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RESEARCH ARTICLE

CYSTATIN C A REAL-TIME BIOMARKER FOR GLOMERULARFILTRATION RATE IN CRITICALLY-ILL PATIENTS WITH END STAGE LIVER DISEASE

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ABSTRACT

Introduction: Parameters allowing regular evaluation of renal function in critically-ill patients such as serum creatinine and blood urea are not optimal. Sudden changes in glomerular filtration rate (GFR) are not followed by parallel changes in serum creatinine and are at risk of developing renal dysfunction.
The aim of study: was to analyze the utility of serum cystatin C as a real-time biomarker of renal function in critically-ill patients with end stage liver disease (ESLD).
Patients and Methods: serum creatinine, cystatin c and 24 hours creatinine clearance were determined daily to 300 patients (220 male and 80 female) critically-ill patients with ESLD. The serum levels of creatinine and cystatin c were correlated with the creatinine clearance daily. The diagnostic value of serum creatinine and cystatin c to identify GFR under 80 ml/min per 1.73 m^2 was evaluated using receiver operating characteristic (ROC) curve analysis.
Result: Thirty out of 300 patients (10 %) had serum creatinine above the upper limit of normal, while 85 out of 300 patients (28.3 %) had serum cystatin c above the upper limit of normal. Statistically the ability of serum cystatin c to identify a creatinine clearance rate 80 ml/min per 1.73 $m^2$was better than that of serum creatinine (areas under the ROC curve: for cystatin c 0.925, and for creatinine0.613).
Conclusion: serum cystatin c is an accurate easy and useful marker, better than serum creatinine to detect early renal dysfunction in real-time before acute renal injury in critically-ill patients.

INTRODUCTION

Renal impairment is a common finding in patients with chronic liver disease; it has a huge impact on the patient's survival (Salerno *et al*., 2007). Moreover, the severity of renal dysfunction increases with the advancement of liver cirrhosis and portal hypertension. Therefore, close follow-up of the renal function in patients with liver cirrhosis is mandatory and markers of early renal impairment are priceless in these patients (Coresh *et al*., 2003). Patients with end-stage liver disease (ESLD) are prone to develop the hepatorenal syndrome, in wich sever renal vasoconstriction leads to a decrease in GFR, Resulting in acute tubular necrosis (ATN) (Salerno *et al*., 2007). Patients with end-stage liver disease (ESLD) are prone to ischemic acute tubular necrosis (ATN) secondary to hypovolemia from large-volume paracentesis, gastrointestinal bleeding, or infections (Nash *et al*., 2002).

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Diuretics to mobilize ascites may induce prerenal azotemia and profound hypo-albuminemia and third spacing with splanchnic vasodilatation may lead to intravasculardepletion secondary to hypovolemia from large-volume paracentesis, gastrointestinal bleeding, or infections (Tseng et al., 2008; Orlando et al., 2002). Both acute and chronic renal diseases are prevalent. Worse chronic renal disease affects approximately 11% of adults over the age of 65 years (Coresh *et al*., 2003). Acute renal insufficiency was noted in 7.1 % of hospital admission, and in 30% of patients admitted to intensive care unit (Nash *et al*., 2002). Most patients with kidney disease are asymptomatic, necessitating the need for routine screening of all critically-ll patients in intensive care units, who are at risk of developing kidney disease. Early detection of kidney disease changes management and development of more efficacious therapy with improved outcomes (Tseng *et al*., 2008).Using the current markers and equations of the renal function in cirrhotic patients can be challenging. Serum creatinine, the most widely used marker, may underestimate renal impairment in patients with liver cirrhosis.

