ORIGINAL RESEARCH ARTICLE

Do old urinary biomarkers have a place in the new definition of hepatorenal syndrome in the Egyptian cirrhotic patients? A single-center experience

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Abstract

Background: Hepatorenal syndrome is still a diagnosis of exclusion despite new classification.

Aims: To validate the accuracy of urinary neutrophil gelatinase-associated lipocalin, interleukin-18, and kidney injury molecule-1 in the new diagnostic criteria of hepatorenal syndrome in Egyptian patients with hepatitis C virus-related liver cirrhosis using serum creatinine as a gold standard test for acute kidney injury.

Methods: One-hundred twenty cirrhotic patients with ascites were recruited and divided into two groups depending on the presence or absence of renal impairment, and 40 age- and sex-matched cirrhotic patients without ascites used as controls participated in the study. Urinary biomarkers were measured and compared with conventional biomarkers used to assess kidney function (serum creatinine, estimated glomerular filtration rate).

Results: The mean urinary neutrophil gelatinase-associated lipocalin, interleukin-18, and kidney injury molecule-1 were statistically significantly higher in patients with hepatorenal syndrome and were found to be helpful in the early detection with cutoff values of 125 ng/ml, 34.8 pg/ml, and 3.1 pg/ml, respectively.

Conclusions: Urinary neutrophil gelatinase-associated lipocalin, interleukin-18, and kidney injury molecule-1 levels are higher in patients with cirrhotic ascites complicated by HRS-AKI using the new definition of HRS, but IL-18 has lower sensitivity and specificity for the prediction of HRS-AKI as compared to NGAL and KIM-1.

Keywords: Cirrhosis, HCV, Hepatorenal syndrome, Organ dysfunction, New biomarkers in AKI

Background

Renal dysfunction is a common complication of liver cirrhosis, occurring in approximately 20–50% of all patients with cirrhosis admitted into hospital [1]. Most of these cases are due to episodes of acute kidney injury (AKI). Two-thirds of these AKI episodes are functionally related to hemodynamic changes in cirrhosis, consisting of splanchnic and systemic arterial vasodilatation with resultant reduction in effective arterial blood volume [2], whereas the remainder of AKI episodes are related to renal structural damage, more commonly tubular than glomerular [3]. The most severe form of AKI is type 1 hepatorenal syndrome (HRS-AKI), which, if left untreated, has a median survival of 7–10 days [4]. The use of vasoconstrictors in HRS-AKI has led to improvement in renal function in only one-third of patients for unclear reasons [5]. This may be attributed to the delayed initiation of vasoconstrictor therapy in HRS-AKI, in which many patients may have crossed the "no return"

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threshold. This delay may be attributed to the use of serum creatinine to assess renal function which tends to overestimate the glomerular filtration rate in decompensated cirrhosis [6]. Also, the increase in serum creatinine often lags (48–72 h) behind the onset of injury [7]. Prolonged ischemia secondary to renal vasoconstriction in late-presenting HRS-AKI can also result in structural damage, such as acute tubular necrosis (ATN), thus blurring the line between structural and functional causes of AKI in cirrhosis [6]. Moreover, ATN and HRS-AKI can present with the same features, thus making the differentiation of the different types of AKI and treatment decisions difficult.

To overcome some of these issues, the International Ascites Club and the Acute Dialysis Quality Initiative have proposed the following diagnostic criteria for the HRS-AKI: acute rise in serum creatinine ≥ 0.3 mg/dL within 48 h or ≥ 50% increase from baseline within 7 days irrespective of the final serum creatinine level in patients with acute or chronic liver disease with advanced liver failure and portal hypertension in the absence of any other apparent cause for the AKI [8]. However, HRS-AKI patients diagnosed with these new diagnostic criteria still had a worse survival, possibly reflecting delayed treatment intervention [9].

With the discovery and validation of candidate AKI biomarkers, we aimed in this study to compare the diagnostic value of three out of these biomarkers of tubular injury in the diagnosis of HRS which is up until now a diagnosis of exclusion with challenging differential diagnosis and to correlate these biomarkers with morbidity and mortality using different scoring systems. These biomarkers include neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), and kidney injury molecule-1 (KIM-1). The primary objective of this study was to determine whether these biomarkers may provide early diagnosis of HRS in patients with HCV-related liver cirrhosis.

Methods

Study participants

The study was reviewed and approved by Minia University Faculty of Medicine Research ethics committee and was conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all study participants, and the study protocol was approved by the institute's committee on human research. This study was an observational, prospective, hospital-based, case-controlled study with the participants recruited from the inpatient service of Internal Medicine Department, Minia University Hospital. The study enrolled one-hundred and sixty (160) patients with HCV-related liver cirrhosis. Patients were classified into three groups:

cirrhotic patients without ascites used as controls (40 patients), cirrhotic patients with ascites but without renal impairment (60 patients), and cirrhotic patients with ascites and with renal impairment diagnosed as having HRS-AKI (formerly, HRS type 1) (60 patients). The inclusion criteria were cirrhotic patients who are HCV positive, with liver cirrhosis diagnosed on liver biopsy or through clinical, radiographic, and biochemistry investigations, and HRS-AKI patients were diagnosed using the revised diagnostic criteria of hepatorenal syndrome (HRS) with rapidly progressive decrease in kidney function, defined as absolute increase in serum creatinine ≥ 0.3 mg/dl within 48 h or relative increase in serum creatinine ≥ 50% from baseline within 7 days and/or decrease in urine output ≤ 0.5 ml/kg ≥ 6 h. The exclusion criteria were HRS-NAKI (formerly HRS type 2) defined as stable or slowly progressive decrease in kidney function with eGFR < 60 ml/min/1.73m^2, serious comorbidities (functional class IV heart failure, O_2-dependent COPD, advanced cancer), chronic kidney disease, other causes of liver cirrhosis, shock, documented established parenchymal kidney disease, obstructive uropathy, use of nephrotoxic drugs in the previous 30 days, urinary tract infection, anuria for 12 h, need for renal replacement therapy, or any solid organ transplantation.

Primary predictors and outcomes

The primary predictors were urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and interleukin-18 (IL-18). Within 2 to 3 h of admission, 10 mL urine was collected from study participants and centrifuged at 2000*g* for 20 min, and the supernatant was frozen at −800 °C and retained for analysis. We measured urinary NGAL, KIM-1, by commercially available enzyme-linked immunosorbent assay (ELISA) kit (BOSTER Biological Technology Co. USA), according to the manufacturer's instructions.

