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VALIDATION OF URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN, INTERLEUKIN-18 AND KIDNEY INJURY MOLOCULE-1 IN THE DIAGNOSIS OF HEPATORENAL SYNDROME IN EGYPTIAN PATIENTS WITH HEPATITIS C VIRUS- RELA

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ABSTRACT Hepatorenal syndrome (HRS) is a functional and potentially reversible form of AKI that occurs in cirrhosis patients with ascites caused by splanchnic vasodilatation , cardiovascular dysfunction and renal hypo-perfusion; all these hemodynamic changes are triggered by portal hypertension. SCr, the clinical standard to define kidney function, poorly discriminates AKI type in cirrhosis so there is urgent need for new biomarkers for early detection and differential diagnosis of AKI in cirrhosis. This study was conducted in the Department of Internal Medicine, Minia University Hospital along the period from January and November . The aim of our study was validate the accuracy of urinary neutrophil gelatinase associated lipocalin (NGAL), interleukin- (IL-) and kidney injury molecule- (KIM-) in the diagnosis of HRS in Egyptian patients with hepatitis C virus (HCV) - related liver cirrhosis with serum creatinine as a gold standard test for kidney injury.

Conclusions Urinary NGAL, KIM- and IL- are valid biomarkers for detection of AKI in hospitalized cirrhotic patients with AKI The studied urinary biomarkers could not differentiate different subtypes of AKI in cirrhotic patients.

KEY WORDS : Hepatorenal Syndrome, Neutrophil Gelatinase, Kidney Injury Molocule, Liver Cirrhosis

Introduction

Cirrhosis is a global health problem (Murray et al., 2012). This may be attributed to the increasing rate of hepatitis C virus (HCV) infection to >185 million infection worldwide (Mohd Hanafiah et al., 2013).In Egypt, the prevalence of HCV is estimated to range from 14.7-22% (Wantuek et al., 2014).

Acute kidney injury(AKI) is a frequent complication of cirrhosis that occurs in approximately 20%of hospitalized patients with advanced disease (du Cheyron et al., 2005). Serum creatinine (sCr) is the most established tool for the diagnosis of AKI in patients with and without cirrhosis (Francoz et al., 2010). A sCr concentration ≥1.5 mg/dl(133umol/l)has been selected in several conferences as a cut-off to define AKI in cirrhosis (Salerno et al., 2007). However ,the use of this fixed value is problematic due to numerous renal and non-renal factors (Sherman et al., 2003), which result in erroneous estimated glomerular filtration rate (eGFR) (Bellomo et al., 2004).

Hepatorenal syndrome (HRS) is a functional and potentially reversible form of AKI that occurs almost exclusively in cirrhosis patients with ascites (Gines and Schrier, 2009). Pathogenesis of HRS is unclear; however, it is postulated that splanchnic vasodilatation reduces effective circulating volume, which in turn leads to cardiovascular dysfunction and renal hypo-perfusion; all these hemodynamic changes are triggered by portal hypertension (Moore and Van Thiel, 2013).

The clinical characteristics of HRS are similar to pre-renal azotemia, but the condition does not respond to volume expansion (Gines and Schrier, 2009). Based on the course of the disease and the presence of trigger(s),HRS is divided into two types. Type 1 HRS is defined as a rapid decline in kidney function with sCr increasing to >2.5 mg/dl within a two week period. It usually follows a precipitating factor and has a worsen survival. Whereas, type 2 HRS is defined as slowly progressive rise in sCr to >1.5mg/dl that almost associated with refractory ascitesTill now, the diagnosis of HRS is one of exclusion due to the lack of the standard test, as kidney biopsy is uncommonly performed in cirrhosis patients due to increased risk of internal bleeding caused by coagulopathy and thrombocytopenia (Tanriover et al., 2008).

Thus, it is obvious that new objective biomarkers which accurately differentiate the structural from functional AKI in cirrhosis patients are urgently needed. In this regard, novel serum/or urinary biomarkers of tubular damage, such as neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18,kidney injury molecule-1 and liver fatty acid-binding protein have been discovered (Ariza et al., 2015).