Decreased hepatic production of creatine, reduced muscle mass, and malnutrition account for an increased gap between serum creatinine levels and the actual renal function (Salerno *et al*., 2007). High serum bilirubin levels may also interfere with the analytical methods of serum creatinine measurement, although this is no more a problem after using the modern Jaffe method auto analyzers (Tseng *et al*., 2008). Inulin clearance, the standard method for measuring the glomerular filtration rate (GFR), is costly and impractical as it requires 24-hour urinary Catheterization (Orlando *et al*., 2002). Isotopic renal scans are not less costly; they cannot be used for repeated measurements that are needed in such patients. Creatinine clearance tends to overestimate the GFR and requires accurate urine volume measurement (Demirtas *et al*., 2001). Based on serumcreatinine, Cockcroft-Gault formula and modification of diet in renal disease (MDRD) equations are of limited valuein cirrhotic patients; they overestimate the GFR as well (Stevens and Levey, 2005). Serum cystatin C (CysC) has been proposed as a novel biomarker of the renal function (Hoste *et al*., 2005). Several studies have reported its value in different sets of patients (Rosner and Bolton, 2006; Huber and Risch, 2005; Villa *et al*., 2005; Perrone *et al*., 1992; Abrahamson *et al*., 1990; Abrahasom *et al*., 1986; Betrosian *et al*., 2007; Wu and Parikh, 2008). However, only few studies have evaluated the role of serum CysC in patients with liver cirrhosis (Huber and Risch, 2005; Villa *et al*., 2005; Perrone *et al*., 1992; Abrahamson *et al*., 1990; Abrahasom *et al*., 1986; Betrosian *et al*., 2007; Wu and Parikh, 2008; Herget *et al*., 2000) and none of these studies have studied its role in detecting early renal impairment in these patients.

Gold standard techniques to assess GFR are based on exogenous substance clearance as inulin, iodine 125 iothalamate, technetium 99m diethylenetriamine-pentaacitic acid (DTPA). However, these methods are difficult to apply in routine practice. The ideal laboratory marker should be of endogenous synthesis, regular production rate, eliminated only by glomerular filtration, and without tubular secretion or reabsorption (Stevens and Levey, 2005). Serum creatinine and creatinine clearance are frequently used in daily practice for estimation of renal function. The serum creatinine is affected by circumstances other than renal ones (for example, muscle mass, protein intake, inflammatory illness, or hepatic disease) and it is secreted by the renal tubules. This leads to an overestimation of GFR, especially when a moderate GFR reduction is present (Stevens and Levey, 2005; Hoste *et al*., 2005; Rosner *et al*., 2006). Moreover, serum creatinine could not detect renal failure until GFR decreases more than 50%, confirming the lower sensitivity of serum creatinine to detect renal dysfunction (Huber and Risch, 2005). In critically-ill patients, there would be a muscle loss, and a relative malnutrition in these cases, serum creatinine could also indicate GFR values higher than the actual levels. Therefore, these parameters allowing regular evaluation of renal function in critically ill patients, in intensive care units are not optimal (Villa *et al*., 2005). To overcome the problems of measuring GFR, an extensive search is being conducted to find a serum marker able to detect renal function impairment, especially at the initial phase. Cystatin c is a nonglycosylated protein that belongs to the cysteine protease inhibitors, cystatin superfamily (Perrone *et al*., 1992). These proteins play an important role in the regulation of proteolytic damage to the cysteine proteases.

Cystatin c is produced at a constant rate by nucleated cells (Abrahamson *et al*., 1990). It is found in relatively high concentrations in many body fluids, especially in the seminal fluid, cerebrospinal fluid, saliva and synovial fluid (Abrahasom *et al*., 1986). Its low molecular weight (13.3 kDa) and positive charge at physiological pH levels facilitate its glomerular filtration. Subsequently, it is reabsorbed and almost completely catabolized in the proximal renal tubules. Its concentration is independent of age, sex, and muscle mass. Moreover, its concentration is not influenced by infections, liver disease, inflammatory diseases; therefore it has been postulated to be an improved marker of GFR compared with serum creatinine (Villa *et al*., 2005).

Aim of the study

Thus the aim of this study was to analyze the utility of serum cystatin c levels as markers of renal function in real-time in critically-ill patients with ESLD.

SUBJECTS AND METHODS

One hundred critically-ill patients with ESLD were studied at national liver institute, Menofeya University, Egypt. Between April2013 untill april 2014.Under written informed consent protocol. All of these patients were hospitalized for their chronic liver disease. They include 90 patients with ascites under frequent paracentesis and 60 spontaneus bacterial peritonitis, patients with ascites,150 Gasatrointestinal bleeding with ascites patients. Twenty patients out of 300 had hepatits B virus (HBV), 150had hepatitis C virus (HCV), 125 had mixed HCV and Bilharzias is and 5 patients out of 300 had autoimmune hepatitis.

Exclusion criteria: Patients with hyperthyroidism, autoimmune diseases, malignancy, rheumatic diseases, patients under corticosteroid therapy and immunosuppressive drugs were excluded from the study (corticosteroid increases cystatin c and immunosuppressive cyclosporin decrease cystatin c). Informed consent was taken from every patient or their relatives and the research approved by the ethical committee of National Liver Institute, Menofeya University.

