Neutrophil Gelatinase-Associated Lipocalin (NGAL): An Early Dual Biomarker in Diagnosis of Ascitic Fluid Infection and Acute Kidney Injury in Liver Cirrhosis in a Tertiary Centre, Egypt

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Authors’ contributions
This work was carried out in collaboration among all authors. Author DKN took responsibility for the practical work, analysis and interpretation of data. Author MFE revised the work. Author MMEN designed the work and drafted the work. Author ESAMK was responsible for the proper sample taken. Author GIB supervised and participated in the practical work and analysis of the data. All authors read and approved the final manuscript.

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ABSTRACT

Background: This study aimed to evaluate the ascitic Neutrophil gelatinase-associated lipocalin (NGAL) as a diagnostic biomarker for bacterial translocation or bactDNA translocation in ascites of cirrhotic patients and to evaluate the urinary NGAL for identifying renal dysfunction in patients with liver cirrhosis.

Methods: This study included 44 patients with ascites. Study participants were divided into two groups. Group (1) had 22 patients with neutrocytic ascites and/or positive ascitic fluid (AF) culture while group (2) included 22 patients with culture negative non neutrocytic ascites (CNNNA).
recruited as controls. AF and urine samples were collected from all studied participants. ELISA kit was used for measurement of NGAL levels in AF and urine. DNA extraction and PCR were done.

**Results:** Values of NGAL in ascitic fluid were statistically significantly higher in bactDNA positive group. At cutoff 143.9 ng/ml, ascites NGAL had 86.4% sensitivity, 45.5% specificity, 61.3% positive predictive value (PPV) and 76.9% negative predictive value (NPV). When comparing ascites NGAL between bactDNA positive and negative group, NGAL has a sensitivity of 86.2% and a specificity of 46.7%, PPV of 75.8%, NPV of 63.6%. As for urine NGAL, patients with AKI had statistically significant higher levels of NGAL. Urine NGAL achieved sensitivity of 77.3%, specificity of 50%, PPV of 60.7%, NPV of 68.75%.

**Conclusion:** Early and accurate diagnosis of BT or bactDNA translocation can be aided by utilizing NGAL especially in ruling out infection in those reported to have negative culture results, besides, it can help in early detection of complication of cirrhosis and infection especially AKI helping in the improving the prognosis of cirrhotic patients. It is an advantage that a single marker can detect both infection and kidney injury.

**Keywords:** Neutrophil gelatinase-associated lipocalin; liver cirrhosis; bacterial translocation; renal dysfunction.

**ABBREVIATIONS**

AKI : Acute Kidney Injury
AF : Ascitic Fluid
AUC : Area Under the Curve
AF : Ascitic Fluid
BT : Bacterial Translocation
CNNNA : Culture Negative Non Neutrocytic Ascites
HRS : Hepatorenal Syndrome
NPV : Negative Predictive Value
NGAL : Neutrophil gelatinase-associated lipocalin
PMNL : Polymorph Neutrophil Leucocyte
PPV : Positive Predictive Value
ROC : Receiver Operating Characteristic
SBP : Spontaneous Bacterial Peritonitis

**1. INTRODUCTION**

Major complications of liver cirrhosis include ascites, hepatic encephalopathy, hepatocellular carcinoma, bacterial infections and acute kidney injury (AKI), of which the latter two are common and severe complication in patients with cirrhosis [1].

Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication in cirrhotic patients with ascites [2]. It can be asymptomatic in up to 30% of patients with mortality rate ranging from 40 to 70% which is related to delayed diagnosis and treatment [3]. It also has high recurrence rate and cause rapid deterioration of liver and renal dysfunction [4].

Diagnosis of SBP is based on ascitic fluid (AF) polymorph neutrophil leucocyte (PMNL) count ≥250/mm³ or positive AF culture [5]. However, ascites culture takes several days for identification of the causative pathogen and it is negative in 60% of neutrocytic patients even with bedside inoculation of the AF samples in blood culture bottles [6].

To achieve higher survival rates in patients with decompensated liver cirrhosis, there is an urgent need for an accurate and rapid biomarker that can be used in screening for SBP allowing for early diagnosis and treatment in order to improve the outcome of SBP patients [4].

