Comparison Of GFR Determined By Using Serum Cystatin C And Serum Creatinine Concentration Alone And In Combination For Diagnosis Of Hepatorenal Syndrome

Abstract

Introduction: Renal dysfunction is common in patients with liver cirrhosis, occurs about 19% of hospitalized patients with cirrhosis which have a huge impact on prognosis. Serum creatinine(Cr) is a widely used but less reliable marker to estimate glomerular filtration rate(GFR).Serum cystatin C(CysC) is a good endogenous marker to determine early renal impairment . Combined cystatin C and creatinine isan effective reflection of GFR. This study aimed to validate renal function by estimation of GFR using serum cystatin C and serum creatinine individually and combinedly.**Methods**: This was an observational cross sectional study, conducted in Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Thirtypatients were cirrhosis with hepatorenal syndrome (HRS) and thirtywere cirrhosis without HRS. **Result:**Mean value of serumcreatinine, serum cystatin C, GFR by creatinine, GFR by cystatin C and GFR by (cre-cys) were statistically significant ($p<0.05$) between two groups.All the study population were in Child Pugh B and C. Association of mean values of creatinine, cystatin C, GFR by creatinine, GFR by cystatin C and GFR by (cre-cys) with Child Pugh B and Cwere statistically significant in both groups. Based on ROCcurves at cut-off value of 1.29 mg/ml cystatinC had sensitivity 96.7% and specificity 76.7% for detectingHRS. Coefficient of GFR by creatinine was -0.01 (CI -0.01 to 0.00) which was not statistically significant.Coefficient of GFR by cystatin C was -0.02 (CI -0.03 to 0.00) and GFR by (cre-cys) was 0.04 (CI 0.01 to 0.06) which were statistically significant for diagnosis of HRS.**Conclusion:** Combined serum creatinine and cystatin C based GFR showed significant association to discriminate early renal impairment in patients with cirrhosis of liver.

Keywords: Serum Cystatin C, Serum Creatinine.

Introduction

Impairment of renal function in patients with liver cirrhosis is common; it has a huge impact on the patients'survival [1]. Moreover the severity of renal dysfunction usually progresses in parallel with advancement of the cirrhosis and portal hypertension. Therefore parameters of impaired renal function are valuable and close monitoring of renal function is of great clinical importance.Hepatorenal syndrome is a major complication of cirrhosis of liver with ascites.HRS is themost advance stage of the various pathophysiological derangement that

take place in cirrhosis [2].Acute renal dysfunction occurs in 15-20% of hospitalized patient with cirrhosis. HRS is found in 10-13% of such patients and appears to be an extention of pathophysiology of prerenal azotaemia and therefore potentially reversible. Annual frequency of HRS in patient with cirrhosis 8% and some reports as high as 40% [3].HRS is a functional renal failure caused by intrarenal vasoconstriction which occur in patient with end stage liver disease and circulatory dysfunction [4]. It is characterized by splanchnic vasodilatation with a relatively low cardiac output leading to effective hypovolaemia.GFR is universally considered as a measure of overall function of kidney. Serum creatinine is most widely used marker for noninvasive GFR estimation in clinical practice. However it is a poor guide to estimate GFR. Decrease hepatic production of creatinine, muscle atrophy, reduced protein intake especially in patient with liver cirrhosis account for an increased gap between serum creatinine level and actual renal function [5]. Cirrhotic patients often have normal creatinine level in presence of moderate or severe renal impairment.As a result it may overestimate GFR compared with actual renal dysfunction. So, its role is thus limited for the detection of mild or moderate renal injury in cirrhotic patient [6].Serum cystatin C is a non-glycosylated low molecular weight protein, almost never influenced by external factors such as age,gender, muscle mass, inflammation.It has been proposed as a novel biomarker of the renal function [7].It reflects GFR more accurately than serum creatinine. CystatinC can help to detect early renal injury as it increases earlier than creatinine when GFR declines [8,9].In patients with cirrhosis of liver serum cystatin C may be more effective than serum creatinine in measurement of renal function [10]. So, cystatin C is considered as a good parameter to estimate GFR for detection of HRS development and to assess survival in these patients.