Urine NGAL assay was performed according to the following steps: the microtiter plate was coated with monoclonal anti-NGAL antibody. A total of 100 μl of standards or samples are added to the appropriate microtiter plate and incubated for 90 min at 37 °C. Remove the liquid of each well, add 0.1 ml of a biotin-conjugated polyclonal anti-NGAL antibody to each well, and incubate for 1 h at 37 °C. Aspirate each well and wash with wash buffer, repeating the process three times for a total of three washes, followed by the addition of 100 μl of avidin conjugated to horseradish peroxidase (HRP) to each microplate and incubated for 30 min at 37 °C. Color development was achieved using a 90 μl TMB substrate solution is added to each well and incubated for 30 min in the dark at 37 °C. Only those wells that contain

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biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of 100 µl sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. Serial dilutions of recombinant human NGAL were used to establish a standard curve. The detection limit of NGAL with this research assay is 15.63–1000 pg/ml. The intra-assay coefficients of variations (CVs) were 3.9% and 6.2% at 1246 ± 48.6 and 6827 ± 423.3 pg/ml, respectively. Inter-assay CVs were 8.1% and 6.9% at 1522 ± 123.3 and 7133 ± 492.2 pg/ml, respectively.

Urine KIM-1 assay was performed according to the following steps: the microtiter plate was coated with monoclonal anti-KIM-1 antibody. A total of 100 µl of standards and samples are added to the appropriate microtiter plate and incubated for 90 min at 37 °C. Remove the liquid of each well, add 100 µl of a biotin-conjugated polyclonal anti-KIM-1 antibody to each well, and incubate for 1 h at 37 °C. Aspirate each well and wash with wash buffer, repeating the process three times for a total of three washes, followed by the addition of 100 µl of avidin conjugated to HRP to each microplate and incubated for 30 min at 37 °C. Color development was achieved using a 90 µl TMB substrate solution is added to each well and incubated for 30 min in the dark at 37 °C. Only those wells that contain biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of 100 µl sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. Serial dilutions of recombinant human KIM-1 were used to establish a standard curve. The detection limit of KIM-1 with this research assay is 31.2–2000 pg/ml. The intra-assay CVs were 4.6% and 6.1% at 253 ± 11.64 and 1220 ± 74.42 pg/ml, respectively. Inter-assay CVs were 6.4% and 7.9% at 317 ± 20.3 and 1458 ± 115.2 pg/ml, respectively.

Urine IL-18 assay was performed according to the following steps; the microtiter plate was coated with monoclonal anti-IL-18 antibody. A total of 100 µl of standards and samples are added to the appropriate microtiter plate and incubated for 90 min at 37 °C .Remove the liquid of each well, add 100 µl of a biotin-conjugated polyclonal anti-KIM-1 antibody to each well, and incubate for 1 h at 37 °C. Aspirate each well and wash with wash buffer, repeating the process three times for a total of three washes, followed by the addition of 100 µl of avidin conjugated to HRP to each microplate and incubated for 30 min at 37 °C. Color development was achieved using a 90 µl TMB substrate solution is added to each well and incubated for 30 min in the dark at 37 °C. Only those wells that contain biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of 100 µl sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. Serial dilutions of recombinant human IL-18 were used to establish a standard curve. The detection limit of IL-18 with this research assay is 15.6–1000 pg/ml. The intra-assay coefficients of variations (CVs) were 4.3% and 5.5% at 135 ± 5.8 and 762 ± 41.9 pg/ml, respectively. Inter-assay CVs were 5.2% and 5.8% at 141 ± 7.3 and 776 ± 45.1 pg/ml, respectively.

Other measurements

Demographic data, history of any serious comorbidities, diuretic therapy (dose and duration), chronic kidney disease, other causes of liver cirrhosis, shock, documented established parenchymal kidney disease, obstructive uropathy, use of nephrotoxic drugs in the previous 30 days, urinary tract infection, anuria for 12 h, need for renal replacement therapy, solid organ transplantation, history of complications such as hematemesis, melena, hepatic encephalopathy, or spontaneous bacterial peritonitis, and urine output were obtained at enrollment. Complete blood count (CBC) was measured. Another sample on red topped plane tube was used. Serum was separated from whole blood by centrifugation at 3000 rpm for 10 min and was used for measuring albumin, calcium, phosphorus, total cholesterol, triglycerides, urea, creatinine, and uric acid levels. Anti-HCV and HBsAg were detected using ELISA (Sanofi Diagnostic Pasteur, Marne-la-coquette, France), with the use of BIOELISA HCV kit, (BIO kit, SA Barcelona). Complete urine analysis with examination of urine sediment and spot morning protein to creatinine ratio were performed to all patients. Abdominal ultrasonography was done using General Electric Ultrasound, USA, and transducer with a frequency of 3.5 megahertz (MHz).

Estimated GFR (eGFR) was derived using the Modification of Diet in Renal Disease (four variables MDRD equation). The fractional excretion of sodium (FeNa) was calculated by the formula $(SCr \times UNa)/(SNa \times UCr)$. The fractional excretion of urea (FeUrea) was calculated by the formula $(SCr \times U_{Urea})/(S_{Urea} \times UCr)$. We assess the severity of the underlying liver disease by MELD-Na score and Child-Turcotte-Pugh (CPT) score. We assess organ dysfunction, morbidity, and mortality using the sequential organ failure assessment score (SOFA score), APACHE II score, and Glasgow coma scale (GCS).

Statistical analyses

All the analyses were performed with Statistical Package of Social Science (SPSS), version 25. Qualitative data were expressed as frequency (%), and quantitative data

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were summarized as mean $\pm$ standard deviation (SD). Qualitative data were analyzed by chi-square test (χ^2) and Fisher exact test as appropriate, while quantitative results were compared with either Student t-test or analysis of variance (ANOVA) followed by Bonferroni correction. Kruskal-Wallis test for nonparametric quantitative data followed by Mann-Whitney test and Dunn-Bonferroni. Also, simple and multiple logistic regression analyses were used to detect independent predictors. For all analyses, statistical significance was defined as p-values ≤ 0.05.

Results

Demographic characteristics of the study groups

Demographic, clinical, and laboratory characteristics of the three groups of patients are shown in Table 1. The three groups were age- and sex-matched.

Urinary biomarkers in study groups

Urinary NGAL, KIM-1, and IL-18 levels are shown in Table 2. Patients with cirrhotic ascites complicated by HRS-AKI (group III) had statistically significant higher levels of all three biomarkers as compared to the other 2 groups (Figs. 1, 2, and 3).

