



Hepatorenal Syndrome: A Way for Early and Accurate Diagnosis

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Abstract

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BACKGROUND: Hepatorenal syndrome (HRS) is a devastating consequence of liver cirrhosis that is clinically categorized into two subtypes. Acute malfunction of renal role, as measured by an elevation in blood creatinine, significantly underestimates the loss in renal function in cirrhotic individuals; more accurate biomarkers are desperately required in cirrhotic patients.

AIM: The present study set out to uncover new biomarkers for the early prediction of AKI in cirrhotic cases. A comprehensive panel of biomarkers was investigated to get a clear insight into the pathogenesis of HRS.

PATIENTS AND METHODS: Participants in this study were 70 individuals from the hepatogastroenterology unit of the Theodor Bilharz Research Institute (TBRI). Detailed medical data and a physical examination were recorded. Three groups of patients have been identified; Group 1: 30 cases with compensated liver cirrhosis and normal kidney functions. Group 2: 20 cases with decompensated liver cirrhosis and normal kidney functions. Group 3: 20 cases with decompensated liver cirrhosis proved hepatorenal syndrome Type 2 h. The following biomarkers were detected in serum using the sandwich-ELISA method: Human L-arginine ELISA kit, human neutrophil gelatinase related lipocalin (NGAL), human noradrenaline (NA), human asymmetrical dimethylarginine (ADMA), human symmetric dimethylarginine (SDMA), human nitric oxide (NO), and human renin.

RESULTS: There was a highly significant difference between Groups 1 and 2 in NITRIC and ADMA. Significant differences between Groups 2 and 3 in NGAL, noradrenalin, and SDMA were observed. There was a significant difference (Group 2 vs. Group 3) in renin, NITRIC, ADMA, and L-ARGININE. There was highly significant differentiation (Group 2 vs. Group 3) in NGAL, noradrenalin, and SDMA. There was highly significant variation as per odd ratio and confidence interval between (Group 3 vs. Group 2) in NGAL.

CONCLUSION: Assessment of renal biomarkers in individuals with decompensated cirrhosis gives critical information on the etiology of AKI. Further, it may aid in the diagnosis and prognosis of AKI. Renin, NITRIC, ADMA, and L-ARGININE could be used as biomarkers to indicate HRS in individuals with advanced cirrhosis.

Introduction

HRS is a serious side effect of liver cirrhosis related to a raised risk of death and disease, glomerular filtration rate decreases because of anomalies in the renal circulatory system that exceed compensatory measures. As cirrhosis, alcoholic hepatitis, or metastatic tumors are the most common causes of portal hypertension, people with this condition might suffer from fulminant hepatic failure for any reason [1], [2]. A liver transplant or the utilization of vasoconstrictor medicines can enhance renal function by maintaining enough renal blood flow. The hepatorenal syndrome was categorized into two clinical subtypes: Type 1 is a rapid decline in renal function manifested by a doubling of initial serum creatinine to at least 2.5 mg/dL or a 50% decrease in initial 24-h creatinine clearance to < 20 mL/min at < 2 weeks and Type 2 is as a progressive decline in renal function that did not meet the Type 1 criteria [3].

HRS Type 1 was dubbed HRS-AKI by the international ascites Club (IAC). HRS-NAKI (non-AKI) is a term used to describe functional kidney injury in persons with cirrhosis but does not match HRS-AKI guidelines [4].

Type 1 and Type 2 HRS are separate manifestations of the same underlying illness [5]. Although the disease's pathogenesis is complex, difficult, and incompletely understood, its hallmark features include splanchnic vasodilation, which results in effective central hypovolemia, which results in cardiovascular abnormalities with vasoconstriction and renal hypoperfusion. Thus, HRS is a functional illness defined by the renal arteries' significant vasoconstriction due to hyperactivation of various vasoconstrictor systems to compensate for the systemic vasodilation generated by the initial splanchnic vasodilation. HRS is generally associated with advanced circulatory failure and is followed by hyponatremia and ascites [1]. HRS can advance in the presence of infection, most commonly after spontaneous bacterial peritonitis

(SBP), resulting from the sepsis-induced exacerbation of circulatory dysfunction [6]. HRS can also develop with large-volume paracentesis (LVP) [7]. Bile cast (or choleric) nephropathy has been found in cirrhosis cases and increased serum bilirubin levels over a long time [8].