Bacterial translocation (BT) is a key factor for occurrence of complications in end-stage liver disease. It was proved that cirrhotic patients with positive bacterial DNA have a higher risk of developing SBP and hepatorenal syndrome (HRS)[7].

Serum creatinine is not sensitive in diagnosing renal dysfunction in cirrhotic patients. Therefore, there is an urgent need for diagnostic biomarkers
for early diagnosis of kidney dysfunction in cirrhotic patients to avoid mortality, improve prognosis, and reduce the cost and the length of hospital stay [8].

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2, a member of lipocalin family of extracellular proteins, is a 25-kDa glycoprotein that was originally identified to be linked with neutrophil gelatinase in the specific granules of human PMNLs. It can also be synthesized by tubular cells of the kidney [9]. Also, upon nephrotoxic and/or ischemic injury, NGAL levels are highly increased in urine, peaking earlier than other traditional markers of kidney injury making it one of the most promising markers for diagnosis of kidney injury [10].

Microarray analysis identified NGAL as one of the earliest induced genes and proteins in the kidney that was easily detected in urine soon after kidney injury making it an early, non-invasive marker of AKI [11].

This study attempted to look for an easy-to-be-detected and time-saving biomarker for early diagnosis of SBP and AKI by investigating the utility of ascitic NGAL as an early and rapid diagnostic method for the detection of bacterial or bactDNA translocation in ascites of cirrhotic patients and evaluation of urinary NGAL to identify renal dysfunction in cirrhotic patients.

2. MATERIALS AND METHODS

2.1 Study Participants’ Selection

This is a prospective study that recruited forty-four adult hospitalized patients with cirrhosis and ascites, admitted to Mansoura Specialized Hospital, Egypt, from May 2018 to October 2019. Study participants were divided into two groups. Group (1) had 22 patients with neutrocytic ascites and/or positive AF culture (SBP) while group (2) included 22 patients with culture negative non neutrocytic ascites (CNNNA), recruited as controls. Group (1) were further classified according to AF bacterial culture results into: group (1a) culture positive neutrocytic ascites (No=3) and group (1b) culture negative neutrocytic ascites (No=19). According to ICA-AKI criteria, all patients were also classified into 22 cirrhotic patients with AKI, 22 cirrhotic patients without AKI. This study protocol was approved by the Institutional Review Board of Mansoura University and all subjects provided informed consent.

SBP diagnosis was based on PMNL count in the AF ≥ 250 cells /mm³ with or without positive culture in the absence of clinical, radiological or laboratory data suggesting secondary peritonitis [12]. Exclusion criteria included patients with prior liver transplant, secondary peritonitis, prior kidney transplant, advanced chronic kidney disease, acute or chronic renal replacement therapy at the time of enrollment and UTI. Patients included in this study were subjected to full history, complete clinical examination and investigations. Routine investigations as liver and kidney function, INR and CBC were obtained from patients' medical records.

2.2 Samples Collection

AF and urine samples were collected from all studied participants. AF sample (20 ml) was collected by diagnostic paracentesis under aseptic while the patient was in the supine position and the puncture site in the left or right lower quadrant [13]. The sample was subjected to PMNL counting, bacterial culturing, measuring of NGAL levels by ELISA and detection of bactDNA by PCR.

Ten ml aliquot was inoculated directly into blood culture medium bottle (Egyptian Diagnostic Media, Cairo, Egypt) at bedside [14]. Then, the bottle was incubated aerobically at 37°C up to 7 days. Inspection and subculture on blood, chocolate and MacConkey agar plates (Oxoid) were done daily. Microbiological identification was done by colonial morphology, Gram stained films and standard biochemical reactions. Disc diffusion method for determining antimicrobial susceptibility was performed according to CLSI, 2018 guidelines [15].

The other 10 ml were used for first for PMNL counting using a haemocytometer, then the sample was centrifuged for 3 min at 3000 rpm. The supernatant was transferred to a fresh tube and stored at -20°C for subsequent measurement of NGAL levels using ELISA kit and the precipitate was transferred to a fresh tube, stored at -20°C for subsequent DNA extraction.