Predictors of hepatorenal syndrome in cirrhotic patients

To evaluate the independent role of each risk factor of HRS-AKI, a simple logistic regression analysis was performed using all parameters that differed significantly between HRS patients and patients without renal impairment as shown in Table 3. It revealed that older age ($OR = 1.2$, 95 $CI = 1.09–1.31$, $p < 0.001$), prolonged hospital stay ($OR = 1.83$, $CI = 1.37–2.44$, $p < 0.001$), lower SBP ($OR = 0.89$, $CI = 0.82–0.96$, $p = 0.003$), lower MAP ($OR = 0.88$, $CI = 0.79–0.97$, $p = 0.013$), encephalopathy ($OR = 21$, $CI = 5.51–79.99$, $p < 0.001$), higher direct bilirubin ($OR = 1.66$, $CI = 1.15–2.41$, $p = 0.007$), higher INR ($OR = 4.49$, $CI = 1.55–13$, $p = 0.006$), higher CHILD score ($OR = 2.58$, $CI = 1.69–3.95$, $p < 0.001$), higher MELD score ($OR = 1.89$, $CI = 1.37–2.62$, $p < 0.001$), higher MELD-Na score ($OR = 1.87$, $CI = 1.39–2.5$, $p < 0.001$), higher serum urea ($OR = 1.007$, $CI = 1.001–1.012$, $p = 0.013$), higher serum creatinine ($OR = 1.64$, $CI = 1.17–2.32$, $p = 0.005$), higher NGAL ($OR = 1.003$, $CI = 1.001–1.005$, $p < 0.001$), higher KIM-1 ($OR = 1.4$, $CI = 1.12–1.76$, $p = 0.003$), higher IL-18 ($OR = 1.05$, $CI = 1.01–1.09$, $p = 0.010$), higher SOFA score ($OR = 2.83$, $CI = 1.78–4.49$, $p < 0.001$), higher APACHE II score ($OR = 1.82$, $CI = 1.39–2.38$, $p < 0.001$), and lower fractional excretion of sodium ($OR = 6.96$, $CI = 1.6–30.12$, $p = 0.009$).

The diagnostic cutoff values of the different urinary biomarkers

Table 4 and Fig. 4a show the receiver-operating characteristic (ROC) curve analysis of the urinary biomarkers as predictors of HRS-AKI. NGAL with a cutoff value > 125 pg/ml has 100% sensitivity, 100% specificity, PPV 100%, NPV 100%, and 100% accuracy, 95% confidence index was 0.96–1.0, and area under the curve (AUC) was 1 (Fig. 4b). Also, KIM-1 at a cutoff value of > 3.1 pg/ml was found to have 100% sensitivity and 68% specificity and 80% accuracy, confidence index was 0.67–0.86, positive predictive value was 62.5%, negative predictive value was 100%, and area under the curve was 0.780 (Fig. 4c). On the contrary, IL-18 was found to have lower sensitivity (50%), lower specificity (76%), and lower accuracy (66.25%), 95% confidence index was 0.51–0.73, positive predictive value 66, negative predictive value 56, and area under the curve was 0.624 at cutoff value of > 34.8 (Fig. 4d).

Value of urinary biomarkers in the diagnosis of stage 1 HRS-AKI

Table 5 and Fig. 5a show the receiver-operating characteristic (ROC) curve analysis of the urinary biomarkers with the use of new diagnostic criteria of HRS. NGAL at cutoff value > 125 pg/mL has 100% sensitivity, 100% specificity, and 100% accuracy, 95% confidence index was 0.96–1.0, and area under the curve was 1 (Fig. 5b). IL-18 at cutoff value of > 34.8 pg/mL has lower sensitivity (51.72%), lower specificity (76%), and lower accuracy (67.09%), 95% confidence index was 0.52–0.74, positive predictive value 55.6, negative predictive value 73.1, and area under the curve was 0.641 (Fig. 5c). KIM-1 at cutoff value of > 3.1 has 100% sensitivity, 68% specificity, and 79.75% accuracy, confidence index was 0.68–0.86, positive predictive value was 64.4%, negative predictive value was 100%, and area under the curve was 0.783 (Fig. 5d).

Discussion

Acute kidney injury (AKI) is frequent in patients with advanced cirrhosis occurring in up to 20–50% of hospitalized patients. AKI is associated with higher mortality, which increases with increasing severity of AKI [10]. The definition of AKI in cirrhosis has undergone significant changes over the past several years with the use of relative changes in serum creatinine instead of absolute cutoffs due to the limitations of serum creatinine [11]. The incidence of hepatorenal syndrome is increasing and mirrors that of chronic liver disease, but this may be attributed in part to change of diagnostic criteria over time [12].

Table 1 Demographic, clinical, and laboratory characteristics of the study groups

