%)	10 (23.8%)	
Ascitic culture	**E. Coli**	30 (65.2%)	42 (!00%)	**<0.001***
	Staph aureus	13 (28.3%)	0 (0.0%)	
	Staph epidermidis	1 (2.2%)	0 (0.0%)	
	Strept viridans	2 (4.3%)	0 (0.0%)	

Median and IQR: Nonparametric test, mean ± SD, or frequency (%), *: Significant, SBP: spontaneous bacterial peritonitis, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transpeptidase, ALB: serum albumin, INR: international normalized ratio, Hb: hemoglobin, WBCs: white blood cell, PMN: polymorphonuclear neutrophils, MELD: end-stage liver disease.

There was insignificant difference between SBP subgroups received and did not receive Vit-D and non-SBP control group regarding serum level of AST, GGT, total bilirubin, direct bilirubin, ALB, INR, creatinine, Na+, K+, and Hb. There was significant deference between SBP subgroups received and did not receive Vit-D and non-SBP control group regarding serum Vit-D, Vit D deficiency, ALP, but no significant difference between SBP receiving Vit-D and SBP not receiving groups. Regarding ALT, (there was significant difference between SBP receiving Vit-D and non-SBP control group), urea (there was significant difference between SBP not receiving Vit-D and non-SBP control group), platelets (there was significant difference between SBP not receiving Vit-D and non-SBP control group), WBCs and ascitic PMN count (there was no significant difference between SBP receiving Vit-D and SBP not receiving groups, otherwise the difference was significant in the other comparisons). The comparison between clinicopathological parameters in SBP subgroups received and did not receive Vit-D and non-SBP control group showed non-significant difference regarding liver and spleen sizes and MELD score. On the other hand, Child-Pugh classification, ascites, and ascetic culture results revealed significant statistical difference. On multiple comparisons, no significant difference was obtained except for ascetic culture results that revealed statistical difference when non-SBP control group was compared to both subgroups receiving and not receiving Vit-D (GI a and GI b) **(Table 3)**.

Table (3): Laboratory investigations and clinicopathological parameters in SBP cases and non-SBP control group

		GI a SBP not receiving vit D (n= 23)	GI b SBP receiving vit D (n= 23)	GII Non SBP (n= 42)	P-value	Multiple comparisons
Laboratory investigations						
Vit-D (ng/mL)		10.59 (6.12)	23.00 (6.50)	17.58 (10.14)	**<0.001***	**p1<0.001 * p2<0.001 * p3<0.001 ***
Vit D deficiency		22 (95.7)	5 (21.7)	25 (59.5)	**<0.001***	**P1<0.001* P2=0.006 * P3=0.009***
ALT (U/L)		31.00 (17.00)	34.00 (18.00)	41.00 (21.00)	**0.014 ***	p1=0.774 **p2=0.018*** p3=0.198
AST (U/L)		41.00 (20.00)	35.00 (19.00)	37.00 (41.50)	0.935	---
ALP (U/L)		167.43 ± 40.49	174.87 ± 21.88	134.26 ± 33.42	**<0.001***	p1=0.797 **p2=0.005*** **p3=0.001 ***
GGT (U/L)		52.00 (25.00)	53.00 (13.00)	51.00 (28.00)	0.856	--
Total bilirubin (mg/dL)		3.20 (2.20)	3.10 (2.70)	2.80 (1.63)	0.298	--
Direct bilirubin (mg/dL)		1.30 (1.40)	1.40 (1.10)	1.23 (0.73)	0.212	--
ALB (g/dL)		2.20 (0.60)	2.10 (0.60)	2.55 (0.80)	0.117	--
INR		1.90 (0.50)	1.91 (0.45)	1.80 (0.62)	0.545	--
Urea (mg/dL)		57.00 (29.00)	42.00 (10.00)	48.00 (30.25)	**0.017***	**p1=0.012 *** p2=0.795 p3=0.090
Creatinine (mg/dL)		1.20 (0.30)	1.20 (0.40)	1.20 (0.40)	0.680	--
Na+ (mmol/L)		123.00 (7.00)	125.00 (8.00)	127.00 (7.25)	0.058	--
K+ (mmol/L)		4.56 ± 0.43	4.57 ± 0.53	4.41 ± 0.50	0.348	--
HB (g/dL)		8.88 ± 1.84	8.47 ± 1.16	9.25 ± 1.68	0.179	--
Platelets (10^3 cell/µL)		87.00 (37.00)	76.00 (20.00)	106.00 (41.75)	**0.002***	p1=0.718 p2=0.093 p3=0.003 *
WBCs (10^3 cell/µL)		14.80 (4.70)	16.00 (5.00)	7.25 (1.75)	**<0.001***	p1=0.882 p2<0.001 * p3<0.001 *
Ascitic PMN count (cell/µL)		378.0 (323.00)	310.0 (152.00)	175.0 (51.25)	**<0.001***	p1=0.209 p2<0.001 * p3<0.001 *
Clinical parameters						
Liver size (cm)		12.50 (1.00)	12.50 (2.00)	12.50 (1.10)	0.410	--
Spleen size (cm)		19.00 (3.50)	19.00 (1.00)	19.00 (2.70)	0.659	--
MELD score		19.65 ± 3.69	19.00 ± 4.23	17.93 ± 4.42	0.262	--
MELD ≤ 15		2 (8.7%)	3 (13.0%)	13 (31.0%)	0.083	--
MELD > 15		21 (91.3%)	20 (87.0%)	29 (69.0%)		
Child Pugh score	**B**	3 (13.0%)	3 (13.0%)	17 (40.5%)	0.014*	P1=1.000 P2=0.065 P3=0.065
	C	20(87.0%)	20(87.0%)	25 (59.5%)		
Ascites	**Mild**	1 (4.3%)	0 (0.0%)	9 (21.4%)	**0.018***	P1=0.988 P2=0.101 P3=0.065
	Moderate	10(43.5%)	12(52.2%)	23 (54.8%)		
	Marked	12(52.2%)	11(47.8%)	10 (23.8%)		
Ascitic culture	**E. Coli**	10(43.5%)	12(52.2%)	0 (0.0%)	**<0.001***	P1=1.000 **P2<0.001 *** **P3<0.001***
	Staph aureus	12(52.2%)	11(47.8%)	0 (0.0%)		
	Staph epidermidis	1 (4.3%)	0 (0.0%)	0 (0.0%)		
	Strept viridans	1(43.5%)	12(52.2%)	0 (0.0%)		