As for urine sample, 10 ml were collected. Part was used for culture on CLED agar plates in order to exclude UTI. Plates were incubated at 37°C for 24-48 hours aerobically, and then the number of colonies was estimated. The other part was centrifuged 5000 × g for 10 minutes and
stored at −20°C for measuring of NGAL levels by ELISA

Measurement of NGAL. The ELISA kit (Bioassay technology Laboratory) was used for measurement of NGAL levels in AF and urine.

DNA extraction was done according to the manufacturer's instructions (Quick-DNA™ Miniprep Plus Kit, Zymo Research).

PCR was done according to the protocol of Such et al [16]. The following primers for 16S rRNA gene were used: 5′-AGA GTT TGA TCA TGG CTC AG-3′ and 5′-ACC GCG ACT GCT GCT GGC AC-3′. The amplicon size was 540 bp fragment.

Each reaction mixture was 25 μL which contained 5 μL DNA template, 12.5 μL masterMix, 1 μL of each primer, 5.5 μL nuclease free water. Using PTC-100™ (MJ Research, INC.), the PCR reaction was done according to the following conditions: initial denaturing step at 94°C for 3 min, then 35 PCR cycles: for 30 s at 94°C; for 30 s at 55°C; and for 60 s at 72°C. Then, a final extension step at 72°C for 5 min was done. PCR products were visualized by running it on a 1.5% agarose gel in Tris-borate buffer.

2.3 Statistical Analysis

The SPSS version 21 (SPSS Inc., Chicago, Illinois, USA) program was used. Data were presented as means and SD or medians, as appropriate. χ² test or Fisher's exact test was used for comparison between groups, in addition to independent-samples Student t-test and Mann Whitney test. Receiver operating characteristic (ROC) curve analysis was carried out to determine the sensitivity, specificity, and area under the curve (AUC) for the NGAL. A P value less than 0.05 was considered significant.

3. RESULTS

This prospective study started from May 2018 till October 2019. It was carried out on 44 cirrhotic patients with ascites recruited from Mansoura Specialized Medical Hospital, Mansoura University. Demographic and clinical data of the study groups showed that mean age of group 1 was 59.23 ± 7.59 years whereas the means calculated for group 2 were 57.55 ± 8.28 years. Male predominance was recorded with statistically significant difference between groups. Etiology of liver cirrhosis was attributed to presence of positive HCV antibodies in 86.4% of both groups. As for comorbidities, DM was present in 31.8% and 45.5% of group 1 and group 2 respectively. HCC was diagnosed in 40.9% of group 1 and 22.7% of group 2 while half of the patients had AKI in both groups. No statistically significant differences were detected for demographic and clinical data between group 1 and group 2 except for gender (Table 1).

AF culture was positive in 3 (13.6%) of the cases (group 1a). All isolated organisms were Gram negative bacteria including two E. coli and one Pseudomonas aeruginosa. The isolated bacteria had (100%) sensitivity to amikacin, gentamycin and piperacillin-tazobactam while resistant to 3rd generation cephalosporins. Meanwhile, all SBP patients of group 1b had negative AF culture, but their AF PMNLs was ≥ 250/mm³ (culture negative neutrocytic ascites= CNNA).

As for laboratory parameters, group 1 cases had significantly higher ascites WCCs, ascites PMNL count, positive detection of bactDNA by PCR, ascites NGAL by ELISA whereas no significant difference was detected between both groups regarding other laboratory parameters (Table 2).
BactDNA was positive in 29 patients (65.9%) while negative in 15 patients (34.1%). Thus, the studied groups were classified according to results of bactDNA into; bactDNA positive group included 29 patients and bactDNA negative group compromised 15 cirrhotic patients. Higher values of NGAL in AF yielded statistical significance on comparing bactDNA positive group versus bactDNA negative group [160.4 (144.95-183.4) vs. 149.7 (123.7-164.3) ng/ml respectively, p =0.03].