Acute renal impairment, as defined by an elevated in serum creatinine, underestimates the decrease in renal function observed in cirrhotic individuals because of impaired hepatic generation of creatine (creatinine precursor), inaccurate measurement of creatinine by calorimetric techniques in elevated serum bilirubin, and decreased muscle mass, and creatinine tubular secretion. Consequently, more precise indicators are required in persons with cirrhosis [9]. Tubular proteins upregulated in response to injury (neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid-binding protein, and kidney injury molecule-1), tubular proteins secreted throughout cellular damage (N-acetyl- β -D-glucosaminidase, α -glutathione S-transferase), inflammation markers (interleukin-18), and plasma proteins with diminished tubular reabsorption (retinol-binding protein, α -1-microglobulin, and β -2-microglobulin) [10]. Individuals with cirrhosis have the most investigated biomarker, NGAL, differentiating ATN from AKI-HRS with the maximum diagnostic accuracy. In addition, urine NGAL levels are a powerful predictor of sudden death in the short-term. Nonetheless, the findings are encouraging and need additional investigation [11].

Aim of the work

This study targeted identifying novel biomarkers for the diagnosis of HRS in cirrhotic individuals. A comprehensive panel of biomarkers was investigated to get a clear insight into the pathogenesis of HRS.

Patients and Methods

Study population and demographic information

This work was conducted at Hepato-Gastroenterology Department, Theodor Bilharz Research Institute, Egypt, and all subjects signed a written informed permission form under the 1975 declaration of Helsinki's ethical standards. This work was approved by the ethics committee at TBRI.

Between January 2019 and March 2022, this trial enrolled a total of 70 individuals. All individuals involved in this study underwent a full thorough history and clinical examination. All individuals were hospitalized in the clinic for gastroenterology in TBRI. All subjects had liver cirrhosis. HRS was diagnosed in

20 patients with cirrhosis, while 50 patients did not have HRS. The hepatorenal syndrome was diagnosed by the latest criteria suggested by the International Ascites Club. The criteria included: Cirrhosis with ascites, low glomerular filtration, serum creatinine over 133 μ mol/L (over 1.5 mg/dL), proteinuria < 500 mg/day, absence of shock, absence of bacterial infection, loss of fluid, impaired kidney function after cessation of diuretic treatment (serum creatinine value which remains at the level of \geq 133 μ mol/L for at least 48 h, after administration of albumin dose 1–100 g/kg a day), treatment without nephrotoxic drugs, and absence of parenchymal renal disease (patient does not have proteinuria > 500 mg/day, no microhematuria >50 erythrocytes, and no pathological findings of ultrasound examination of the kidneys).

Individuals were categorized into three groups: Group 1: 30 cases with compensated liver cirrhosis and normal kidney functions. Group 2: 20 cases with decompensated liver cirrhosis and normal kidney functions. Group 3: 20 cases with Type 2 h.

Sample collection and storage

About two vacutainer tubes were used to withdraw venous blood samples through a single aseptic venipuncture from patients. 2 ml were collected into EDTA vacutainer for CBC. 2 ml were collected into a serum separator vacutainer tube for blood chemistry and special investigations. Blood was permitted to clot by keeping it undisturbed for 10–20 min at room temperature. Centrifugation at 2000–3000 rpm for 20 min was used to dislodge the clot. The supernatant was carefully collected and stored at -20°C until used for viral marker assays.

Laboratory investigations involving

Qantas, an automated cell counter made by Boule Diagnostics in Sweden, was used to get a complete blood picture. Blood chemistry such as (serum albumin, aspartate aminotransferase (AST), urea, alanine aminotransferase (ALT), and creatinine) was conducted on Olympus AU480 Chemistry Analyzer, Beckman Coulter, USA. The viral markers such as hepatitis B surface antigen (HBsAg), and HCV immunoglobulin G (HCV IgG) were carried out on ADVIA Centaur CP Immunoassay System, Siemens, Germany.

Special investigations

The biomarkers of interest were detected in serum using the sandwich-ELISA method. Human L-arginine ELISA kit (Cat No In-Hu4073), Human Neutrophil Gelatinase Associated Lipocalin (NGAL) ELISA kit (Cat No In-Hu3931), Human Noradrenalin (NA)

ELISA kit (Cat No In-Hu3281) all from Bioneovan Co., Ltd, China (www.bioneovan.com). Human asymmetrical dimethylarginine (ADMA) ELISA kit (Cat No BYEK2502), Human symmetric dimethylarginine (SDMA) ELISA kit (Cat No BYEK 2917), Human Endothelin 1(ET1) ELISA kit (Cat No BYEK 1310), Human Nitric oxide (NO) ELISA kit (Cat No BYEK 2618), and Human Renin ELISA kit (Cat No BYEK 2619) all from Biospes, Co., Ltd, China (www.biospes.com). The manufacturer's instructions and procedures were strictly followed. Standard and test samples' optical densities (OD) were assessed using a microplate reader (Tecan, Sunrise™Japan). The validity of the assays was confirmed according to the OD criteria of standards. Data were processed using Magellan™ software to generate a log/log standard curve from which the concentrations of tested samples were calculated by multiplying the dilution factor.