Median and IQR: Non parametric test, mean ± SD, or frequency (%), *: Significant

A positive highly significant correlation between Vit-D and serum albumin was observed in both subgroups and control. Additionally, a negative correlation between Vit-D level and INR was found to be highly significant in subgroup (GI a) not receiving Vit-D and control group. Similar results were found regarding MELD score in subgroup (GI a) not receiving Vit-D and control group and ascitic PMN count (cell/µL) in both SBP subgroups (GI a and b). No significant correlation was observed between serum Vit-D level and any of the remaining parameters in both subgroups and control group **(Table 4)**.

Table (4): Correlation between serum Vit-D (ng/mL) and various parameters in both SBP subgroups receiving and not receiving Vit-D and non-SBP control group

	Vit-D (ng/mL)					
	SBP not receiving vit D		**SBP receiving vit D**		**Non SBP**	
	r	**P-value**	***r***	**P-value**	***r***	**P-value**
Age (years)	0.07	0.751	-0.12	0.573	0.00	0.999
ALT (U/L)	-0.10	0.635	-0.08	0.708	-0.14	0.387
AST (U/L)	-0.05	0.839	-0.14	0.531	-0.08	0.626
ALP (U/L)	-0.05	0.815	-0.08	0.707	-0.08	0.629
GGT (U/L)	-0.05	0.806	0.03	0.900	-0.03	0.841
Total bilirubin (mg/dL)	-0.22	0.314	-0.10	0.640	-0.18	0.262
Direct bilirubin (mg/dL)	-0.24	0.273	-0.19	0.390	-0.16	0.302
ALB (g/dL)	**0.67***	**0.001 ***	**0.50***	**0.014 ***	**0.44***	**0.004 ***
INR	**-0.66***	**0.001 ***	-0.38	0.077	**-0.42***	**0.006 ***
Urea (mg/dL)	-0.26	0.236	-0.27	0.205	-0.08	0.595
Creatinine (mg/dL)	-0.20	0.371	-0.16	0.457	-0.20	0.196
Na+ (mmol/L)	0.11	0.626	0.13	0.566	0.03	0.843
K+ (mmol/L)	0.08	0.726	0.02	0.912	0.03	0.858
Hemoglobin (g/dL)	0.20	0.356	0.12	0.579	0.02	0.897
Platelets (10^3 cell/µL)	0.11	0.622	0.29	0.187	0.11	0.473
WBCs (10^3 cell/µL)	-0.31	0.147	-0.12	0.573	0.14	0.378
Ascitic PMN count (cell/µL)	**-0.61***	**0.002 ***	**-0.61***	**0.002***	-0.22	0.161
Liver size (cm)	-0.17	0.437	-0.37	0.080	-0.17	0.293
Spleen size (cm)	0.04	0.846	-0.03	0.902	0.05	0.735
MELD score	**-0.51***	**0.012***	-0.29	0.182	**-0.37***	**0.016 ***

