RESEARCH ARTICLE

Ascitic fluid calprotectin and ratio of calprotectin to total protein in spontaneous bacterial peritonitis

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Abstract

Objective: To evaluate the diagnostic and prognostic role of ascitic fluid calprotectin and its ratio to total protein in spontaneous bacterial peritonitis cases.

Method: The prospective study was conducted at Kafrelsheikh University Hospital, Egypt, from November 2019 to December 2020, and comprised cirrhotic patients of either gender with ascites. Diagnostic abdominal paracentesis was performed for all patients and ascetic fluid calprotectin was measured. Patients were followed for development of spontaneous bacterial peritonitis or mortality. Data was analysed using SPSS 20.

Results: Of the 90 patients, 61(67.7%) were males and 29(32.2%) were females. There were 67(74.4%) patients with spontaneous bacterial peritonitis; 48(71.6%) males and 19(28.3%) females with mean age 60.42±8.3 years. The remaining 23(25.5%) did not have spontaneous bacterial peritonitis; 13(56.5%) males and 10(43.4%) females with mean age 59.7±7.4 years. The patients had significantly higher calprotectin, and calprotectin/total protein ratio ($p<0.05$). Logistic regression identified ascitic fluid calprotectin as a significant predictor of mortality ($p=0.05$). The non-survivors had significantly higher ascitic fluid calprotectin and calprotectin/total protein ratio compared to the survivors ($p<0.05$).

Conclusion: Ascites calprotectin level and its ratio to total protein was found to be accurate diagnostic and predictive biomarkers for spontaneous bacterial peritonitis.

Keywords: Ascitic fluid, Ascites, Gastroenterology, Prognosis, Paracentesis, Peritonitis, Liver cirrhosis, Communicable diseases, Morbidity. **DOI: 10.47391/JPMA.EGY-S4-6**

Introduction

Numerous chronic liver conditions that result in liver cirrhosis are linked with high rates of morbidity and mortality.[1] Patients with cirrhosis and ascites are at risk for spontaneous bacterial peritonitis (SBP) which affects 10-30% of cirrhotic patients who are hospitalised with ascites, with a mortality rate that can exceed 30%.[2]

A polymorphonuclear leukocyte (PMNL) count of more than 250/mm^3 in the ascitic fluid (AF) is required for the diagnosis of SBP. To manage the effects of SBP and lower mortality rates, early and accurate diagnosis is crucial.[3] A zinc- and calcium-binding protein, called calprotectin, is mostly found in neutrophils, and the quantity of it in body fluids is correlated with the number of neutrophils.[4]

Calprotectin in AF is related to PMNL, suggesting that it may be a suitable candidate for SBP diagnosis.[5]

The current study was planned to evaluate the diagnostic and prognostic value of AF calprotectin and calprotectin/total protein ratio in SBP.

Patients and Methods

The prospective study was conducted at Kafrelsheikh University Hospital, Egypt, from November 2019 to December 2020. After approval from the institutional ethics review committee, the sample was raised by a convenient technique, from among adult cirrhotic patients of either gender having ascites who had not received any antibiotic treatment for SBP. Patients associated with malignancy or infections were excluded, and so were those who received antibiotics within the receding 2 weeks. Data was collected after taking written informed consent from all the subjects who were then subjected to detailed history, clinical examination and appropriate imaging studies. Laboratory investigations included complete blood count (CBC) using an automated cell counter (Pentra XL80, Horiba, France), liver functions test (LFT), renal functions and lactate dehydrogenase (LDH) using automated chemistry analyser (Pentra C 400, Horiba, France).

Diagnostic abdominal paracentesis was performed for all cirrhotic patients with ascites when admitted as a routine procedure. AF sample was obtained from every patient at the time of admission. It was converted into 3 samples of

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5cm each, and evaluated for physical examination (colour and turbidity), biochemical properties (total protein, albumin, glucose, LDH), cytological examination (total leukocytic count [TLC] and red blood cells [RBC]) and microbiological examination. One of the samples was stored at -20°C for calprotectin measurement using an enzyme-linked immunosorbent assay (ELISA) (Catalogue No E4010, Bioassay Technology Lab, China).