Imaging study (renal duplex)

Ultrasonographic examination on renal vessels will be performed using a 3.5–5 MHz high-resolution probe to detect renal resistive index.

Statistical analysis

Data were assessed by IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA) and MedCalc® version 19.1. The mean, standard deviation (SD), lowest, and maximum values for quantitative variables were calculated. Qualitative variables were represented by the percentages (percent) and numbers (N).

Comparisons among quantitative variables were performed after normality data were evaluated by the Kolmogorov–Smirnov normality test. In contrast, the Student's t-test (parametric testing) was utilized for the means of two groups. When the test findings demonstrated that the data were not normal, the Mann–Whitney test was utilized to contrast them (non-parametric tests).

The Chi-square test (χ^2) was used to contrast qualitative variables. Fisher's exact test was employed rather than the Chi-square test when one or more cells were predicted to be ≤ 5 . All findings were represented in p: $p < 0.05$ was regarded as significant, $p < 0.001$ was regarded as highly significant, and $p > 0.05$ was deemed non-significant.

Results

There was significant variation among (Group 2 vs. Group 1) ($p < 0.05$) in hemoglobin, neutrophil, lymphocyte, monocyte, bilirubin, renin, NITRIC, and noradrenalin, and there was a highly significant difference between (Group 2 vs. Group 1) ($p < 0.01$) in RBCs, hematocrit, platelet, AST, ALT, albumin, creat, urea, INR, and ADMA. There was no significant difference between (Group 2 vs. Group 1) in L-ARGININE, SDMA, NGAL, eosinophil, TLC, age, and sex (Table 1 and 2).

Our findings showed a significant difference between (Group 3 vs. Group 1) ($p < 0.05$) in neutrophil, bilirubin, L-ARGININE, SDMA, and there was a highly significant difference between (Group 3 vs. Group 1) ($p < 0.01$) in RBCs, hemoglobin, hematocrit, lymphocyte, PLT, AST, ALT, albumin, creat, urea, INR, renin, NITRIC, NGAL, noradrenaline, and ADMA. However, there was no significant variation between (Group 3 vs. Group 1) in eosinophil, monocyte, and TLC (Table 1 and 2). These results demonstrated a significant difference between (Group 3 vs. Group 2) ($p < 0.05$) in hemoglobin, hematocrit, bilirubin, renin, NITRIC, L-ARGININE, and ADMA. In addition, there was a highly significant difference between (Group 3 vs. Group 2) ($p < 0.01$) in monocyte, PLT, albumin, creatinine, urea, INR, SDMA, NGAL, and noradrenaline. Table 1a and b showed no significant difference between (Group 3 vs. Group 2) in

Table 1: Demographic data and laboratory investigations

Parameters	Group 1 N = 30	Group 2 N = 20	Group 3 N = 20	p-value		
				Group 2 versus Group 1	Group 3 versus Group 1	Group 3 versus Group 2
Demographic data						
Age	40.2 ± 14.1	43.6 ± 13.5	53.3 ± 12.7	0.4		
Sex						
Female	21 (70.0%)	15 (71.4%)	14 (70.0%)	0.9	0.9	0.9
Male	9 (30.0%)	5 (28.6%)	6 (30.0%)			
Laboratory investigations						
Hgb	12.1 ± 2.0	10.8 ± 1.7	9.3 ± 2.3	0.04*	0.001**	0.04*
RBCs	4.9 ± 0.5	3.5 ± 0.7	3.2 ± 1.0	0.001**	0.001**	0.3
Hct	37.8 ± 5.9	32.0 ± 4.5	26.1 ± 8.6	0.001**	0.001**	0.01*
TLC	7.4 ± 1.8	8.2 ± 4.7	7.2 ± 3.3	0.5	0.8	0.5
Neutrophil	57.4 ± 12.1	68.0 ± 12.2	70.3 ± 15.3	0.01*	0.01*	0.6
Lymph	36.9 ± 11.3	26.0 ± 12.1	22.6 ± 11.9	0.01*	0.001**	0.4
Mono	3.7 ± 1.1	5.1 ± 1.9	3.2 ± 1.2	0.02*	0.2	0.001**
Eoso	2.2 ± 0.6	2.3 ± 0.9	1.9 ± 0.6	0.8	0.07	0.2
Platelets	274.5 ± 63.0	163.8 ± 49.3	76.3 ± 20.5	0.001**	0.001**	0.001**
AST	19.8 ± 7.4	77.4 ± 58.6	65.6 ± 14.2	0.001**	0.001**	0.4
ALT	17.6 ± 11.2	55.7 ± 38.9	52.0 ± 30.2	0.001**	0.001**	0.7
S. albumin	4.1 ± 0.4	3.0 ± 0.7	2.2 ± 0.6	0.001**	0.001**	0.001**
Creatinine	0.8 ± 0.2	1.2 ± 0.2	5.7 ± 1.3	0.001**	0.001**	0.001**
Urea	26.6 ± 9.0	39.7 ± 9.2	181.6 ± 55.1	0.001**	0.001**	0.001**
INR	1.1 ± 0.1	1.7 ± 0.3	2.7 ± 0.5	0.001**	0.001**	0.001**
BIL	0.4 ± 0.2	3.5 ± 5.5	7.9 ± 11.4	0.03*	0.02*	0.01*