r: Pearson correlation coefficient, * S: Significant

The relation between Vit-D and clinicopathological parameters in addition to gender was explored by comparing median levels of Vit-D in categories of these parameters. Vit-D median levels showed a significant decrease in female, MELD score > 15, Child C class, and marked ascites suggesting an association of Vit-D deficiency with the progression of liver disease **(Table 5)**.

Table (5): Relation between Vit-D and clinicopathological parameters in study population

		Vit-D (ng/mL)		**P-value [a]**
Gender	**Male**	47 (53.4%)	21.10 (10.91)	**0.037 ***
	Female	41 (46.6%)	14.90 (10.61)	
MELD score	**≤ 15**	18 (20.5%)	21.30 (9.58)	**0.026 ***
	>15	70 (79.5%)	15.90 (11.51)	
Child Pugh classification	**B**	23 (26.1%)	20.96 (6.80)	**0.008 ***
	C	65 (73.9%)	14.90 (12.21)	
Ascites	**Mild/ moderate**	55 (62.5%)	19.50 (9.90)	**0.016 ***
	Marked	33 (37.5%)	13.14 (13.43)	

Data are presented as median and IQR, *: Significant,

Vit-D concentration was significantly associated with decreased risk (protective effect) of SBP development by 0.16 times for each additional unit of Vit-D. Additionally, Child-Pugh classification revealed significant increase in the estimated risk of having SBP by 3.53 times as Child class C was compared to class B. Similarly, when marked ascites was compared to mild/moderate ascites, a significant increased risk for SBP of 2.49 times was observed. Potential confounders and other clinical variables did not associate significantly with SBP not receiving Vit-D. They included age, female sex, MELD score, liver size, and spleen size. Further multivariable logistic regression analysis was conducted to assess independent association with SBP. P-value ≤0.1 was set as a criterion for variable inclusion in multivariable model. Three variables, Vit-D, Child C class, and marked ascites were included into the model where Vit-D was the only variables to be significantly and independently associated with a decreased risk of SBP of 0.14 times in cirrhotic patients not receiving Vit-D of the study population. Although Child C and marked ascites showed slightly independent increased risk of SBP on multivariable model but their effect was not significant **(Table 6)**.

Table (6): Risk factor independently associated with SBP in cirrhotic cases did not receive Vit-D

Variables	SBP not receiving Vit-D (n= 23)	Non SBP (n= 42)	Univariable analysis	Multivariable analysis
			P value	Adjusted P-value [b]
Gender (female)	12 (52.2)	21 (50.0)	0.867	--
Age (years)	60.09 ± 5.65	61.26 ± 6.18	0.447	---
Vit-D (ng/mL) *	11.56 ± 4.51	17.35 ± 6.41	**<0.001** *	**0.015***
MELD score *	19.65 ± 3.69	17.93 ± 4.42	0.120	---
Liver size (cm)**	12.50 (1.00)	12.50 (1.10)	0.325	---
Spleen size (cm)*	19.54 ± 2.13	19.23 ± 1.95	0.543	---
Child (C vs. B)	20 (87.0%)	25 (59.5%)	**0.022***	0.585
Ascites (Marked vs. mild/moderate)	12 (52.2%)	10 (23.8%)	**0.021***	0.576

Data are presented as median and IQR, mean ± SD, or frequency (%), *: Significant,

The ROC analysis estimated a sensitivity of 78.3%, a specificity of 69.0%, a PPV of 58%, a NPV of 85.3% and an accuracy of 73.7% at the best cutoff value of ≤13.96 (ng/mL). The AUC was 0.76 with a highly significant P-value < 0.001 **(Figure 1)**.

