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Original article

Urinary Neutrophil Gelatinase Associated Lipocalin (NGAL) as a Non-Invasive Biomarker for Diagnosis of Spontaneous Bacterial Peritonitis

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Abstract:

Background: Spontaneous bacterial peritonitis (SBP) is a severe complication in cirrhotic patients with ascites, necessitating early and accurate diagnosis. Urinary Neutrophil Gelatinase Associated Lipocalin (NGAL) has emerged as a potential non-invasive biomarker for SBP diagnosis. The aim of the study was to assess the diagnostic value of urinary NGAL in cirrhotic patients with ascites for early non-invasive detection of SBP. **Methods:** This cross-sectional study was conducted on 80 cirrhotic patients with ascites, divided into two groups: 40 with SBP and 40 without SBP. Urinary NGAL levels were measured, and their diagnostic performance was evaluated using receiver operating characteristic (ROC) analysis. **Results:** Urinary NGAL levels were significantly higher in the SBP group (2803.7 ± 1048.2 ng/dL) compared to the non-SBP group (1044.5 ± 377 ng/dL) ($p < 0.001$). ROC analysis revealed that NGAL had a sensitivity of 85%, specificity of 97.5%, and an area under the curve (AUC) of 0.96 at a cutoff value of >1696.5 ng/dL for diagnosing SBP. Additionally, a strong positive correlation was found between ascitic neutrophil count (ANC) and urinary NGAL levels ($r = 0.81$, $p < 0.001$). **Conclusion:** Urinary NGAL is a promising non-invasive biomarker for the early diagnosis of SBP in cirrhotic patients, demonstrating high sensitivity and specificity. Its incorporation into routine clinical practice could enhance the timely detection and management of SBP, improving patient outcomes.

Keywords: Spontaneous Bacterial Peritonitis; Cirrhosis; Neutrophil Gelatinase-Associated Lipocalin.

Introduction

Cirrhotic patients with ascites are at high risk of developing spontaneous bacterial peritonitis (SBP). In hospitalized patients, the prevalence of SBP in patients with ascites from end-stage liver disease is in the range of 1-30% [(1)].

The mortality rate exceeds 80% in patients with cirrhosis who develop septic shock secondary to SBP. In addition, each hour of delay in appropriate antimicrobial therapy increases the in-hospital mortality rate by 1.86 times. Therefore, it is critical to improve the survival rate in patients with decompensated liver cirrhosis by early diagnosis, appropriate treatment choice and close monitoring [(2)].

Unfortunately, there is a lack of unique clinical manifestations for SBP. Only one-third of patients have typical abdominal symptoms and some patients show intractable ascites and hepatic encephalopathy as the first presentation [(3)].

According to the standard available guidelines, SBP is diagnosed if the ascitic neutrophil count (ANC) ≥ 250/ mm3 after exclusion of any finding suggestive of secondary peritonitis regardless of ascitic fluid culture [(4)].

Although a ascitic neutrophil count of 250 cells/mm3 is widely used as a sensitive diagnostic marker in clinical practice, there are still some patients with high-risk factors who cannot be diagnosed according to ascitic neutrophil count. Many research groups are attempting to find markers and/or methods for the accurate diagnosis of SBP, including urinary reagent strips [(5)].

There are several known risk factors for SBP in patients with cirrhosis and ascites, including upper gastrointestinal bleeding, low ascetic protein concentration (<1.5 g/dL) and a history of prior episodes of SBP [(6)].

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein with many functions including response to injury, as in the case of acute renal tubular damage, as well as involvement in the innate immune response to infection [(7)].

NGAL was first discovered inside the specific granules of neutrophils, but further studies revealed that it is secreted by various cells of the body including epithelial cells (lungs, liver, bowel, prostate, kidney, etc.), monocytes, macrophages and adipocyte [(8)].

There is a low baseline production of NGAL that maintains its serum concentration to around 20 ng/ mL, but various stimuli that induce epithelial damage can increase this baseline level [(9)].

Increased NGAL levels in plasma and ascitic fluid have showed significant correlation with SBP, but still requires an invasive sampling technique [(10)]. Urinary NGAL could independently predict the development of SBP- as liver cell failure parameters and scoring systems were not able to predict SBP independently- but urinary NGAL could [(11)].