	Group I (*n* = 40)	Group II (*n* = 60)	Group III (*n* = 60)	*P*-value		
[a]**Age (years)**						
Range	(40–60)	(38–63)	(41–62)	0.943		
Mean ± SD	52.2 ± 5.11	49.7 ± 7.31	53.8 ± 5.6			
[c]**Sex**						
Male	14 (70%)	21 (70%)	22 (73.3%)	0.950		
Female	6 (30%)	9 (30%)	8 (26.7%)			
[a]**SBP (mmHg)**				**0.007***		
Range	(100–120)	(100–120)	(100–120)	I vs II	I vs III	II vs III
Mean ± SD	109.75 ± 7.15	110.16 ± 6.08	105.16 ± 6.22	1	**0.047***	**0.010***
[a]**DBP (mmHg)**						
Range	(60–80)	(60–80)	(60–80)	0.145		
Mean ± SD	72 ± 6.15	70.66 ± 5.21	69 ± 4.81			
[a]**MAP (mmHg)**				**0.026***		
Range	(73–93)	(73–93)	(73–93)	I vs II	I vs III	II vs III
Mean ± SD	84.5 ± 5.87	83.66 ± 4.8	80.86 ± 4.62	1	**0.043***	0.102
[b]**Hospital stay (days)**				**< 0.001***		
Median	4	6	8.5	I vs II	I vs III	II vs III
IR	(0–5)	(4–7)	(7–11)	0.092	**< 0.001***	**0.001***
[d]**Hematemesis (*n*, %)**				**0.014***		
No	9 (45%)	3 (10%)	5 (16.7%)	I vs II	I vs III	II vs III
Yes	11 (55%)	27 (90%)	25 (83.3%)	**0.007***	**0.029***	0.706
[d]**Encephalopathy (*n*, %)**				**< 0.001***		
No	17 (85%)	18 (60%)	3 (10%)	I vs II	I vs III	II vs III
Yes	3 (15%)	12 (40%)	27 (90%)	0.069	**< 0.001***	**< 0.001***
[d]**Spontaneous bacterial peritonitis (*n*, %)**				**0.008***		
No	20 (100%)	27 (90%)	21 (70%)	I vs II	I vs III	II vs III
Yes	0 (0%)	3 (10%)	9 (30%)	0.265	0.007*	0.104
[a]**Hb (gm/dl)**				**0.005***		
Range	(6.8–14.8)	(5.4–14.4)	(5.1–16.5)	I vs II	I vs III	II vs III
Mean ± SD	11.63 ± 1.81	10.29 ± 1.97	9.58 ± 2.46	0.097	**0.004***	0.606
[a]**HCT (%)**				**0.004***		
Range	(22.5–42.5)	(16.5–45)	(14.5–47.9)	I vs II	I vs III	II vs III
Mean ± SD	34.18 ± 4.73	30.75 ± 6.37	27.83 ± 7.39	0.206	**0.003***	0.249
[a]**WBCs (cells/cmm^3) (× 10^3)**						
Range	(1–10.5)	(1–16)	(1–21)	0.493		
Mean ± SD	5.98 ± 2.34	6.86 ± 3.73	7.27 ± 4.5			
[a]**Platelets (cells/cmm^3) (× 10^3)**				**0.029***		
Range	(54–423)	(39–270)	(29–210)	I vs II	I vs III	II vs III
Mean ± SD	150.45 ± 88.02	114.4 ± 54.84	102.83 ± 45.42	0.141	**0.028***	1
[a]**Serum albumin (gm/dl)**				**< 0.001***		
Range	(1.9–4.7)	(1.6–3.9)	(1.3–3.4)	I vs II	I vs III	II vs III
Mean ± SD	3.12 ± 0.96	2.54 ± 0.56	2.2 ± 0.57	**0.015***	**< 0.001***	0.165
[a]**Total protein (gm/dl)**				**< 0.001***		
Range	(3.9–8.5)	(3.5–7.7)	(3.8–7.6)	I vs II	I vs III	II vs III
Mean ± SD	6.63 ± 1.08	6.12 ± 1.01	5.57 ± 1.05	0.289	**0.002***	0.131
[b]**Total bilirubin (mg/dl)**				**< 0.001***		
Median	1.4	2.65	3.85	I vs II	I vs III	II vs III
IR	(0.7–2.35)	(1.57–3.3)	(2.75–5.57)	0.148	**< 0.001***	**0.020***
[b]**Direct bilirubin (mg/dl)**				**< 0.001***		
Median	0.9	1.2	2.15	I vs II	I vs III	II vs III
IR	(0.23–1.39)	(0.67–1.6)	(1.2–4.22)	0.376	**< 0.001***	**0.006***
[b]**ALT (IU/L)**						
Median	41.05	28.5	37.75			

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Table 1 (continued)

	Group I (*n* = 40)	Group II (*n* = 60)	Group III (*n* = 60)	P-value		
IR	(28.25–78.42)	(20.75–48.25)	(24.75–58.75)	0.267		
[b]AST (IU/L)						
Median	56	43.5	57.85	0.317		
IR	(38.8–92.65)	(33.25–89.25)	(45–97.5)			
[a]INR				**0.005***		
Range	(1–3.9)	(1.2–2.8)	(1.2–2.9)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	1.48 ± 0.63	1.64 ± 0.37	1.94 ± 0.49	0.779	**0.006***	0.061
[a]CPT score				**< 0.001***		
Range	(5–12)	(6–13)	(9–14)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	6.85 ± 1.75	9.56 ± 1.69	11.7 ± 1.41	**< 0.001***	**< 0.001***	**< 0.001***
[c]CPT class (*n*, %)						
Class A	11 (55%)	1 (3.3%)	0 (0%)	**< 0.001***		
Class B	8 (40%)	10 (33.3%)	2 (6.7%)	**I vs II**	**I vs III**	**II vs III**
Class C	1 (5%)	19 (63.3%)	28 (93.3%)	**< 0.001***	**< 0.001***	**0.010***
[a]MELD score				**< 0.001***		
Range	(6.4–27)	(9.3–23.2)	(19–41)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	11.48 ± 4.63	15.18 ± 3.65	27.52 ± 4.73	**0.012***	**< 0.001***	**< 0.001***
[a]MELD-Na score				**< 0.001***		
Range	(9.4–30)	(13.2–25.7)	(22.1–41)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	14.79 ± 4.62	19.36 ± 3.03	29.85 ± 4.09	**< 0.001***	**< 0.001***	**< 0.001***
[a]Serum Na (mEq/l)				**< 0.001***		
Range	(132–142)	(125–143)	(114–139)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	136.5 ± 2.61	135.43 ± 4.08	131.96 ± 5.05	1	**0.001***	**0.006***
[a]Serum K (mEq/l)						
Range	(3.5–4.9)	(2.9–5.4)	(2.4–5.7)	0.750		
Mean ± SD	4.31 ± 0.32	4.21 ± 0.52	4.17 ± 0.91			
[a]Serum creatinine (mg/dl)				**< 0.001***		
Range	(0.4–0.7)	(0.4–0.7)	(1.5–4)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	0.56 ± 0.07	0.56 ± 0.07	2.31 ± 0.61	1	**< 0.001***	**< 0.001***
[a]Serum urea (mg/dl)				**< 0.001***		
Range	(25–57)	(16–60)	(78–252)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	37.49 ± 8.55	40.74 ± 11.65	143.33 ± 54.73	1	**< 0.001***	**< 0.001***
[a]GFR (ml/min/1.73m^2)				**< 0.001***		
Range	(106–176)	(102–219)	(16–44)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	140.05 ± 18.36	144.56 ± 26.97	26.1 ± 6.62	1	**< 0.001***	**< 0.001***
[b]FE$_{Na}$(%)						
Median	0.41	0.35	0.73	0.054		
IR	(0.21–0.68)	(0.22–0.81)	(0.31–0.92)			
[b]FE$_{Urea}$(%)				**0.001***		
Median	20.25	22.75	10.78	**I vs II**	**I vs III**	**II vs III**
IR	(16.09–25.67)	(10.77–30.5)	(5.67–18.68)	1	**0.018***	**0.001***