Also, according to ICA-AKI criteria, all patients were also classified into 22 cirrhotic patients with AKI with age of 58.9 ± 8.2 years of which 18 were males (81.8%) and 22 cirrhotic patients without AKI with mean age of this group was 57.8 years with standard deviation of 7.7 years. This group included 15 males (68.2%). There were no statistically significant differences between AKI and Non-AKI groups as regards: age, gender, presence of HCV antibodies, presence of DM or HCC.

As for laboratory parameters, creatinine and urinary NGAL were statistically significantly higher in AKI group than non- AKI group while other parameters were non-significant (Table 3).

A ROC curve was plotted for determining the cutoff value of ascites NGAL for diagnosis of AF infection. At cutoff 143.9 ng/ml, ascites NGAL had 86.4% sensitivity, 45.5% specificity, 61.3% PPV and 76.9% NPV (Fig. 1a). When comparing ascites NGAL between bactDNA positive and bactDNA negative groups, at a cutoff value of 138 ng/ml, NGAL has a sensitivity of 86.2% and a specificity of 46.7%, PPV of 75.8%, NPV of 63.6% (Fig.1b). Out of 44 AF samples tested, 33 patients (75 %) were diagnosed as having AF infection by one or more of the following three tests (PMN count, culture and PCR) while the rest (25%) were negative for any test. Due to presence of various approaches while there is no true gold standard in diagnosing AF infections, we attempted calculation of validity of NGAL and a ROC curve for NGAL was done (Fig. 1c). At cutoff 126.3 ng/ml, ascites NGAL had 100% sensitivity, 45.5% specificity, 84.6% PPV, 100% NPV in diagnosing AF infection.

A ROC curve was done for determining the cutoff value of urine NGAL for diagnosis of AKI. At cutoff 133 ng/ml, urine NGAL achieved sensitivity of 77.3%, specificity of 50%, PPV of 60.7%, NPV of 68.75%, making it a suitable marker to diagnose AKI in cirrhotic patients (Fig. 2).

Table 2. Laboratory parameters among study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (No=22)</th>
<th>Group 2 (No= 22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites WCC/ mm³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>700 (500-1700)</td>
<td>100 (100-200)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ascites PMNLs/ mm³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>456 (344-1056)</td>
<td>80 (64-130)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TLC / mm³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>6650 (4450-10575)</td>
<td>6100 (3475-9525)</td>
<td>0.48</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>9.8 ± 1.5</td>
<td>9.7 ± 1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Platelets (1000/ mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>99 (72.3-135.5)</td>
<td>97.5 (75.25-194)</td>
<td>0.43</td>
</tr>
<tr>
<td>RBCs (x10⁶/mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>3.2 (3.365)</td>
<td>3.5 (2.9 - 4)</td>
<td>0.48</td>
</tr>
<tr>
<td>Prothrombin time/ sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>16.3 (14.2-19.9)</td>
<td>16.9 (15.5-18.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>INR Median (IQR)</td>
<td>1.3 (1.2-1.6)</td>
<td>1.37 (1.2-1.5)</td>
<td>0.88</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1.2 (0.9-1.7)</td>
<td>1.25 (0.8-1.8)</td>
<td>0.7</td>
</tr>
<tr>
<td>AST (U/L) Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 (25- 82.25)</td>
<td>50.5 (24.75-70)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>ALT U/L Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.5 (14.75-36.25)</td>
<td>24 (17.25-30)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Bilirubin mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2.2 (0.9-5.4)</td>
<td>0.9 (0.7-5.9)</td>
<td>0.14</td>
</tr>
<tr>
<td>Positive BactDNA</td>
<td>18 (81.8%)</td>
<td>11 (50%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Ascites NGAL ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>162.8 (147.9-189.2)</td>
<td>148.1 (127.2-167.7)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Table 3. Laboratory parameters among study groups according to presence of AKI