For all cases, the level of serum creatinine and cystatin c were estimated and correlated with creatinine clearance daily for seven days after Paracentesis, gastrointesinal bleeding and spontaneous bacterial peritontis. A 24 hours urine collection for creatinine clearance after adjustment to adult body surface area.The diagnostic value of serum creatinine and cystatin c to identify GFR under 80 ml /min per 1.73 m2 was evaluated using Receiver Operating Curve (ROC) analysis.Serum and urine creatinine levels were measured spectrophotometrically using standard laboratory method (kinetic alkaline picrate). Renal dysfunction was defined as creatinine clearance less than 80 ml/min per 1.73 m2. Serum cystatin c levels were determined by nephlometry in a BN-II device (Dade Behring Marburg GmH, Germany). Statistical analysis was performed using SPSS 11(SPSS inc., Chicago, IL, USA) and EPIDAT 3.0 (Xunta de Galicia, Galicia, Spain and World Health Organization,2003) for windows. Data are expressed as a mean value and 95% confidence interval (CI) unless indicated otherwise. Inverse of Cr and cystatin C were corrleted with Cr C. Correlations between age and Cr C, Cr and Cystatin C were

perfotmed. The diagnostic value of serum Cr and serum cystatin C for identifying Cr C less than 80 ml/min per 1.73 m^2 was evaluated using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity and specificity were calculated by the positive predictive value (PPV) and negative predictive value (NPV). A *P*. value of less than 0.05 was considered statistically significant.

RESULTS

The mean creatinine clearance was 77.6±26.4 ml/min (50-130 ml /min) with 95 % CI. The mean serum creatinine was 1.34 ±0.64 mg /dL(0.6-3.2 mg/dl with 95 % CI. The mean serum cystatin C was 2.8 ± 0.78 mg/L (0.4-4.5 mg/L) with 95 % CI. Seventy patients (23.3%) had CrC less than 80 ml/min and 230 patients (76.66%) had CrC more than 80 ml/min. Thirty patients out of 300 (10%) had serum creatinine above the upper limit of normal (1.2ml/dL); while 85 patients out of 100 (85%) had serum Cystatin C above the upper limit of normal (1.5mg/L).

The areas under the RCO curve were 0.613 for creatinie and 0.925 for cystatin C. Patients with end-stage liver disease (ESLD) are prone to ischemic ATN secondary to hypovolemia from large-volume paracentesis, gastrointestinal bleeding, or infections (Betrosian *et al*., 2007). Diuretics to mobilize ascites may induce prerenal azotemia and profound hypo-albuminemia and third spacing with splanchnic vasodilatation may lead to intravascular depletopn (Wu and Parikh, 2008). Monitoring of renal function is extremely important in the management of critically ill patients with ESLD. The earlier recognition of kidney disease and successful intervention may improve outcome. The National Kidney Foundation, through its Kidney Disease Outcome Quality Initiative (K/DOQI), and other national institutions recommend GFR for definition, classification, screening and monitoring of chronic kidney disease (Villa *et al*., 2005). Creatinine clearance the most widely used clinical marker of kidney function, is now recognized as unreliable measure of GFR because serum creatnine is affected by age, weight, muscle mass,race various medications ,eating meat and extra glomerular elimination (Finnney *et al*., 2000).

Table 1. Creatinine clearance <80mL/min in relation to serum creatinine and serum cystatin C

Range		Cr C< 80mL/min per 1.73m^2		
		Range	Mean ± DS	%
Serum Creatinine (mg/dL)	> 1.2mg/dL(n=30)	1.3 – 3.2	1.89 ± 0.33	42.86
	< 1.2mg/dL(n=40)	1.0 – 1.2	1.18 ± 0.41	57.14
Serum cystatin C (mg/L)	> 1.5mg/L(n=70)	1.6 – 4.5	3.3 ± 0.81	100
	< 1.5mg/dL(n=0)	0	0	0

Table 2. Comparison of groups with creatinine clearance lower and higher than 80mL/min per 1.73m^2

	Cr C< 80mL/min per 1.73m^2			Cr C> 80mL/min per 1.73m^2			p.value
	N	Range	Mean ± DS	N	Range	Mean ± DS	
Cr C (mL/min per 1.73m^2)	70	50-78	69.9-14.6	230	80-130	96.25 + 28.2	<0.01
Serum Creatinine (mg/dL)	70	1.0-3.2	1.48±1.2	230	0.6-1.2	1.02 ± 0.62	NS
Serum cystatin C	70	2.0-4.5	3.3±1.3	230	0.4-2.5	1.64 ± 0.68	<0.01