Bold values indicate significant results

SBP Systolic blood pressure, *DBP* Diastolic blood pressure, *MAP* Mean arterial pressure, *Hb* Hemoglobin, *Hct* Hematocrit, *WBCs* White blood cells, *ALT* Alanine aminotransferase, *AST* Aspartate transaminase, *INR* International normalized ratio, *CPT* Child-Pugh-Turcotte score, *MELD* Model for end-stage liver disease, *MELD-Na* Model for end-stage liver disease-sodium, *Na* Serum sodium, *K* Serum potassium, *GFR* Glomerular filtration rate, *Fe$_{Na}$* Fractional excretion of sodium, *FE$_{Urea}$* Fractional excretion of urea

*Significant difference at *p*-value < 0.05

[a] One way ANOVA test for parametric quantitative data between the three groups followed by post hoc Bonferroni correction between each two group

[b] Kruskal-Wallis test for nonparametric quantitative data between the three groups followed by Mann-Whitney test between each two groups and Dunn-Bonferroni post hoc in case of significant Kruskal-Wallis

[c] Chi-square test for qualitative data between groups

[d] Fisher exact test for qualitative data between groups

Table 2 Urinary biomarkers levels in the study groups

	Group I (*n* = 40)	Group II (*n* = 60)	Group III (*n* = 60)	*P*-value		
[a]NGAL (pg/ml)				< 0.001*		
Range	(20–66.8)	(24–125)	(139–1317)	I vs II	I vs III	II vs III
Mean ± SD	40.07 ± 14.61	62.6 ± 29.65	732.9 ± 304.78	1	< 0.001*	< 0.001*
[a]IL-18 (pg/ml)				0.011*		
Range	(10.2–49)	(12.6–56.4)	(19.2–98)	I vs II	I vs III	II vs III
Mean ± SD	32.87 ± 9.84	30.32 ± 9.52	41.67 ± 20.66	1	0.018*	0.012*
[b]KIM-1 (pg/ml)				< 0.001*		
Median	1.4	2.35	4.55	I vs II	I vs III	II vs III
IR	(0.3–5.02)	(2.2–4.55)	(3.97–5.72)	1	< 0.001*	0.002*

Bold values indicate significant results

*Significant difference at *p*-value < 0.05

[a] One way ANOVA test for parametric quantitative data between the three groups followed by post hoc Bonferroni correction between each two group

[b] Kruskal-Wallis test for nonparametric quantitative data between the three groups followed by Mann-Whitney test between each two groups and Dunn-Bonferroni post hoc in case of significant Kruskal-Wallis
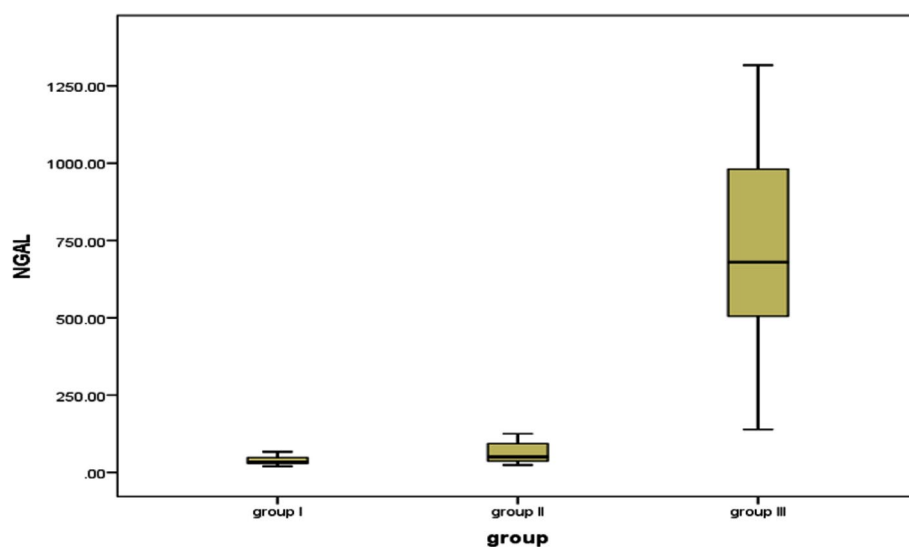
Fig. 1 Box plot of urinary NGAL levels according to study groups

Early identification of the phenotype of AKI is important as management differs according to the etiology which may affect recovery. One of the most challenging issues in patients with advanced cirrhosis is the ability to differentiate HRS from ATN. Serum creatinine (SCr), creatinine clearance, and SCr-derived equations tend to overestimate GFR in cirrhosis which affect their utility in the early diagnosis of AKI. Conventional tools such as urine output, fractional excretion of sodium or urea, or proteinuria have been shown to have significant limitation in patients with advanced cirrhosis as well as poor correlation with biopsy findings in candidates for liver transplantation [6]. Emerging literature continues to validate and refine the use of functional and damage biomarkers for the diagnosis and prognosis of AKI in liver cirrhosis.