The aim of this study was to investigate the value of Urinary neutrophil gelatinase associated lipocalin (NGAL) for diagnosis of (SBP).

Patients and methods:

This cross-sectional study was conducted on 80 adult patients with cirrhotic ascites with and without SBP spontaneous bacterial peritonitis- who were admitted to Department of Hepatology, Gastroenterology and Infectious diseases of Benha University & Hospital and Department of Hepatology, Gastroenterology and Infectious Diseases; Al Mahalla Hepatology Teaching Hospital, from May 2023 to May 2024. Patients were divided into two groups: group I; included 40 patients with cirrhotic ascites complicated by SBP while group II; included 40 patients with cirrhotic ascites with no evidence of SBP. The study was approved by the Research Ethics Committee, Faculty of Medicine, Benha University and an informed written consent was obtained from the patients (M.S.28.4.2023).

We excluded patients with any grade of renal impairment, patients with ascites due

to causes other than cirrhosis and portal hypertension, patients who received recent antibiotic, patients with evidence of malignancy, patients with secondary peritonitis, patients with hemorrhagic ascites and patients with other infections (skin, chest, urinary tract infections, meningitis, dental infections, gastroenteritis and biliary tract infections).

The diagnosis of liver cirrhosis was based on a combination of physical examination, laboratory and ultrasound findings. The Child-Pugh and the widely used MELD scores- were used to assess the severity of every patient's liver disease.

All patients had laboratory investigations including: complete blood count, liver and kidney function tests, viral markers, and diagnostic abdominal paracentesis

Midstream clean-catch urine samples were collected from all patients on the same day as the paracentesis samples, and the urinary NGAL levels were determined using a commercially available ELISA (Antibody Shop, Gentofte, Denmark).

Data analysis was performed by SPSS software, version 24 (SPSS Inc., PASW statistics for windows version 25. Chicago: SPSS Inc.). Qualitative data were described using number and percentage. Quantitative data were described using median (minimum and maximum) for non-normally distributed data and mean± Standard deviation for normally distributed data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at the (≤0.05) level. Chi-Square , Fisher exact test , Monte Carlo tests were used to compare qualitative data between groups as appropriate. ROC curve Receiver Operating Characteristic Curve was used to detect cutoff value, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Results: (SBP = group I & Non SBP = group II)

The study included 40 patients with cirrhotic ascites and SBP and 40 patients with cirrhotic ascites without SBP. The majority of patients of the studied groups were male (75%) with mean age 63.8 ± 7.6 years and there was no significant deference between the 2 studied groups regarding age, sex, residence, smoking, presence of DM or hypertension. There was statistically significant difference between studied groups regarding fever, abdominal pain and hepatic encephalopathy (P-value < 0.001, <0.001 and 0.002 respectively). High statistically significant difference was observed between the studied groups regarding abdominal tenderness (P-value < 0.001).

There was statistically significant increased percentage of jaundice, pallor, disorientation, palmer erythema and flappy tremor in group I when compared with group II (P-value 0.001, < 0.001, 0.002, 0.043 and 0.002 respectively). There was a statistically significant difference between two studied groups regarding hemoglobin and WBCs (P-value 0.003 and <.001 respectively). Total leucocytic count was statistically higher in group I. There was statistically significant deference between the 2 studied groups as regard total bilirubin, direct bilirubin and INR (P-value < 0.001, 0.021 and 0.009 respectively). Baseline characteristics of the studied groups are shown in Table (1).

There was high statistically significant increased ANC and urinary NGAL in group I when compared with group II (p-value <0.001 and <0.001 respectively, Table (1).

Using ROC curve, it was shown that: ANC can be used to discriminate between SBP group and non-SBP group at a cutoff level of > 240, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001). In addition, urinary NAGL can be used to discriminate between SBP group and non-SBP group at a cutoff level of > 1696.5 ng/dl, with 85% sensitivity, 97.5% specificity, 97.1% PPV and 86.7% NPV (AUC = 0.96 & p-value < 0.001), Table (2) and Figure (1).

As regard urinary NAGL correlations in SBP group there were: a statistically highly significant (p-value < 0.001) positive correlation (r = 0.81) between ANC and U. NAGL. No statistically significant (p-value > 0.05) correlation between U. NAGL and other studied data. While in non-SBP group there were: a statistically highly significant (p-value < 0.001) positive correlation (r = 0.64) between ANC and U. NAGL. No statistically significant (p-value > 0.05) correlation between U. NAGL and other studied data, Table (3).