NGAL is a small 25 KDa glycoprotein that released at the presence of inflammatory and ischemic insults from different cells including renal tubules (Kjeldson et al., 1993). Basically, it is undetectable in urine. Whereas, in renal dysfunction it presents and increases proportionally with the severity of the disease. Moreover, it appears in urine prior the rise of sCr levels; therefore, it could be helpful in early prediction of AKI in cirrhosis (Parikh et al., 2011). The

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preliminary studies showed that uNGAL level either alone (Verna et al., 2012 and Belcher et al., 2014) or in a panel of a aforementioned biomarkers (Ariza et al., 2015) could differentiate functional and structural causes of renal dysfunction in patients with advanced cirrhosis , however, there remains a need to clarify its potential role for the identification of patients at increased risk for HRS.

Kidney injury molecule 1 (KIM-1) is a 38.7-kDa type I transmembrane glycoprotein with an extracellular immunoglobulin-like domain topping a long mucin-like domain (Ichimura et al., 1998). It is expressed at low levels in the normal kidney as well as in other organs, but its expression is dramatically up-regulated in the kidney post-ischaemia/reperfusion injury in rats (Ichimura et al., 1998) as well as in rodent models of drug-induced AKI (Amin et al. 2004, Prozialeck et al. 2007). The expression is mainly upregulated in proximal tubule cells both in rodents (Ichimura et al., 2004) and in humans (Han et al., 2002). Studies in human had beenreported that KIM-1 was an efficient novel urinary biomarker in diagnosis of AKI within 24 hours after kidney injury, especially in the diagnosis of ischemic ATN (Huang et al., 2011).

Patients and Methods

The current prospective, hospital-based, case-controlled study; with the participants recruited from the patients admitted to the Internal Medicine Department, Minia University Hospital for the treatment of cirrhosis-related complications. The study was conducted in the period from January 2015 and November 2015 on a group of patients with HCV-associated liver cirrhosis complicated with HRS .This group of patients was compared with two other cirrhosis groups: a group without ascites and a group with ascites but without impairment of kidney function. These groups were used to represent the different stages of natural history of cirrhosis (Moore and Van Thiel, 2013)

Diagnosis of chronic HCV was considered by presence of anti –HCV and detectable serum HCV- RNA for six months or more. Established diagnosis of liver cirrhosis was based on a combination of clinical, biochemical ,ultrasonographic and endoscopic findings.

The study groups included:

Group I: It included 20 cirrhotic patients without ascites their age ranged from 40-60 years. They were 14 (70%) males and 6 (30%) females.

Group II: It included 30 cirrhotic patients with ascites but without renal impairment, their ages ranged from 38-63years . They were 21 (70%) males and 9(30%) females.

Group III : It included 30 cirrhotic patients with ascites and with renal impairment diagnosed as having hepatorenal syndrome, their ages ranged from 48-68 years they were 22 (73.3%) males and 8 (26.7%) females

This group was subdivided into:

Subgroup III-a : Patients with type 1 hepatorenal syndrome. (23 (76.6 %) patients) their ages ranged from 48-68 years including 18 (78.3%) males and 5 (21.7%) females. Subgroup III-b Patients with type 2 hepatorenal syndrome(7 (23.3 %) patients) their ages ranged from 52-65 years including 4 (57.1%) males and 3 (42.9%) females.

HRS was diagnosed according to criteria reported by Salerno et al. (2007) ; including:

a. Presence of cirrhosis with ascites.

b. Serum creatinine > 1.5 mg/dL.

c. No improvement in serum creatinine level (decrease to 1.5 mg/dL) after at least 2 days with diuretic withdrawal and volume expansion with albumin. The recommended dose of albumin is a single infusion of 1 g/kg of body weight (maximum, 100 g).

d. Absence of shock.

e. No current or recent treatment with nephrotoxic drugs.

f. Absence of parenchymal kidney disease as indicated by proteinuria > 500 mg/d, microhematuria (>50 red blood cells/high-power field), and/or abnormal renal ultrasonography.

The two clinical types of HRS were defined according to criteria of Salerno et al. (2007) as follows :

Type 1: Rapidly progressive renal disease in cirrhotic patients. It was defined as a 100% increase in sCr to a final value > 2.5 mg/dl in < 2 weeks. The clinical presentation is usually that of acute kidney failure.

Type 2: Stable or slowly progressive renal disease in cirrhotic patients that didn't meet the criteria of type 1. The clinical picture is that of ascites refractory to diuretic therapy.