Age, Hgb, RBCs, Hct, TLC, Neutrophil, Lymph, Mono, Eoso, Platelets, AST, ALT, S. Albumin, Creatinine, Urea, INR, and BIL are expressed as Mean ± SD. While sex is expressed as frequency and percent. * $p < 0.05$ is significant, ** $p < 0.01$ is greatly significant.

Table 2: Studied biomarkers

Studied biomarkers	Group 1 N = 30	Group 2 N = 20	Group 3 N = 20	p-value		
				Group 2 versus Group 1	Group 3 versus Group 1	Group 3 versus Group 2
RENIN	4.5 (1.3–120.1)	43.9 (4.9–144.4)	133.1 (52.2–202.0)	0.03*	0.001**	0.02*
NITRIC	5.1 (3.6–16.0)	20.1 (7.0–77.5)	86.2 (46.6–140.6)	0.01*	0.001**	0.02*
NGAL	27.7 (16.3–63.5)	45.0 (23.5–130.5)	150.5 (87.7–211.9)	0.1	0.001**	0.001**
Noradrenalin	58.8 (14.6–98.5)	84.3 (59.4–129.9)	143.1 (129.9–424.7)	0.04*	0.001**	0.001**
SDMA	2.9 (2.0–5.7)	2.9 (2.1–4.2)	4.5 (3.8–8.3)	0.9	0.01*	0.001**
ADMA	80.8 (40.0–281.5)	259.9 (175.1–549.2)	450.8 (334.2–653.7)	0.002**	0.001**	0.03*
L-ARGININE	27.3 (3.6–75.0)	50.5 (24.1–115.8)	128.9 (24.7–206.0)	0.1	0.01*	0.01*

Renin, Nitric, NGAL, NA, SDMA, ADMA, and L-ARGININE are expressed as median with interquartile range (25–75%). *p<0.05 is significant, **p<0.01 is highly significant.

Table 3: Diagnostic performance

Studied markers	Studied markers	Cutoff	Sn.	Sp.	PPV	NPV	Accuracy	AUC	95%CI	p
Group 2 vs Group 1	RENIN	>11.3	71.43	66.67	60.0	76.9	38.10	0.676	0.531–0.800	0.02*
	NITRIC	>4.2	100.00	46.67	56.8	100.0	46.67	0.732	0.589–0.846	0.001**
	NGAL	>107.9	38.10	96.67	88.9	69.0	34.76	0.627	0.480–0.758	0.1
	Noradrenalin	>21.2	100.00	36.67	52.5	100.0	36.67	0.675	0.529–0.799	0.02*
	SDMA	>3.3	23.81	56.67	27.8	51.5	19.52	0.510	0.366–0.652	0.9
	ADMA	>99.6	100.00	63.33	65.6	100.0	63.33	0.759	0.619–0.867	0.001**
Group 3 vs Group 2	L-ARGININE	>15.8	100.00	43.33	55.3	100.0	43.33	0.634	0.488–0.765	0.08
	RENIN	>61.5	75.00	71.43	71.4	75.0	46.43	0.710	0.547–0.840	0.01*
	NITRIC	>23.8	85.00	61.90	68.0	81.2	46.90	0.714	0.552–0.844	0.01*
	NGAL	>145.88	50.00	100.00	100.0	67.7	50.00	0.795	0.640–0.905	<0.0001**
	Noradrenalin	>116.4	90.00	71.43	75.0	88.2	61.43	0.838	0.690–0.934	<0.0001**
	SDMA	>3.3	90.00	76.19	78.3	88.9	66.19	0.793	0.638–0.903	0.0001**
ADMA	>237.7	100.00	42.86	62.5	100.0	42.86	0.690	0.527–0.825	0.03*	
L-ARGININE	>119.7	65.00	90.48	86.7	73.1	55.48	0.727	0.566–0.854	0.01*	

PPV: Positive predictive p-value, Sn: Sensitivity, Sp: Specificity, NPV: negative predictive value, AUC Area under curve, and CI: 95% Confidence Interval. *p < 0.05 is significant, **p < 0.01 is greatly significant.