As per the recommendations of the European Association for the Study of Liver Disease (EASL) guidelines,[6] all patients underwent the prescribed course of treatment. Evaluation of the patients after 48 hours was done to determine whether proper antibiotic treatment was warranted. Patients were followed up with clinical examination, radiological assessment and calprotectin measurement for SBP development to detect recurrence or mortality.

Data was analysed using SPSS 20. Quantitative variables were reported as mean and standard deviations (SD) or median and range, and were compared using t test or Mann-Whitney U test, as appropriate. Categorical variables were expressed as frequencies and percentages, and were compared using chi-square or Fisher's exact test, as appropriate. Spearman's rank correlation coefficient was used to assess strength and direction of a linear relationship between two variables. Receiver operating characteristic (ROC) curve was used to determine the best cut-off value for a certain diagnostic marker for a certain health problem. Binary logistic regression analysis was used to identify predictors of mortality. P<0.05 was considered statistically significant.

Results

Of the 90 patients, 61(67.7%) were males and 29(32.2%) were females. There were 67(74.4%) patients with SBP; 48(71.6%) males and 19(28.3%) females with mean age 60.42±8.3 years. The remaining 23(25.5%) did not have SBP; 13(56.5%) males and 10(43.4%) females with mean age 59.7±7.4 years. TLC and all AF parameters were significantly higher among the patient group (p<0.05). The only exception was LDH (p>0.05). Among the patients, 15(22.4%) had recurrent SBP and 6(8.9%) died, while none of the patients without SBP died (Table 1).

Within the SBP group, patients with recurrent SBP had significantly higher median values for AF calprotectin 1900ng/ml (range: 1740-2870ng/ml) versus 1038ng/ml (range: 630-2530ng/ml) (p<0.001) and calprotectin/total protein ratio 1056.8ng/ml (range: 890.0-1137.7ng/ml) versus 701.52ng/ml (range: 270.0-947.1ng/ml) (p<0.001) when compared with patients without SBP. The non-survivors had significantly higher median values for AF calprotectin 1782.0ng/ml (range: 1740.0-2640.0ng/ml) versus 1160ng/ml (range: 630-143.5ng/ml) (p=0.049) and calprotectin/total protein ratio 972.8ng/ml (range: 713.6-1083.6ng/ml) versus 723.4ng/ml (range: 270.0-1137.8ng/ml) (p=0.015) when compared with survivors.

There was significant direct correlation between AF calprotectin and TLC (r=0.14, p<0.001), serum albumin (r=0.66, p<0.001), AF albumin (r=0.66, p<0.001), AF LDH (r=0.25, p=0.017), AF TLC (r=0.94, p<0.001), AF total protein (r=0.77, p<0.001), PMN count (r=0.96, p<0.001) and AF calprotectin/total protein ratio (r=0.76, p<0.001). Almost similar correlations were found between AF calprotectin/total protein ratio and biochemical parameters (Table 2).

ROC curve identified AF calprotectin level of 639ng/ml as the cut-off with the best diagnostic performance for SBP with 97.0% sensitivity, 95.7% specificity, 98.5% positive predictive value (PPV) and 91.7% negative predictive value (NPV) (Figure). Logistic regression identified ascitic fluid calprotectin as a significant predictor of mortality (Table 3). Patients with higher AF calprotectin levels had significantly shorter survival compared to patients with lower levels (p<0.05).

Table-1: Clinical, laboratory data and outcome parameters (n=90).