<table>
<thead>
<tr>
<th>Variable</th>
<th>AKI (No=22)</th>
<th>Non-AKI (No= 22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC / mm² Median (IQR)</td>
<td>6450 (3875- 9625)</td>
<td>5600(4125-9925)</td>
<td>0.6</td>
</tr>
<tr>
<td>Hemoglobin (g/dL) Mean ± SD</td>
<td>9.58 ± 1.3</td>
<td>9.95 ± 1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Platelets (1000/ mm³) Median (IQR)</td>
<td>90 (78-137.75)</td>
<td>114 (64-156)</td>
<td>0.5</td>
</tr>
<tr>
<td>RBCS (x10⁹/mm³) Median (IQR)</td>
<td>3.15 (2.9- 3.6)</td>
<td>3.5 (3-4.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>Prothrombin time /sec Median (IQR)</td>
<td>16.9 (15.3-21.3)</td>
<td>16.2 (14.7-18.3)</td>
<td>0.3</td>
</tr>
<tr>
<td>INR Median (IQR)</td>
<td>1.37 (1.27-1.7)</td>
<td>1.3 (1.2-1.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>Creatinine (mg/dL) Median (IQR)</td>
<td>1.8 (1.5- 2.2)</td>
<td>0.9 (0.8-1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (U/L) Median (IQR)</td>
<td>45 (25-70.75)</td>
<td>56 (28.5- 58.25)</td>
<td>0.5</td>
</tr>
<tr>
<td>ALT (U/L) Median (IQR)</td>
<td>26.5 (14.75- 30)</td>
<td>22 (20-33)</td>
<td>0.5</td>
</tr>
<tr>
<td>Bilirubin (mg/dL) Median (IQR)</td>
<td>1.4 (0.7- 6.5)</td>
<td>0.9 (1.2- 4.2)</td>
<td>0.9</td>
</tr>
<tr>
<td>Albumin (g/dL) Mean ± SD</td>
<td>2.58±0.5</td>
<td>2.68 ±0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Urine NGAL (ng/ml) Median (IQR)</td>
<td>171.5 (132.3- 208.98)</td>
<td>133.05 (115.98-170.93)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Fig. 1a. ROC curve of ascites NGAL between groups 1 and 2

Fig. 1b. ROC curve of ascites NGAL between bactDNA positive and negative groups
4. DISCUSSION

Cirrhotic patients are prone to infections due to many factors such as immune dysfunction, bacterial translocation and altered gut microbiome [17].

The diagnosis of SBP is challenging as there is no single, rapid and accurate test to confirm the diagnosis with detection of the causative agent [18].

This study was to investigate the utility of ascitic NGAL as a diagnostic biomarker of bacterial or bactDNA translocation in ascites of cirrhotic patients and evaluation of urinary NGAL to identify renal dysfunction in cirrhotic patients.

In this study, there were no statistically significant differences between group (1) and group (2) regarding: age, presence of HCV antibodies, presence of DM, HCC or AKI. This was in agreement with [19, 20] studies. In disagreement to our results, Omar and Mohammed [21] reported significantly higher age and presence of DM in non SBP group.

The majority of the study (86.4%) had chronic hepatitis C-related cirrhosis. These results are in agreement with [20,22] who concluded that HCV infection, which is endemic in Egypt, is the commonest cause of cirrhosis in Egypt.

There was a statistically significant difference between the two groups regarding gender as this study had 33 male patients of all 44 patients...
included in the study (75%). These results are in accordance with [2, 23]. In contrast, [21, 24] reported no significant difference between hepatic patients with and those without bacterial infection regarding gender.

SBP is more common in males than females [25]. The male predominance in this study agrees with Mueller et al. [26] who concluded that cirrhosis with portal hypertension is usually a complication of alcoholism or chronic hepatitis C virus infection, and both of these conditions are more prevalent among men. Also, male patients tend to seek medical advice more than female patients with the same clinical complaints [27].

In group 1, the bacterial culture of AF was positive only in 13.6% of the patients. Similar detection rates of 12.5% and 13.12% were reported by some previous studies [27,28]. While higher detection rates of 30%, 34% were reported by [20,29] studies respectively.

In contrast, none of ascitic samples of SBP group were culture positive [30]. This variation in isolation rates is mainly due to the relatively low concentration of bacteria in AF [31] as colony count in spontaneously infected ascites is approximately one microorganism per mL [28]. Additionally, discrepancies of culture results may be due to difference in culture techniques as some studies didn’t use blood culture bottles [32].