NGAL is a small 25 KDa glycoprotein detected in proliferating nuclear antigen-positive proximal tubular cells. The urine usually does not contain NGAL in significant amounts in healthy individuals, and its appearance in urine indicates either a failure to reabsorb filtered NGAL caused by proximal tubular structural damage or induction of renal tubular NGAL production in more distal tubular injury, as opposed to the functional impairment marked by increases in serum urea, creatinine, and cystatin C [13]. We found that cirrhotic patients
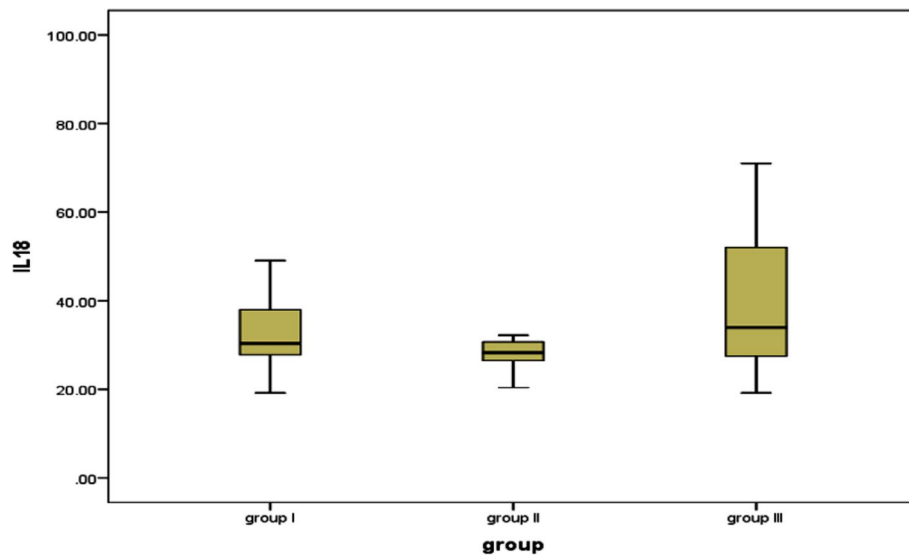
Fig. 2 Box plot of urinary IL-18 levels according to study groups
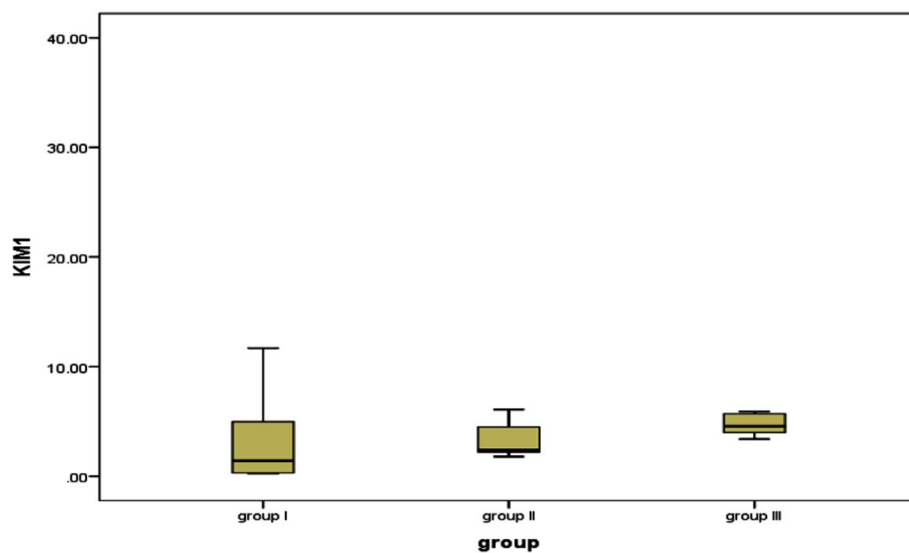

Fig. 3 Box plot of urinary KIM-1 levels according to study groups

with HRS had significantly higher urinary NGAL, compared to cirrhotic patients with normal renal function with or without ascites. Similar results have been reported in patients with cirrhosis and AKI with urinary NGAL higher in patients with impaired kidney function than patients without AKI irrespective of the presence of ascites and are markedly higher in patients with ATN when compared to those with HRS-AKI, prerenal azotemia, or CKD. Urinary NGAL was significantly higher in the subset of patients with HRS-AKI associated

with concomitant infections [14]. We demonstrate that urinary NGAL could predict HRS at a cutoff point of > 125 ng/ml. Similar findings have been reported in other studies which demonstrated that patients with HRS had urinary NGAL levels intermediate between prerenal azotemia and intrinsic AKI but with different cutoff points [15, 16]. However, NGAL has several limitations in general and in patients with liver cirrhosis. In general, urinary NGAL level increases during other conditions such as anemia, hypoxia, malignancies, inflammation

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Table 3 Best fitting simple linear regression predictors of renal impairment in cirrhotic patients

Variable	OR	95% *CI*	*P*-Value
Age (years)	1.2	1.09–1.31	< 0.001*
Length of hospital stay (days)	1.83	1.37–2.44	< 0.001*
Encephalopathy (*n*, %)	21	5.51–79.99	< 0.001*
Spontaneous bacterial peritonitis (*n*, %)	6.71	1.65–27.34	0.008*
Total bilirubin (mg/dl)	1.44	1.11–1.87	0.006*
Direct bilirubin (mg/dl)	1.66	1.15–2.41	0.007*
INR	4.49	1.55–13	0.006*
CPT score	2.58	1.69–3.95	< 0.001*
CPT class (*n*, %)			
B	1		
C	12.6	2.62–60.54	< 0.001*
MELD score	1.89	1.37–2.62	< 0.001*
MELD NA score	1.87	1.39–2.5	< 0.001*
Urea (mg/dl)	1.007	1.001–1.012	0.013*
Creatinine (mg/dl)	1.64	1.17–2.32	0.005*
NGAL (pg/ml)	1.003	1.001–1.005	0.001*
KIM (pg/ml)	1.4	1.12–1.76	0.003*
IL 18 (pg/ml)	1.05	1.01–1.09	0.010*
SOFA score	2.83	1.78–4.49	< 0.001*
APACHE II score	1.82	1.39–2.38	< 0.001*
FE_{Na}(%)	6.96	1.61–30.12	0.009*

*Significant level at *P* value < 0.05

Bold values indicate significant results

INR International normalized ratio, *CPT* Child-Turcotte-Pugh score, *MELD* Model for end-stage liver disease, *MELD-Na* Model for end-stage liver disease-sodium, FE_{Na} Fractional excretion of sodium, *SOFA score* Sequential Organ Failure Assessment, *APACHE II score* Acute Physiology and Chronic Health Evaluation II score

Table 4 ROC curve analysis of urinary biomarkers for prediction of HRS

Variable	AUC	*P*-value	95% *CI*
NGAL (pg/ml)	1	< 0.001*	0.96–1.0
IL-18 (pg/ml)	0.624	0.067	0.51–0.73
KIM-1 (pg/ml)	0.780	< 0.001*	0.67–0.86

*Significant level at *P* value < 0.05

Bold values indicate significant results

NGAL Neutrophil gelatinase lipocalin, *IL-18* Interleukin-18, *KIM-1* Kidney injury molecule-1, *AUC* Area under the curve, *95% CI* 95% confidence index

whether chronic and acute, and CKD [17]. In patients with cirrhosis, NGAL should be interpreted with caution for several reasons. Firstly, NGAL liver synthesis increases during sepsis [18]. Secondly, there is a significant overlap between ATN and HRS groups, which is more pronounced with plasma NGAL levels [14]. Finally, the diagnosis of AKI is based on clinical criteria, as the

gold standard of biopsy for AKI diagnosis cannot be used in almost all patients with cirrhosis [19].