Exclusion criteria include:

1. Class IV heart failure, O2 dependent chronic obstructive pulmonary disease (COPD).

2. Chronic kidney disease with serum creatinine persistently > 4 mg/dL and/or eGFR<60 ml/min/1.73m2 for > 3 months, using MDRD.

3. Other causes of liver cirrhosis.

4. Shock as defined by American College of Chest Physicians (Levy et al., 2003)

5. Established parenchymal kidney disease.

6. Obstructive uropathy based on ultrasound examination.

7. Use of nephrotoxic drugs in the previous 30 days.

8. Urinary tract infection.

9. Anuria for 12 hours or more.

10. Need for renal replacement therapy.

11. Any solid organ transplantation.

12. Diabetic patients.

Results

The current prospective, hospital-based, case-controlled study was conducted in the Internal Medicine Department, Minia University Hospital in the period from January 2015 and November 2017.

The study groups included:

Group I: It included 20 cirrhotic patients without ascites. They were 14 (70%) males and 6 (30%) females. their ages ranged from 40 to 60 years with a mean ± SD of 52.2 ± 5.11 years

Group II: It included 30 cirrhotic patients with ascites but without renal impairment. They were 21 (70%) males and 9(30%) females. their ages ranged from 38 to 63years with a mean ± SD of 49.7 ± 7.31 years.

Group III: It included 30 cirrhotic patients with ascites and with renal impairment diagnosed as having hepatorenal syndrome, they were 22 (73.3%) males and 8 (26.7%) females. Their ages ranged from 48 to 68 years with a mean ± SD of 57.8 ± 5.6 years. This group was subdivided into:

III –a Patients with type 1 hepatorenal syndrome. (23 (76.6 %)

patients) their ages ranged from 48 to 68 years with a mean ± SD of 57.9 ± 5.9 years. They included 18 (78.3%) males and 5 (21.7%) females.

III –b Patients with type 2 hepatorenal syndrome(7 (23.3 %) patients) their ages ranged from 52 to 65 years with a mean ± SD of 57.28 ± 4.85 years. They included 4 (57.1%) males and 3 (42.9%) females.

The three groups were sex-matched ,however, patients with HRS were older when compared to either group I or II (mean ± SD of 57.8 ± 5.6 years vs. 52.2 ± 5.11 years vs. 49.7 ± 7.31 years with p < 0.007 and < 0.001 respectively) .They had the lowest SBP (mean ± SD of 105.16±6.22 mmHg vs. 109.75±7.15 mmHg vs. 110.16±6.08 mmHg with p = 0.047 and 0.010 respectively), the highest prevalence of hepatic encephalopathy (90% vs. 15% vs. 40% with p ≤ 0.001) , the longest hospital stay (median 8.5 (7-11) days vs. 4(0-5) days vs. 6 (4-7) days , p ≤ 0.001) , the highest prevalence of spontaneous bacterial peritonitis (30% vs. 10% vs. 0% , p = 0.007 and 0.104 respectively) ,lowest GCS score (mean ± SD of 14.06 ± 1.25 vs. 14.85 ± 0.67 vs. 14.56 ± 0.93,p =0.028,0.183 respectively), the highest SOFA score (median of 6 (5-7) vs. 1 (0.25-3.75) vs. 3 (2-4),p< 0.001) and the highest APACHE II score (mean ± SD of 13.3 ± 2.36 vs. 8.8 ± 2.54 vs. 9.6 ± 3.03,p< 0.001)