RBCs, neutrophils, lymphocytes, TLC, eosinophil, AST, and ALT.

The results indicated a significant difference between (Group 2 vs. Group 1) ($p < 0.05$) regarding renin (cutoff >11.3 , sensitivity 71%, specificity 66%) and in noradrenalin (cutoff >21.2 , sensitivity 100%, specificity 36%) (Table 3, Figures 1 and 2). Our findings demonstrated a highly significant variation between (Group 2 vs. Group 1) ($p < 0.01$) in NITRIC (cutoff > 4.2 , sensitivity 100%, specificity 46%), and ADMA (cutoff > 99 , sensitivity 100%, specificity 63%) (Table 3, Figures 1 and 2). As shown in (Table 3, Figures 1 and 2), there was no considerable difference between (Group 2 vs. Group 1) in the remaining markers.

(Table 3, Figures 1 and 2) demonstrated a significant variation between (Group 2 vs. Group 3) ($p < 0.05$) regarding renin (cutoff >61.5 , sensitivity 75%, specificity 71%), NITRIC (cutoff >23.8 , sensitivity 85%, specificity 61%), ADMA (cutoff >237 , sensitivity 100%, specificity 42%) and L-ARGININE (cutoff >119.7 , sensitivity 65%, specificity 90%). (Table 3, Figures 1 and 2) indicated a greatly significant variation between (Group 2 vs. Group 3) in NGAL (cutoff >145 , sensitivity 50%, specificity 100%) and in noradrenalin (cutoff >116 , sensitivity 90%, specificity 71%), and in SDMA (cutoff >3.3 , sensitivity 90%, specificity 76%).

Table 4: Prognostic performance of studied biomarkers

Studied biomarkers	Group 2 versus Group 1		Group 3 versus Group 2	
	OR (95% CI)	p value	OR (95% CI)	p value
Renin	1.00 (1.001-01-)	0.3	1.01 (1.00–1.02)	0.02*
Nitric Oxide	1.01 (1.00–1.02)	0.1	1.01 (1.00–1.03)	0.03*
NGAL	1.02 (1.00–1.03)	0.03*	1.02 (1.01–1.03)	0.001**
Noradrenalin	1.00 (1.00–1.01)	0.7	1.01 (1.00–1.03)	0.02*
SDMA	0.95 (0.821-11-)	0.5	1.47 (1.07–2.02)	0.02*
ADMA	1.00 (1.00–1.00)	0.5	1.00 (1.00–1.01)	0.07
L-Arginine	1.00 (0.99–1.01)	0.7	1.01 (1.00–1.02)	0.02*

CI: Confidence Interval, OR: Odd Ratio, p value measured based on logistic regression analysis. *p < 0.05 is significant, **p < 0.01 is highly significant.

Table 4 showed a greatly significant difference regarding NGAL ($p < 0.01$) between (Group 3 vs. Group 2) according to the odd ratio and confidence interval.

As demonstrated in (Table 4), there was a significant difference regarding renin, NO, noradrenalin, SDMA, and L-Arginine ($p < 0.05$) between (Group 3 vs. Group 2) according to odd ratio and confidence interval. There was a significant variation regarding NGAL ($p < 0.05$) between (Group 2 vs. Group 1), as per the odd ratio and confidence interval (Table 4).

Proteomic bioinformatics analysis of studied biomarkers

STRING network. Every different colored line that attaches proteins demonstrates a separate proof channel for the particular interaction, including text mining (green), experiments (magenta), and databases (blue) (Figure 3).

Discussion

Hepatorenal syndrome (HRS) is a disorder in which persons with severe liver disease have reduced kidney function. People with hepatorenal syndrome do not have a known cause of kidney impairment, and their kidneys are structurally normal [2], [3]. This distinguishes HRS as a distinct pathophysiological condition that enables the study of the interaction of vasoconstrictor and vasodilator systems in renal circulation [4], [8], [12]. The present study enrolled