Table 3. Frequency of renal impairment in the 3 groups

	Cr > 1.2mg/dL	Cr C <80ml/min	SerumCystatin C>1.5mg/dL
Frequent paracentesis (N=90)	6	10	16
SBP (N=60)	9	24	36
GIT bleeding with ascites (N=150)	15	36	43

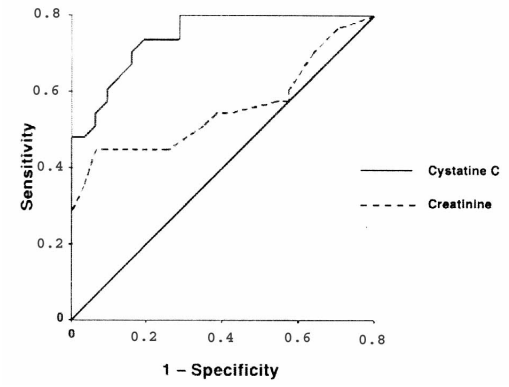

Fig. 1. Receiver operating characteristic (ROC) curves to diagnose sensitivity and specificity of S. creatinine and S.cystatine C

This figure showed thatthe capacity of cystatine C to detect acute renal insufficiency was better than that of serum creatinine in patients with creatinine clearance < 80 ml/min per 1.73m^2.

Creatinine production changes significantly according to the muscle mass of the body and dietetic factors. It is filtered by the glomeruli , but it is also secreted by the renal tubules. This tubular secretion contributes approximately 20% of the total creatinine excretion by the kidney, and it can increase as GFR

decreases. All of these factors explain why serum creatinine concentration may not be a good parameter for accurate determination of GFR, especially at lower rates (Levey *et al*., 1988). Cystain C production in the body is a stable process that is not influenced by renal conditions, increased protein catabolism, or dietetic factors. Moreover, it does not change with age or muscle mass like creatinine does. Its biochemical characteristics allow free filtration in the renal glomerulus, and subsequent catabolism by the proximal tubules. For these reasons, serum cystatin C has been suggested to be an ideal endogenous marker of GFR (Finnney *et al*., 2000; Herget-Rosenthal *et al*., 2000; Risch *et al*., 2001). In the present study, the mean serum creatinine in the 40 critically ill patients was 1.34 mg/dL which is slightly increased than the upper limit of normal (1.2 mg/dL) and the mean serum creatinine in the group of patients with creatinine clearance M 80 mL/min per 1.73 m^2 also slightly elevated to 1.48 mg/dL. On the other hand the mean serum cystatin C in the 40 critically ill patients was 2.8 mg/L which is about double the upper limit of normal (1.5mg/dL); and the mean serum cystatin C in the group of patients with Cr C <80mL/min/1.73 m^2 was 3.3 mg/L which is more than double the upper limit of normal (1.5 mg/L). In the group of patients with Cr C >80mL/min/1.73 the serum creatinine was within normal 1.02mg/dL while serum cystatin C was elevated to 1.64 mg/L which is more than the upper limit of normal (1.5 mg/L). This finding indicate that serum cystatin C detetmild and moderate acute kidney injury better than serum creatinine. Our results coincide with that reported by (Coll *et al*., 2000; Grubb and Cystatin, 2000; John *et al*., 2003; Delanaye *et al*., 2004). These authors reported that cystatin C values was abnormally high when GFR decreases to 88 mL/min per 1.73 m^2 (Coll *et al*., 2000; John *et al*., 2003). Therefore, cystatin C could detect renal dysfunction one to two days before creatinine (Herget Rosenthal *et al*., 2004). some authors reported that cystaine C was also superior tp cretinine in the children and patients with muscle loss (Le Bricon *et al*., 2005; Filler *et al*., 2005) We found CrC less than 80 mL/min per 1.73m^2 in 70% of critically-ill patients which is similar to other studies [8,11]. All our patients were ale, but other studies find gender differences in cystatin C levels (Hoste *et al*., 2005; John *et al*., 2003; Kezama *et al*., 2009; Foster *et al*., 2006).

Conclusion

The results of the present study indicate that serum cystatin C is a good real-time marker of GFR in critically-ill patients. The simplicity of serum cystatin C determination and its reasonable cost suggest that this test may soon replace creatinine clearance as the biochemical marker of choice for monitoring GFR in a routine practice, especially in critically-ill patients.

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