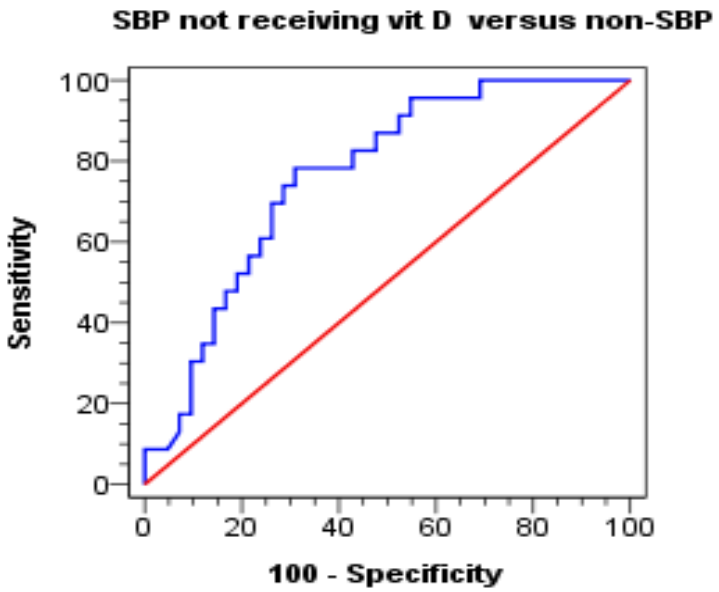

Figure (1): ROC curves of Vit-D for discrimination between SBP not receiving Vit-D subgroup and Non SBP control groups.

DISCUSSION

SBP is an AF infection that develops on its own when there is no intra-abdominal infection source. SBP is present in 1.5–3.5% of cirrhotic patients with ascites and around 10–30% of hospitalised patients [9]. The 1-year survival rate with SBP is just 30–40%, and the death rate is significant, ranging from 20 to 30% [10].

SBP is linked to a higher risk of infection and mortality and is more prevalent in MELD patients. Although the results of AF culture are taken into consideration, the current gold standard for the diagnosis of SBP is predicated on a PMN leukocyte count of 250 cells/mm^3 [11].

Vit-D insufficiency occurs commonly in cirrhotic patients as they have an impaired ability for Vit-D biotransformation in the liver, which is considered a critical organ that can produce 25-hydroxyVit-D3 from Vit-D [12].

It was shown that in individuals with cirrhosis and ascites, a low level of AF Vit-D is linked to a higher risk of AF bacterial infection [13]. Higher death rates among cirrhosis patients as a result. In fact, bacterial infections shorten the patients' lives when they have cirrhosis [7].

The release of stored WBCs from the bone marrow is stimulated by SBP, which sets off the systemic immunological response. Vitamin D also acts as a mediator with anti-inflammatory properties. Researchers discovered a connection between severe inflammation and progressive fibrosis in cirrhotic individuals with low 25(OH)D levels. Thus, vitamin D administration may help to lower inflammation and bring the WBC equilibrium back [14].

Our results revealed that cirrhotic patients in both the SBP and non-SBP control groups were vitamin D deficient, with no statistically significant difference between the two groups. This finding is compatible with **Jamil *et al.*** [15] who demonstrated that only 12% of cirrhotic patients had adequate stocks of vitamin D, whereas many cirrhotic patients had either low or inadequate amounts of the vitamin. This suggests that, regardless of the severity of ascites or SBP infection, Vit-D deficiency is associated to liver cirrhosis.

In the current study, the liver profiles of all participants were evaluated. Both SBP subgroups receiving and not receiving Vit-D had significantly higher levels of ALP than the control group. In agreement with our result **Zeid *et al.*** [16] who showed a significant increase in ALP activity among cirrhotic individuals with SBP compared to individuals with simple ascites**.**

Furthermore, our results revealed that ALT was considerably lower in SBP receiving Vit-D compared to non-SBP control group. This result aligns with the findings of **Komolmit *et al.*** [17]**,** who demonstrated a substantial reduction in ALT levels following Vit-D treatment. The alteration may indicate a reduction in inflammation and hepatic fibrogenesis.

The renal function was also assisted in our biochemical investigations and an elevation of urea level in the SBP not received Vit-D subgroup was observed compared to the SBP received Vit-D subgroup. Our findings are consistent with those of **Keryakos *et al.*** [18]**,** who found that cirrhotic individuals with SBP had higher blood urea levels than those without SBP.