Interleukin 18 (IL-18) is a proinflammatory cytokine overexpressed in proximal tubule and released in urine following AKI [20, 21]. We found that patients with HRS-AKI had significantly higher urinary IL-18, compared to cirrhotic patients with normal renal function with or without ascites. Based on the receiver-operating characteristic analysis, at a cutoff point of > 34.8 pg/ml, IL-18 is helpful in diagnosing HRS. In another study of IL-18 in patients with liver cirrhosis but not limited to patients with HRS, they have found significantly higher urinary IL-18 levels in patients with a clinical diagnosis of ATN compared to non-ATN AKI [22].

Kidney injury molecule-1 (KIM-1) is a transmembrane protein, which is upregulated by the renal epithelial cells after ischemic injury [23] and is a marker of proximal tubular injury [24]. We found that patients with HRS had significantly higher urinary KIM-1, compared to cirrhotic patients with normal renal function with or without ascites. Based on receiver-operating characteristic analysis, KIM-1 could predict HRS-AKI at a cutoff point of > 3.1 pg/ml. Previous studies have found that urinary KIM-1 to be increased in patients with ATN but not in those with prerenal AKI, urinary tract infections, or CKD [25]. The role of KIM-1 as a diagnostic biomarker in patients with cirrhosis and AKI has been studied in only few studies which demonstrate increase in urinary KIM-1 levels in ATN as opposed to other causes of AKI [16, 22].

The finding of high levels of the three urinary biomarkers in HRS-AKI observed in our study is in agreement with the emerging data of the presence of parenchymal renal disease before hemodynamic derangements that characterize HRS pathogenesis [26]. This is supported by the finding of reflux of proximal convoluted epithelium into Bowman's space in 71.4% of cases of HRS [27]. Also, in another study which compares the level of urinary NGAL to the treatment response in patients with cirrhosis and HRS-AKI, they found that complete responders had the lower value of urinary NGAL when compared to partial responders and nonresponders [28]. Finally, up to 21% in one study and 42% in another study of patients with HRS have persistent renal dysfunction after orthotopic liver transplantation, which may reflect the underlying structural abnormality in HRS [29, 30].

We found in this study that the predictors for HRS occurrence in cirrhosis are encephalopathy (*OR* = 21), higher INR, higher CPT score, higher serum creatinine, higher NGAL, higher KIM-1, higher IL-18, higher SOFA score, higher APACHE2 score, and lower Fe_{Na}. These findings may help to invent a risk score for HRS in cirrhotic patients using some or all these variables.
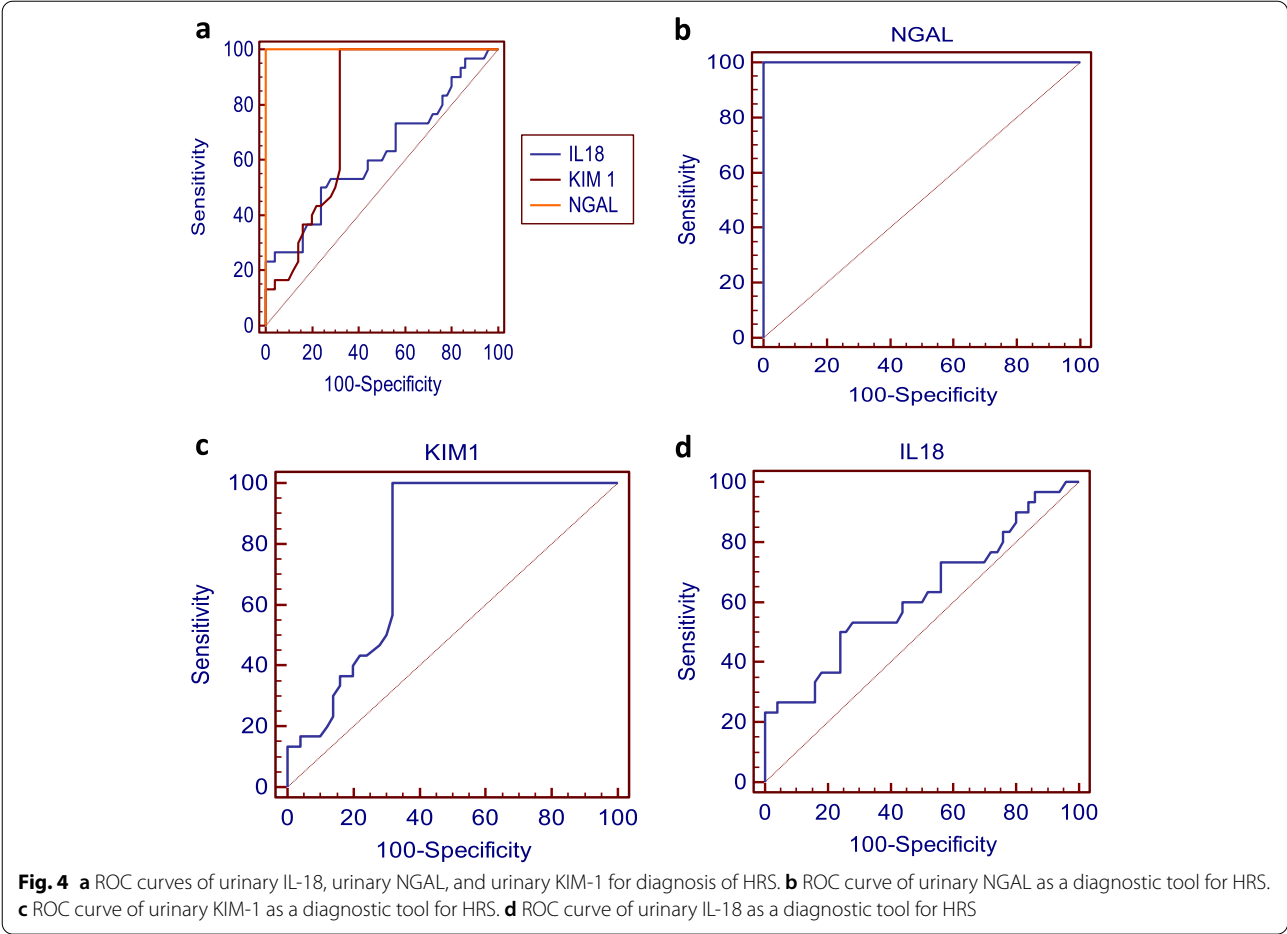
Fig. 4 **a** ROC curves of urinary IL-18, urinary NGAL, and urinary KIM-1 for diagnosis of HRS. **b** ROC curve of urinary NGAL as a diagnostic tool for HRS. **c** ROC curve of urinary KIM-1 as a diagnostic tool for HRS. **d** ROC curve of urinary IL-18 as a diagnostic tool for HRS