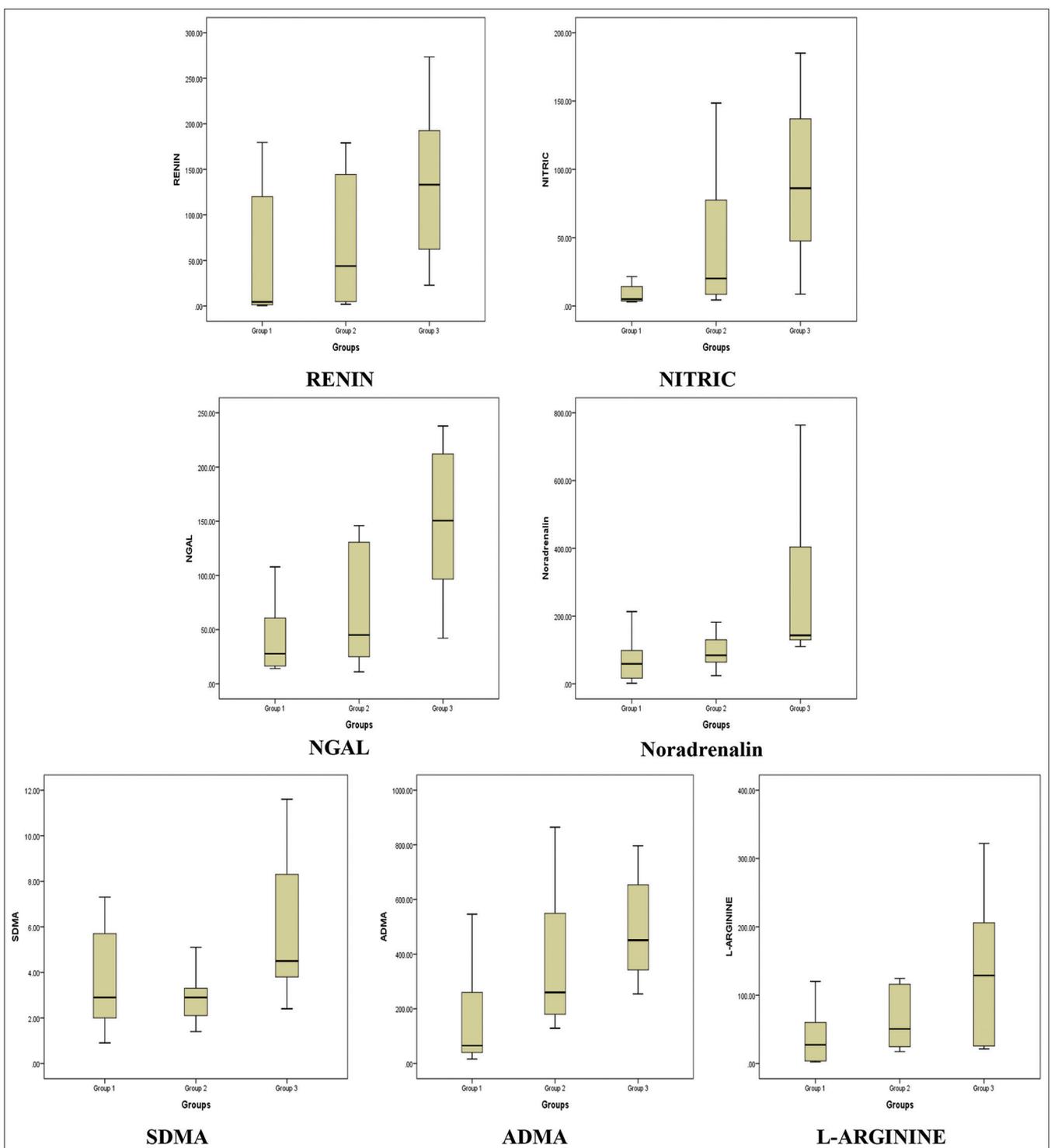


Figure 1: Box plot of the studied biomarkers: Demonstrate HRS markers level between different groups

90 individuals with liver cirrhosis at the TBRI's Hepatogastroenterology Department. They were divided into three groups: Group 1 comprised 30 individuals with compensated liver cirrhosis and normal kidney function; Group 2 comprised 20 individuals with decompensated liver cirrhosis and normal kidney function; and Group 3 comprised 20 cases with decompensated liver cirrhosis and renal impairment.

We investigated many biomarkers and their relationship with HRS in this study. Regarding NGAL,

our result showed a significant difference between Group 3 and Group 1 ($p = 0.001$), and Group 2 ($p = 0.001$). This result is similar to other findings, which indicated another result by Yap and his colleagues, who found that the baseline urinary NGAL was significantly associated with HRS development [13]. Further study showed that urine NGAL is highly effective at identifying ATN from other forms of AKIs in cirrhosis [14].