Table(9): Demographic and clinical characteristics of the study groups

	Group I (n=20)	Group II (n=20)	Group III (n=20)	P value		
[11]Age(years)				< 0.001*		
Range	(40-60)	(38-63)	(48-68)	I vs II	I vs III	II vs III
Mean ± SD	52.2 ± 5.11	49.7 ± 7.31	57.8 ± 5.6	0.499	0.007*	< 0.001*
[12]Sex(n,%)					0.910	
Male	14 (70%)	21 (70%)	22 (73.3%)	I vs II	I vs III	II vs III
Female	6 (30%)	9 (30%)	8 (26.7%)	1	0.767	0.774
[13]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*
[14]DBP(mmHg)					0.145	
Range	(60-80)	(60-80)	(60-80)	I vs II	I vs III	II vs III
Mean ± SD	72 ± 6.15	70.66 ± 5.21	69 ± 4.81	1	0.183	0.685
[15]MAP(mmHg)					0.024*	
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
[16]Hospital stay(days)					< 0.001*	
Median (IQR)	4 (0-5)	6 (4-7)	8.5 (7-11)	I vs II	I vs III	II vs III
				0.092	< 0.001*	0.001*
[17]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
[18]Hepatic					< 0.001*	
Encephalopathy(n,%)				I vs II	I vs III	II vs III
No	17 (85%)	18 (60%)	3 (10%)	0.069	< 0.001*	< 0.001*
Yes	3 (15%)	12 (40%)	27 (90%)			
[19]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
[20]GCS					0.026*	
Range	(12-15)	(12-15)	(11-15)	I vs II	I vs III	II vs III
Mean ± SD	14.85 ± 0.67	14.56 ± 0.93	14.06 ± 1.25	1	0.028*	0.183
[21]SOFA score					< 0.001*	
Median(IQR)	1 (0.25-3.75)	3 (2-4)	6 (5-7)	I vs II	I vs III	II vs III
				0.119	< 0.001*	< 0.001*
[22]APACHE II score					< 0.001*	
Range	(4-16)	(3-18)	(8-20)	I vs II	I vs III	II vs III
Mean ± SD	8.8 ± 2.54	9.6 ± 3.03	13.3 ± 2.36	0.913	< 0.001*	< 0.001*

Discussion

Acute kidney injury (AKI) is frequent in cirrhosis and recurrent episodes of AKI may occur in end-stage cirrhosis. Serum creatinine (sCr), creatinine clearance as well as SCr derived-equations tend to overestimate GFR in cirrhosis which affect their utility in the early diagnosis of AKI. Moreover, early identification of the phenotype may facilitate recovery. One of the most challenging issues is the ability to differentiate HRS from ATN (Francoz et al., 2016).

Several novel biomarkers recently emerged to help in the early diagnosis of AKI and to phenotype AKI. The most promising tubular biomarkers of tubular injury in AKI are (i) neutrophil gelatinase-associated lipocalin (NGAL), (ii) interleukin 18 (IL-18), (iii) kidney injury molecule 1 (KIM 1).

In the current study, patients 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 both animal models and human studies. In animal models, NGAL expression is markedly increased in the kidneys and released in urine following ischemic or nephrotoxic insults. Urinary concentration increases very rapidly (within 2 h) following ischemia (Mishra et al., 2003; Mishra et al., 2004). Human studies have shown that NGAL measurement in either urine or serum might be useful to detect AKI at an early stage in numerous clinical situations such as, sepsis and septic shock, contrast-enhanced imaging, cardiac surgery, polytrauma and hypothermia (Mishra et al., 2005; Trachtman et al., 2006; Wagener et al., 2006; Hirsch et al., 2007; Wheeler et al., 2008; Nickolas et al., 2008; Makris et al., 2009). In addition, NGAL may be useful in monitoring some kidney diseases such as delayed kidney graft function (Lee et al., 2012 and Pianta et al., 2015), kidney allograft rejection (Kohei et al., 2013), lupus nephritis (Torres-Salido et al., 2014) and IgA nephropathy (Ding et al., 2007). Recently, it has been suggested that NGAL may help to identify the cause of AKI in patients with liver disease, especially in differentiating ATN from HRS (Fagundes et al., 2012; Verna et al., 2012; Barreto et al., 2014; Belcher et al., 2014). On average, urinary NGAL is higher in patients with cirrhosis and AKI compared to patients without AKI (Fagundes et al., 2012) and is significantly higher in patients with persistent AKI as compared to patients with transient AKI (Barreto et al., 2014). Among patients with AKI, uNGAL was found to be markedly higher in those with a diagnosis of ATN when compared to those with a diagnosis of type-1 HRS, prerenal azotemia or CKD (Fagundes et al., 2012; Belcher et al., 2014). Among patients with type-1 HRS, uNGAL was significantly higher in those with concomitant infections. Interestingly, two studies suggest that elevated uNGAL was predictive of early mortality in cirrhotic patients with AKI (Verna et al., 2012; Barreto et al., 2014).

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