Table 5 ROC curve analysis and optimal cutoff values of urinary biomarkers for stage 1 HRS-AKI in cirrhotic patients with ascites

Variable	Optimal cutoff	Sensitivity	Specificity	PPV	NPV	AUC	*P-value*	95% CI	Accuracy
NGAL (pg/ml)	> 125	100	100	100	100	1	**< 0.001***	0.96–1.0	100
IL18 (pg/ml)	> 34.8	51.72	76	55.6	73.1	0.641	**0.037***	0.52–0.74	67.09
KIM 1 (pg/ml)	> 3.1	100	68	64.4	100	0.783	**< 0.001***	0.68–0.86	79.75

*Significant level at *P* value < 0.05

Bold values indicate significant results

NGAL Neutrophil gelatinase lipocalin, *IL-18* Interleukin-18, *KIM-1* Kidney injury molecule-1, *AUC* Area under the curve, *95% CI* 95% confidence index, *PPV* Positive predictive value, *NPV* Negative predictive value

We observed that HRS-AKI patients had higher incidence of SBP than patients with normal renal function. Both HRS and SBP have similar chronologic and pathologic connection, and HRS is precipitated by SBP in 28% of cases despite treatment and resolution of infection. This is due to the release of proinflammatory cytokines and endotoxins that leads to systemic vasodilation secondary to increased production of nitric oxide and other vasodilator substances and decreased cardiac output secondary to sepsis-induced cardiomyopathy [31].

We found that serum sodium is lower in patients with HRS as compared to patients with normal renal function. This finding is in accordance with the study conducted by Angeli and his colleagues who observe that the existence of serum sodium < 135 mmol/L was associated with severe ascites, as indicated by high prevalence of refractory ascites, large fluid accumulation rate, frequent use of large-volume paracentesis, and impaired renal function, compared with normal serum sodium levels. Moreover, low serum sodium levels were also associated with greater
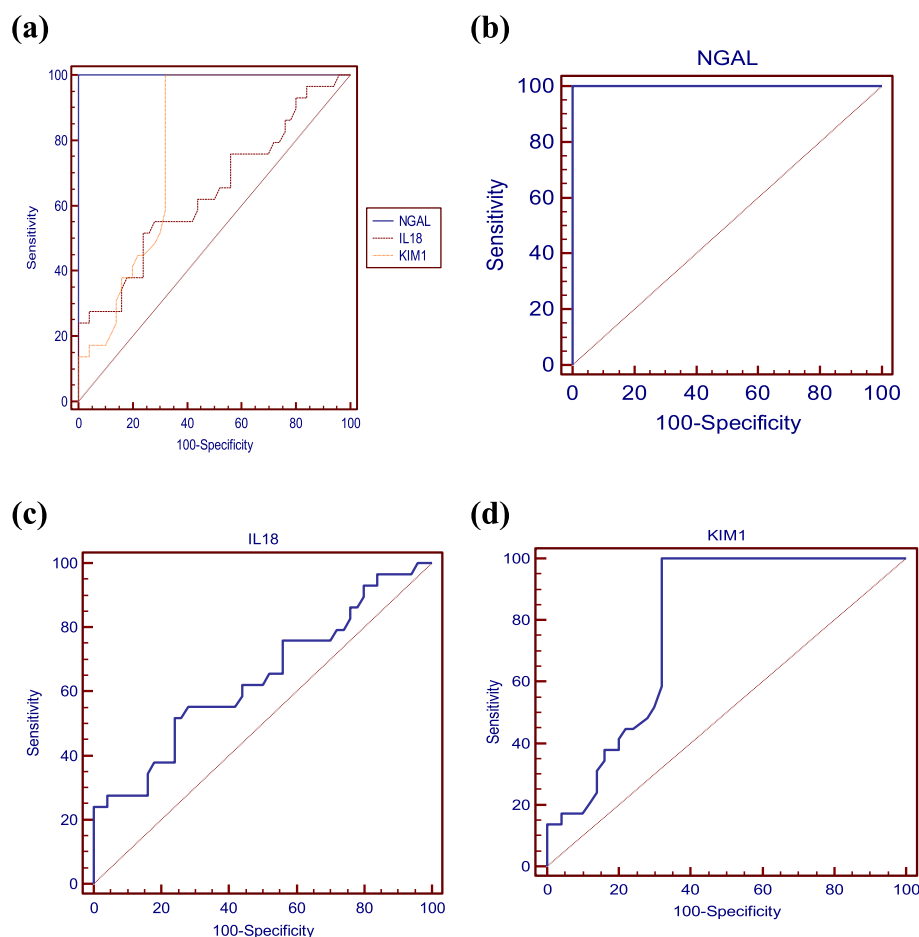
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(a)

(b)

(c)

(d)

Fig. 5 **a** ROC curve to evaluate the capability of urinary IL-18, NGAL, and KIM-1 for diagnosis stage 1 AKI. **b** ROC curve to evaluate the capability of urine NGAL for diagnosis of stage 1 AKI. **c** ROC curve to evaluate the capability of urinary IL-18 for diagnosis of stage 1 AKI. **d** ROC curve to evaluate the capability of urinary KIM-1 for diagnosis of stage 1 AKI

frequency of hepatic encephalopathy, spontaneous bacterial peritonitis, and hepatorenal syndrome, but not gastrointestinal bleeding [32]. Hyponatremia was used to support the diagnosis of HRS in the old diagnostic criteria. Hyponatremia in patients with cirrhosis and ascites is an important predictor of mortality associated with lower survival [33] and also an independent predictive factor for the development of hepatic encephalopathy [34].

The current study is limited by the small number of patients. Another important limitation is the lack of serial measurements of the studied biomarkers to assess their impact on response to therapy and outcome. We also correlate the urine biomarkers with serum creatinine which overestimates kidney function in cirrhosis, but there is no consensus statement on accurate measurement of GFR in cirrhotic patients. Moreover, we did not use kidney biopsy to exclude other diagnoses than HRS which up until now is a diagnosis of exclusion.

Conclusion

Urinary neutrophil gelatinase-associated lipocalin, interleukin-18, and kidney injury molecule-1 levels are higher in patients with cirrhotic ascites complicated by HRS-AKI using the new definition of HRS, but IL-18 has lower sensitivity and specificity for the prediction of HRS-AKI as compared to NGAL and KIM-1.

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Availability of data and materials
Available

Declarations

Ethics approval and consent to participate
The research was conducted in accordance with the ethical standards of Minia University committee on human experimentation and with Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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