In this study, all organisms were Gram negative. Actually, it is stated that Gram-negative organisms, most commonly E. coli and P. aeruginosa, are the predominant cause of bacterial infections in cirrhotic patients as the organisms which cause AF infection are normally found in gastrointestinal tract [33].

On the other hand, an increasing incidence of Gram-positive infections is being reported in cirrhotic patients due to the increased use of invasive procedures and use of antibiotics that alter the intestinal flora leading to translocation of Gram-positive bacteria [34].

As regard laboratory parameters of cirrhotic patients in this study, ascites WCCs and ascites PMNLs count were statistically significantly higher in group 1 than group 2 (P value = <0.001). Whereas other laboratory parameters were comparable between group 1 and group 2 with no significant statistical difference.

In this study, there was a significant increase in AF WCCs, PMNLs in group 1 versus the group 2. These results were in line with [23,35]. In contrast to these results, Jansen [36] stated that, although ascitic WCC is increased in SBP patients, it possesses low specificity as large proportion of sterile ascites patients may have increased WCC. Also, diuretic therapy causes increase of the WCC with concurrent changes in the PMNLs count.

In fact, laboratory investigations are nonspecific and not related to SBP per se (except for AF analysis), but rather, to the underlying liver disease and associated complications. Hence, occurrence of SBP cannot be predicted from clinical and laboratory variables [30,32].

We aimed to study the 16s rRNA PCR testing for more accurate and rapid diagnosis of bacterial translocation. In this study, 81.8% of group 1 were positive for bactDNA while half of group 2 were positive. This is in agreement with [32] who detected bactDNA in 73% of SBP group and 51.3% in CNNNA group.

Similar detection rate of bactDNA in neutocytic ascites (81.8%) was observed in [37] who detected it in 87.5% of neutocytic ascites cases. In patients with CNNNA, bactDNA was detected in 50% which is close to 52% reported by [38] and 41% reported by [39]. Meanwhile, studies reported higher rates of bactDNA positivity in 92% and 100% of CNNNA respectively [40,41]. The presence of bacterial DNA in non-infected cirrhotic patients reflects subclinical bacterial translocation [42]. The difference in detection rates is explained by differences in the patient population and differences in the analytical sensitivity of the different assays due to various sequence of primers and DNA extraction methods [32].

Detection of bactDNA in AF by PCR revealed that 65.9% of all samples were positive for bactDNA. This is closely related to the results reported by [32] study in which 68.5% of samples had positive bactDNA and slightly higher than 41% of [43].

In this study, NGAL measurement in AF samples were statistically significantly higher in group 1 in comparison with group 2. Also, values of NGAL in AF were statistically significantly higher in bactDNA positive group. This is in consistency with Zhang et al. [44] study who reported that
ascites NGAL could identify bactDNA presence in ascites.

At cutoff 143.9 ng/ml, ascites NGAL had 86.4% sensitivity, 45.5% specificity, 61.3% PPV, 76.9% NPV. Our results are similar to Ghoniem et al. [24] study who reported ascites NGAL sensitivity of 90%, specificity of 72%, PPV of 77%, NPV of 88%, in the diagnosis of bacterial infection.

When comparing ascites NGAL between bactDNA positive and negative group, NGAL has a sensitivity of 86.2% and a specificity of 46.7%. Zhang et al. [44] study reported similar sensitivity of NGAL (85.7%) and a slightly higher specificity of 77.8% but the study sample was smaller than ours.

SBP is a life-threatening complication in cirrhotic patients with ascites. Late ormdiagnosed SBP can lead to increased mortality. So, a test with high sensitivity is highly desirable for early diagnosis. This high sensitivity of ascites NGAL would allow recognition of almost all cases of SBP and ruling out SBP in clinically suspected cirrhotic patients at the same time. Meanwhile, this moderate specificity would allow for significant reduction in prescriptions of antibiotics. Also, PPV is acceptable meaning that antibiotic therapy would be over prescribed to only one out of three patients, but that is considered harmless in comparison to missing a truly infected patient. As for NPV, it would confidently help in ruling out SBP.