Additionally, our findings showed that WBCs in group I were significantly higher than the non-SBP control group. Moreover, WBCs were significantly elevated in SBP receiving and not receiving Vit-D compared to non-SBP control group. **Zeid *et al.*** [16] results are similar to ours where a significant increase in WBCs count was observed when comparing patients with SBP to those without SBP.

Likewise, a highly significant thrombocytopenia was recorded as our results in group I compared to group II. Also, a highly significant thrombocytopenia was observed in SBP not receiving Vit-D compared to the non-SBP control group. The present results are in harmony with **Zeid *et al.*** [16] because individuals with SBP showed an extremely substantial thrombocytopenia as compared to the group with simple ascites. Besides, the ascitic PMN count was significantly higher in group I and SBP-receiving or not Vit-D subgroups than in the non-SBP control group. **Zhang *et al.*** [19] reported comparable results in their study that was conducted on 119 patients suffering from chronic liver disorders in order to assess the expression of LL-37 in patients with cirrhosis and SBP after vitamin D administration. The findings demonstrated that all of the SBP patients had significantly higher PMN levels in their ascites.

In addition, a significant difference regarding MELD score category between the SBP and non-SBP control groups was noticed in our investigation, where MELD score >15 was highly significant in group I compared to group II. This result comes in the same line with **Gayatri *et al.*** [20] who confirmed that greater MELD scores resulted in a considerably greater frequency of SBP in liver cirrhotic individuals.

In the current study, MELD score > 15 and Child C class showed a highly negative correlation with Vit-D level in SBP not receiving Vit-D subgroup and control group. These results are matched to those assigned by **Afifi *et al.*** [12], which revealed that the 25(OH)D levels were inversely linked with both the MELD score and the Child-Pugh scoring systems, with the child C class having more 25(OH)D -deficient patients than class B or class A, indicating that as the disease progressed, 25(OH)D levels became more deficient.

In addition, the current investigation found a highly significant positive connection between Vit-D and serum albumin in both subgroups and the control. Furthermore, a significantly significant negative connection was discovered between Vit-D level and INR in SBP subgroups that did not receive Vit-D and

the control group. Our findings were consistent with those of **Afifi *et al.* [12],** who revealed a substantial negative association between INR and serum 25(OH)D levels. Additionally, a high positive correlation was detected between albumin level and serum 25(OH)D level.

Another significant discovery of this study is the result of ROC curve, which was used to identify the diagnostic performance of Vit-D levels for discriminating between SBP cases not receiving Vit-D and non-SBP control groups. Vitamin D with a cutoff value of less than or equal to 13.96 ng/ml demonstrated a high sensitivity and specificity (78.3 and 69%, respectively). Similarly, **Abdel Hafez *et al.* [21]** showed that, based on the ROC curve, the vitamin D level 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 the exclusion of SBP.

In our study, based on univariate analysis of different studied parameters, the risk factors of SBP in cases with SBP who did not receive Vit-D in their treatment regimen (GI a) and non-SBP control group (GII), include lower Vit-D, higher Child–Pugh and marked ascites. These variables when used for multivariate linear regression analysis to assess independent association with SBP, only Vit-D was significantly and independently associated with a decreased risk of SBP.

The risk factors of SBP in ascetic cirrhotic patients include lower platelet counts, higher WBC counts, higher TSB, lower prealbumin (PA), lower serum ALB, higher Child-Pugh and MELD scores, lower Vit-D and LL-37 levels. This observation was also reported by **Zeid *et al.* [16]** based on the univariate analysis of different studied parameters. Only reduced Vit-D levels were shown to be substantially correlated with the incidence of SBP when these factors were employed in a multivariate linear regression analysis.

Our study was limited by the small sample size, the influence of varying vitamin D dosages, and the absence of research on various delivery methods in patients with hepatic cirrhosis.

CONCLUSIONS

The elevated risk of SBP was linked to a MELD score > 15. Additionally, it was demonstrated that Staph aureus and Escherichia coli were the most prevalent bacteria among SBP patients. Furthermore, our research demonstrated a correlation between low serum levels of 25(OH)D3 and systemic inflammation linked to liver cirrhosis, as seen by raised white blood cells and a higher PMN count. Furthermore, it has been discovered that the blood 25(OH)D3 level is a separate prognostic factor for SBP patients; vit D level lower than or equal to 13.96 ng/ml cut-off seems to discriminate patients at higher risk for SBP.

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