Table 1: Base line data of the studied groups.

clinical presentation		SBP group		Non SBP group		
Fever		27	67.5%	4	10%	**< 0.001 HS**
Abdominal pain		40	100%	18	45%	**< 0.001 HS**
Hepatic encephalopathy		27	67.5%	13	32.5%	**0.002 S**
Jaundice		31	77.5%	2	20%	0.001 S
Pallor		32	80%	11	27.5%	< 0.001 HS
Palmar erythema		27	67.5%	18	45%	0.043 S
Flappy tremors		27	67.5%	13	32.5%	0.002 S
Palpable spleen		24	60%	23	57.5%	0.820 NS
Abdominal tenderness		39	97.5%	19	47.5%	< 0.001 HS
Hb (g/dl)	Mean±SD	8.8 ± 1.1		9.7 ± 1.4		0.003 S
	Range	7 - 12		7 - 13		
WBCs	Mean±SD	10.9 ± 4		7 ± 4.4		< 0.001 HS
($x10^3$/ul)	Range	4 - 20		2 - 21		
PLTs	Mean±SD	80.4 ± 30.7		71.5 ± 55.5		0.377 NS
($x10^3$/ul)	Range	37 - 144		8 - 284		
T. bilirubin	Mean±SD	16.87 ± 18.04		3.56 ± 1.49		< 0.001 HS
(mg/dl)	Range	1.1 - 7.5		0.2 - 51		
D. bilirubin	Mean±SD	3.24 ± 3.42		1.91 ± 0.95		0.021 S
(mg/dl)	Range	0.4 - 4.4		0.1 - 14.2		
INR	Mean ±SD	1.88 ± 0.37		1.62 ± 0.48		0.009 S
	Range	1.3 - 2.8		0.9 - 3.5		

Table 2: Diagnostic performance of studied markers (ANC & U. NGAL) in discrimination of studied groups

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
ANC (/mm3)	> 240	1.0	100%	100%	100%	100%	< 0.001
U. NAGL (ng/dL)	> 1696.5	0.96	85%	97.5%	97.1%	86.7%	< 0.001

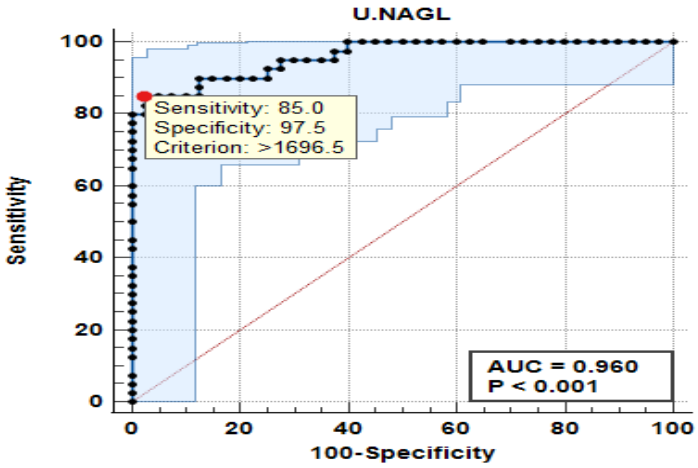

Figure (1): ROC curve between SBP group & non-SBP group as regard u NGAL

Table 3: Correlation between urinary NGAL and laboratory & clinical data in the studied groups

Urinary NAGL (ng/dl)	SBP group		Non SBP group	
	r	p-value	r	p-value
ANC	0.81	< 0.001 **	0.64	< 0.001 **
Age	-0.05	0.757	0.04	0.803
Hb	0.11	0.487	-0.06	0.695
WBCs	-0.23	0.147	0.01	0.94
PLTs	-0.03	0.871	0.15	0.344
ALT	-0.26	0.108	-0.19	0.244
AST	-0.11	0.494	0.10	0.528
ALB	-0.09	0.6	0.05	0.743
Urea	-0.16	0.314	-0.04	0.832
CHILD score	0.021	0.898	0.05	0.767
MILD score	-0.09	0.550	0.03	0.862

Discussion:

The current study showed there was no statistically significant difference between the two study groups regarding age (p=0.140). This is in agreement with Abdel-Razik , et al who reported that there was no significant difference regarding age between SBP and non-SBP patients [(12)].