Nitric oxide (NO) is a vasodilator that is thought to be involved in renal perfusion. Preliminary

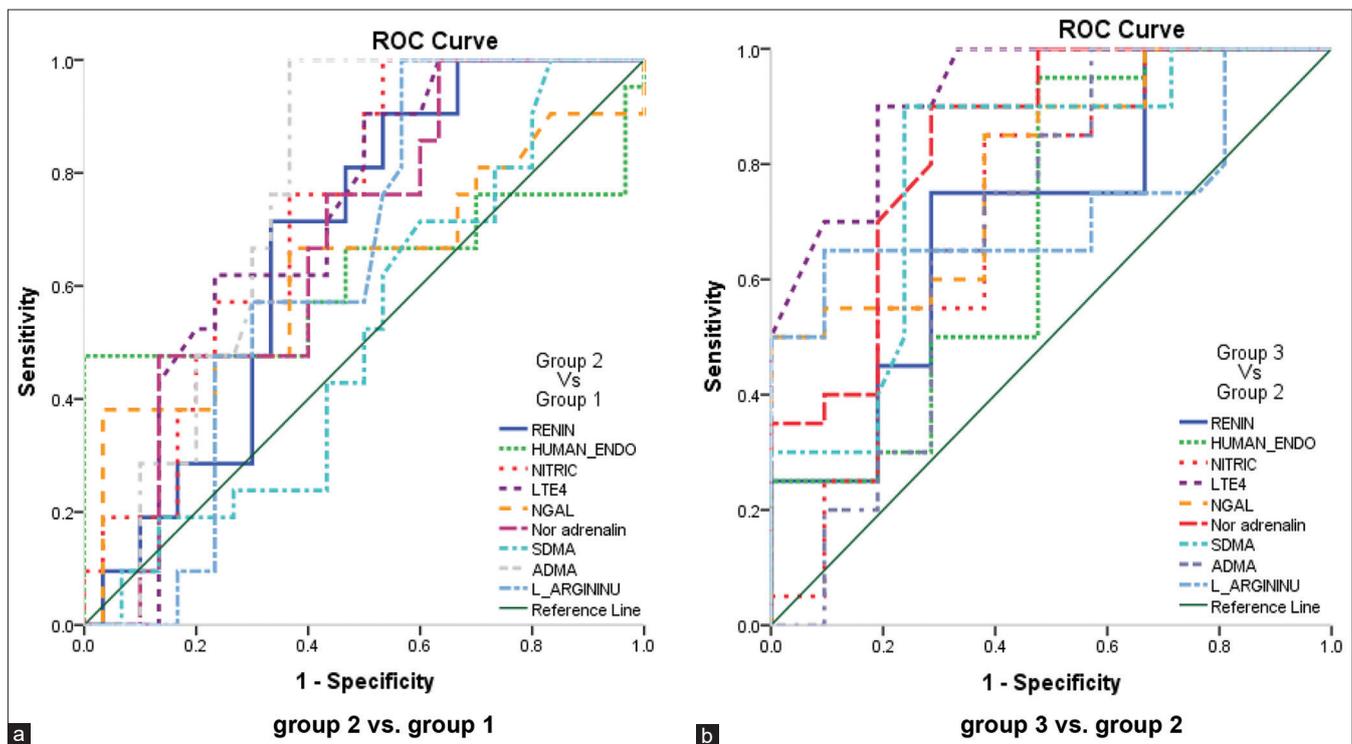


Figure 2: (a and b) ROC Curve analysis of the studied biomarkers in the studied groups

evidence, primarily from animal tests, indicates that persons with cirrhosis produce more nitric oxide, even though NO suppression has no finding in renal

vasoconstriction due to changes implemented in PG synthesis. When both NO and PG generations are suppressed, a significant vasoconstriction of the kidney occurs. Vasoconstrictor action may well be the dominant system in HRS, although it is not clear whether this is due to decreased vasodilatory activity, or the other way around. Our results showed a substantial difference between Groups 3 and 1 ($p = 0.001$), and 2 (0.02). In several individuals with decompensated cirrhosis, systemic endotoxemia is hypothesized to boost NO production in cirrhosis. Increased plasma nitrite/nitrate levels in individuals with decompensated cirrhosis are symptomatic of increased NO generation [15]. Cirrhosis cases and ascites had higher plasma RAAS activity and antidiuretic hormone levels, and a high serum NO level is related to reduced urine salt excretion as well as elevated plasma RAAS activity and antidiuretic hormone concentrations [15], [16].