	SBP n=67	**No SBP n=23**	***p-value***
Mean Age (years)	60.42 ± 8.3	59.7 ± 7.4	0.71
Male/female n	48/19	13/10	0.18
HCV n (%)	64 (95.5)	20 (87.0)	0.16
HBV n (%)	3 (4.5)	3(13.0)	0.16
Child-Pugh score n (%)			
B	57 (85.1)	19 (82.6)	0.75
C	10 (14.9)	4 (17.4)	
Laboratory data			
Haemoglobin (g/dL) mean ± SD	10.6 ± 2.1	10.5 ± 0.7	0.6
TLC (×103 cells/ml) median (range)	15.8 (13.5–55.5)	5.0 (2.3–9.3)	<0.001
Platelet count (×103/ml) median (range)	112.0 (41.0 – 265.0)	115.0 (43.0 – 330.0)	0.8

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Table-1: *continued from previous page*

	SBP n=67	No SBP n=23	*p-value*
INR mean ± SD	1.47 ± 0.2	1.39 ± 0.2	0.13
ALT (IU/L) median (range)	45.0 (12.0 – 124.0)	33.0 (23.0 – 64.0)	0.28
AST (IU/L) median (range)	59.0 (20.0 – 299.0)	45.0 (38.0 – 106.0)	0.71
Bilirubin (mg/dl) median (range)	1.5 (0.6 – 5.6)	1.3 (0.7 – 3.1)	0.38
Albumin (g/dl) mean ± SD	3.2 ± 0.4	3.2 ± 0.2	0.4
Ascitic Fluid parameters			
Albumin (g/dl) mean ± SD	0.9 ± 0.4	0.6 ± 0.2	<0.001
LDH (IU/L) median (range)	198.0 (98.0–420.0)	180.0 (98.0–330.0)	0.72
TLC (cell/ml) median (range)	725.0 (435.0–700.0)	100.0 (30.0–200.0)	<0.001
Total protein (g/dl) median (range)	1.83 (0.87–4.3)	1.01 (0.6–2.1)	<0.001
PMN (cell/ml) median (range)	625.0 (355.0–1350.0)	65.0 (20.0–150.0)	<0.001
Calprotectin (ng/ml) median (range)	1240.0 (630.0–2870.0)	364.0 (266.0–712.0)	<0.001
Calprotectin/total protein ratio median (range)	756.8 (270-1137.7)	348.2 (173.3–1186.7)	<0.001
6-month outcome n (%)			
Recurred SBP	15 (22.4)	-	NA
Mortality	6 (9.0)	-	0.33

HCV: Hepatitis C virus, HBV: Hepatitis B virus, TLC: Total leukocytic count, INR: International normalised ratio, ALT: Alanine transaminase, AST: Aspartate transaminase, LDH: Lactate dehydrogenase, PMN: Polymorphonuclear leukocytes, SBP: Spontaneous bacterial peritonitis, SD: Standard deviation.

Table-2: Correlation of ascitic fluid (AF) calprotectin and calprotectin-to-total protein ratio with the studied parameters.

	AF calprotectin		AF calprotectin/ total protein ratio	
	r-value	*p-value*	*r-value*	*p-value*
Age	-0.002	0.99	0.1	0.37
Laboratory data				
Haemoglobin	0.13	0.23	0.11	0.24
TLC	0.14	<0.001	0.49	<0.001
Platelet count	0.08	0.48	0.21	0.052
INR	0.002	0.99	-0.18	0.086
ALT	0.19	0.07	0.16	0.12
AST	0.15	0.16	0.13	0.23
Bilirubin	-0.04	0.72	0.019	0.86
Albumin	0.66	<0.001	0.011	0.92
AF parameters				
Albumin	0.66	<0.001	0.17	0.12
LDH	0.25	0.017	0.14	0.2
TLC	0.94	<0.001	0.7	<0.001
Total protein	0.77	<0.001	0.24	0.021
PMN	0.96	<0.001	0.7	<0.001
Calprotectin	-	-	0.76	<0.001
Calprotectin/total protein ratio	0.76	<0.001	-	-

TLC: Total leukocytic count, INR: International normalised ratio, ALT: Alanine transaminase, AST: Aspartate transaminase, LDH: Lactate dehydrogenase, PMN: Polymorphonuclear leukocytes.