In this study, SBP was more prevalent in males (75%) compared to females (25%); however, the difference between the two groups was not statistically significant (p = 0.329). This finding is consistent with the study by Abdel Rahman EM., et al. which reported a similar distribution of SBP in male and female patients, with no statistically significant difference between the groups [(13)].

Hepatic encephalopathy (HE) in our study was significantly more common in the SBP group than in the non-SBP group (p = 0.002). However, this contrasts with the findings of Weil D, et al. who found no statistically significant difference between those with SBP and those without regarding HE [(14).] The discrepancy may be attributed to differences in sample size between the studies.

In this study, pallor was significantly more prevalent in the SBP group compared to the non-SBP group (p < 0.001), which aligns with the findings of Lata J, et al. who observed that signs of advanced liver cirrhosis, including pallor and jaundice-

are exacerbated in cirrhotic patients with SBP [15].

Regarding the severity of liver disease, patients in the SBP group had significantly higher rates of Child-Pugh grade C compared to the non-SBP group (80% vs 53%, p = 0.01), similar to the study by Mohamed AA, et al. who found Child-Pugh grade C in 69.9% of SBP patients and 20% of non-SBP patients (16). Additionally, the MELD score was significantly higher in the SBP group (21.09 ± 7.89) compared to the non-SBP group (15.67 ± 5.91) (p = 0.001), which aligns with the findings of Abdel Rahman EM., et al. who also reported higher MELD scores in SBP patients compared to non-SBP patients [13].

Regarding blood and serum parameters in our study, hemoglobin levels were significantly lower in the SBP group than in the non-SBP group (8.8 vs. 9.7, p = 0.003), which aligns with findings of lower hemoglobin in SBP patients versus those without SBP (17). White blood cell counts were significantly higher in the SBP group than the non-SBP group (10.9 vs 7.0, p < 0.001), consistent with results showing elevated white blood cells in SBP patients compared to non-SBP patients, likely due to neutrophilia from bacterial infection [18].

Serum total bilirubin and INR were significantly higher in the SBP group compared to the non-SBP group, with p-values < 0.001 and 0.009, respectively. These findings align with the study by Metwally K, et al. who reported elevated total bilirubin and INR in patients with SBP compared to those without SBP [19].

Regarding ascitic fluid analysis, ascitic fluid total protein (gm/dl) and glucose (mg/dl) levels were significantly lower in the SBP group compared to the non-SBP group (1.47 vs. 1.73, p = 0.0001) and (126.6 vs 134.9, p < 0.001), respectively. These findings are consistent with the study by El-Toukhy N, et al. who reported lower levels of ascitic fluid protein and glucose in SBP patients compared to non-SBP patients. The decreased levels may be attributed to the consumption of glucose and protein by bacteria and white blood cells in the ascitic fluid in spontaneous bacterial peritonitis [20].

In this study, urinary NGAL levels (ng/dL) were significantly higher in the SBP group compared to the non-SBP group (2803.7 vs. 1044.5, p < 0.001). This finding aligns with the study by Fouad TR, et al. who found that urinary NGAL could independently predict the development of SBP (p = 0.001). Elevated NGAL levels reflect infection and inflammation, which are characteristic of SBP [11].

In the present study, regarding ROC analysis for urinary NGAL as a marker for the diagnosis of SBP, the cutoff level of >1696.5 (ng/dL) demonstrated a sensitivity of 85%, specificity of 97.5%, PPV 97.1%, and NPV 86.7% (AUC = 0.96 & p-value < 0.001)- which is in agreement with the study by Fouad TR, et al. who stated that urinary NGAL at a cutoff value of 1225 pg/mL showed a sensitivity of 95% and a specificity of 76%, and is therefore a useful diagnostic tool [11].

Conclusion:

In conclusion, our study demonstrates that urinary neutrophil gelatinase-associated lipocalin (NGAL) is a sensitive and specific biomarker for the diagnosis of SBP in patients with cirrhotic ascites. With high sensitivity and specificity, NGAL levels were significantly higher in patients with SBP compared to those without, indicating its potential as a reliable diagnostic tool. These findings support the incorporation of NGAL testing into routine clinical practice for early (non-invasive) and accurate detection of SBP, thereby achieving the study's objective of identifying an effective diagnostic method for this condition.

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