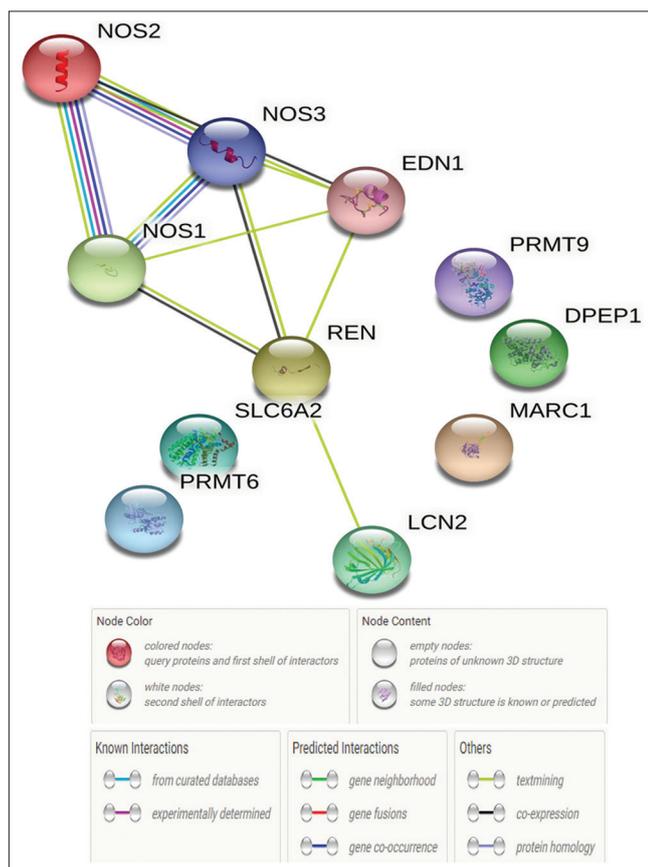


Figure 3: Protein-protein interaction network of the studied biomarkers

antagonist to the high levels of NO in the blood, causing renal vasoconstriction in HRS.

In cases with HRS, the sympathetic nervous system is hyperactive, culminating with renal vasoconstriction and increased salt retention [20]. Our findings indicated a significant difference in noradrenaline levels between Groups 3 and 1 ($p = 0.001$) and 2 ($p = 0.001$). Numerous investigations have demonstrated increased catecholamine release in the renal and splanchnic vascular beds [21]. Since the 1980s, the relevance of hepatorenal innervation has been recognized. The increased intrahepatic pressure enhanced the function of the efferent renal sympathoadrenergic system [22]. Vasoconstriction of the kidney's afferent arterioles decreased renal plasma flow and GFR while increasing sodium and water reabsorption through the tubules. More than half of individuals with decompensated liver disease have activated the renin-angiotensin-aldosterone system (RAAS), which is heightened in those with HRS [23], [24]. Our findings indicated that Group 3 had a significantly greater renin level than Group 2 ($p = 0.02$), Group 1 ($p = 0.001$), as well as Group 2, had a significantly higher renin level than Group 1 ($p = 0.03$). Increased angiotensin II levels protect the kidneys by selectively constricting the efferent glomerular arterioles.

Increased plasma renin release followed by an increase in angiotensin II formation was found in refractory ascites and HRS, indicating a role of RAAS in the development of HRS. Angiotensin II helps to maintain vascular tone in patients with advanced liver disease, but has no role in healthy controls or patients with compensated cirrhosis, suggesting that this mediator contributes to vascular dysfunction in cirrhosis [25].

ADMA is an endogenous direct inhibitor of the enzyme nitric oxide (NO) synthase, which participates in NO synthesis. NO participates in the maintenance of vascular tonus. Increased concentration of ADMA in the blood of patients with decompensated liver cirrhosis reduces the synthesis of NO, whereby intrahepatic vascular resistance is increased [26]. ADMA is hydrolyzed by the action of the enzyme dimethylarginine dimethylaminohydrolase (DDAH). Compared to ADMA, SDMA has indirect inhibitory effect on NO synthase. SDMA can disturb the synthesis by competing in the transport against L-arginine on the level of cell membrane [27], [28].

Our study shows an increased level of ADMA and SDMA in Group 3, compared to Group 2 and Group 1. Some studies [29] have demonstrated that increased ADMA level in blood of patients with decompensated liver cirrhosis is probably the result of DDAH enzyme activity exhaustion. Increased level of ADMA has a causative role in the development of HRS. Accumulation of ADMA in patients with liver cirrhosis causes liver damage. Accumulation of ADMA inhibits NO synthase thereby causing vasoconstriction

of the kidney blood vessels. Thus, blood flow through the kidney is interrupted, in other words, glomerular filtration is reduced and SDMA is retained in the kidney. Compared to ADMA, SDMA is not broken down by the action of DDAH enzyme but is excreted as such through the kidneys [30], [31].

Conclusion

Renin, Nitric Oxide, ADMA, SDMA, and L-arginine may act as biomarkers for advanced cirrhotic patients to indicate HRS. Integrating biomarkers into clinical decision-making can enhance therapy accuracy by identifying patients who have structural injury underlying their AKI. Additional study is required to characterize biomarkers unique to HRS.

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