Table-3: Predictors of mortality in the studied SBP patients

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p-value*	OR	95% CI	*p-value*
Age	0.95	0.96-1.06	0.36	-	-	-
Gender	1.15	0.21-6.38	0.87	-	-	-
CPS	1.73	0.73-4.11	0.22	-	-	-
AF Calprotectin	1.04	1.0-1.06	0.013	1.04	1.0-1.06	0.013

SBP: Spontaneous bacterial peritonitis, CPS: Child-Pugh score, AF: Ascetic fluid, OR: Odds ratio, CI: Confidence interval.

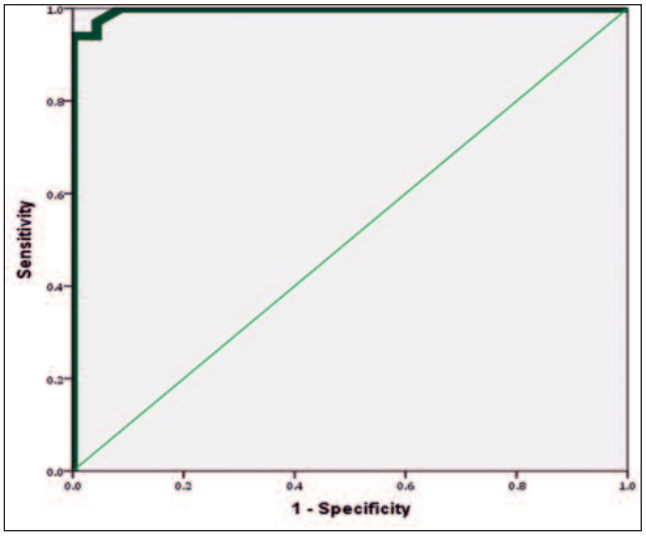

Figure: Receiver operating characteristic (ROC) curve related to the performance of ascitic fluid (AF) calprotectin in the diagnosis of spontaneous bacterial peritonitis (SBP).

Discussion

The current study aimed at establishing the role of AF calprotectin and the ratio of calprotectin to total protein in early SBP detection.

When compared to individuals without SBP, those with SBP showed noticeably greater levels of AF calprotectin. These results are consistent with those demonstrated by previous studies.[7-10] Additionally, it was evident that AF calprotectin had the best performance as a predictor of SBP at a cut-off value of 639ng/ml. Burri et al.[1] reported the best cut-off value of 630ng/ml, while Selim et al.[11] noted a cut-off value of 620ng/ml.

Moreover, the results revealed that AF calprotectin and white blood cell (WBC) count had a significant positive correlation in patients with SBP, which is consistent with Fernandes et al.[10] In the SBP group, a significant positive correlation between AF calprotectin, AF TLC, and PMNLs was detected. Similar findings were reported by Soyfoo et al.[12] and Burri et al.[1] As reported previously[8], a significant positive correlation between AF calprotectin and AF total protein in the SBP group was found in the current study.

In line with Heikal et al.[13] the current study also noted that the ratio of AF calprotectin to total protein was considerably higher in SBP patients than the non-SBP patients. In the current study, patients with higher AF calprotectin levels had significantly shorter survival. Also, logistic regression analysis identified AF calprotectin levels as an important mortality predictor. These findings are in harmony with the conclusions of Nasereslami et al.[14] who highlighted the prognostic value of AF calprotectin in the evaluation of cirrhotic patients.

The current study, to the best of our knowledge, is the first to focus on the prognostic value of calprotectin in SBP.

The current study has its limitations as well. The sample size was not calculated, which could influence the power of the study. Also, AF tuberculosis (TB) infection was not excluded by testing AF adenosine deaminase (ADA). Future studies should include this marker.

Conclusion

Calprotectin and the ratio of calprotectin to total protein in ascetic patients was found to be a useful marker for SBP diagnosis that may additionally provide prognostic value.

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Conflict of Interest: None.

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