Because of diversity of approaches and absence of true gold standard in diagnosing AFI, by utilization of 3 tests (PMNLs count, Culture and PCR), ascites NGAL had 100% sensitivity, 45.5% specificity, 84.6% PPV, 100% NPV in diagnosing AF infections. So, positive tests would identify all infected patients leading to early treatment which would reduce the complication and mortality. Negative tests would help rule out SBP with absolute confidence (100%). Also, PPV value of 84.6 % is acceptable given that only two of every 10 patients will be over treated with antibiotic which is harmless in comparison with the fatal consequences of withholding therapy according to a false-negative result.

As for AKI, no statistically significant differences were detected between AKI and non-AKI groups as regards: age, gender, presence of HCV antibodies, presence of DM or HCC as in agreement with some previous studies [45,46]. In contrast, Lasheen et al. [47] reported higher significance of males with older age in AKI group. This is due to different study population.

All laboratory parameters were comparable between AKI and non-AKI group with no significant statistical difference. Whereas creatinine was statistically significantly higher in AKI group as in [45,48]. In contrast, Treeprasertsuk et al. and Abdel-Hady et al. [49,50] found no difference between the two groups but none of the patients in their studies had serum creatinine more than 1.5 mg/dl which was chosen in many studies as a cut off for diagnosing impairment of kidney function [51].

Urine NGAL was elevated in patients with AKI with statistically significant difference (P= 0.04). In harmony with our results, El-Bassat et al. [51] study stated that urine NGAL levels were significantly higher in cirrhotic patients with impaired kidney function compared with those with normal kidney function. This is also in accordance with some studies [45,48]. In contrast, Singal et al. [52] found no significant difference, but they used another criteria for diagnosis of renal impairment and the study was done on post liver transplant patients.

In our study, at cutoff 133 ng/ml, urine NGAL achieved sensitivity of 77.3%, specificity of 50%, PPV of 60.7%, NPV of 68.75% with AUC 0.68. This is helpful for the early detection of AKI in hospitalized cirrhotic patients who are prone to development of AKI, especially those hospitalized with bacterial infection or acute decompensated cirrhosis. In Abdel-Hady et al. [50] study, the AUC was 0.77, urine NGAL achieved a sensitivity and specificity of 71.4% and 62.5%, respectively, PPV was 62.5% and NPV was 71.4%. These results are highly consistent with our results. Jo et al. [45] reported AUC 0.7 for urine NGAL which is close to our result of (urine NGAL AUC =0.68). But they reported lower sensitivity (47%) and higher specificity (92%). On the other hand, Aljumah et al. [53] reported AUC of 0.64, lower sensitivity of 40%, higher specificity of 84%, comparable PPV of 57% and NPV of 72%. Variable cutoffs have been proposed for NGAL. This is mainly due to: difference in the study design, the degree of disease severity, different sample size, variable definitions of renal impairment in cirrhosis used, and different data analysis. Also, multiple factors are involved which have the ability to affect the assay results such as processing of the sample, threshold of measurement, intra- or inter-
individual variations and different sensitivity of ELISA kits.

There is no single ideal biomarker that had not been identified as the only diagnostic biomarker for SBP or AKI. However, NGAL accomplishes high sensitivity which is highly needed in life threatening conditions with high morbidity and mortality. It should be able to support the physicians' decision for initiation of appropriate therapy without posing a threat to the patient.

One of the strengths of our study was that we excluded patients with UTI to ensure that values of urinary NGAL are related only to the kidney condition. Also, up to our knowledge that is the first study to combine NGAL as a marker for SBP and AKI in cirrhotic patients.

The main limitation in this study was that the sample size was small and from a single center. This can be proven by subsequent large-scale studies from multiple centers before these results can be applied to general population.

5. CONCLUSION

Early and accurate diagnosis of BT or bactDNA translocation can be aided by utilizing NGAL especially in ruling out infection in those reported to have negative culture results. Besides, it can help in early detection of complication of cirrhosis and infection especially AKI helping in the improving the prognosis of cirrhotic patients. It is an advantage that a single marker can detect both infection and kidney injury.

CONSENT

An informative consent was received from each study participant.

ETHICAL APPROVAL

The study protocol acquired an approval from our institutional review board (MS.18.12.303).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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