Methodology

This observational cross sectional study was conducted in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from July 2014 to June 2017. The diagnosis of cirrhosis was based on a combination of clinical,laboratory and ultrasonographic findings. Total 50 patients were enrolled in this study.Cirrhotic patient who met the criteria of hepatorenal syndrome[10]considered as case. Age & sex matched cirrhotic patient without HRS visited outpatient and admitted inpatient department as control.Patients aged18-60 years were eligible for the study.Patients were excluded if they had intrinsic renal disease, cardiac failure, malignancy, thyroid disorder. Sample for serum creatinine and serum cystatin C were sent to Biochemistry and Microbiology department respectively. The local ethical committee approved the study.A written informed consent was obtained from all

participating patients prior to include in the study. Serum creatinine was measured by kinetic jaffe's method on Automated Biochemical analyzer (Olympus AU 400).Serum cystatinC was measured by ELISA method using cystatinC kit on a CX7 analyzer. GFR was calculated using three estimating formula, modification of diet in renal disease(MDRD)by 4 variables,Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) using cystatinC alone (CKD-EPIcys) and both creatinine and cystatinC (CKD-EPIcre-cysC).

GFR was estimated by using formula:

A) Serum creatinine based GFR: MDRD

1.86 creatinine$^{-1.154}$ × age $^{-0.203}$ × 0.742 (F) [11]

B)Serum Cystatin C based GFR: CKD – EPI

127.7 × cystatin C$^{-1.17}$ × age $^{-0.13}$ × 0.91 (F) [12]

C)Serum creatinine – cystatin C based GFR: CKD – EPI

177.6× (s /creatinine in mg/dl)$^{-0.65}$×(s/ cystatin C in mg/l)$^{-0.57}$ ×age$^{-0.2}$ ×0.82 (F) [13]

Statistical analysis was carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Student t-test was used to analyze the categorical variables, shown with cross tabulation. Receiver operating characteristic (ROC) curves was generated to determine the cutoff value for the best sensitivity, specificity, negative and positive predictive values of Creatinine, Cystatin C, GFR by cystatin C, GFR by creatinine and GFR by cre-cys to diagnose HRS. P values <0.05 was considered as statistically significant.

Results

Baseline characteristics data of the enrolled patients are presented in Table 1. The mean age was found 50.2±10.3 years in case group and 43.9±11.2 years in control group. Majority (96%)patients were male in case group and 80.0% incontrol group. Mean serum creatinine was found 2.97±3.05 mg/dl in case group and 0.96±0.25 mg/dl in control group, and mean serum cystatin C was found 2.4±1.04 mg/L in case groupand 1.08±0.35 mg/L in control group. The mean GFR by creatinine was found 34.36±10.43 ml/min in case group and 96.76±34.78 ml/min in control group, mean GFR by cystatin C was 30.6±10.71 ml/min in case group and 82.73±28.49 ml/min in control group. The mean GFR by cre-cyswas found 30.53±9.4 ml/min in case group and 86.9±27.46 ml/min in control group. The mean differences were statistically significant ($p<0.05$) between two groups (Table 2). HBsAg was found in 60.0% cases and 86% controls. Anti HCV was found in 16% cases. In Child Pugh

B(7-9 score), mean creatinine, cystatin C, GFR by creatinine, GFR by cystatin C and GFR by cre-cys were statistically significant ($p<0.05$) between case and control groups. In Child Pugh C(10-13 score), mean creatinine, cystatin C, GFR by creatinine, GFR by cystatin C andGFR by cre-cys were alsostatistically significant ($p<0.05$) between case and control groups (Table 3).

Table 1: Baseline characteristics of the study population.

Variable	Mean	±SD	Min	-max
Age (years)	47.1	±11.1	18.0	-60.0
BMI (kg/m^2)	23.4	±3.0	18.0	-35.4
SBP (mmHg)	98.2	±11.9	40.0	-120.0
DBP (mmHg)	67.6	±7.6	50.0	-80.0
Creatinine (mg/dl)	1.96	±2.37	0.52	-18.0
Cystatin C (mg/L)	1.74	±1.02	0.62	-6.90
GFR by creatinine (ml/min)	65.57	±40.47	9	-180
GFR by cystatin C (ml/min)	56.67	±33.86	5	-126
GFR by cre-cys (ml/min)	58.71	±34.95	7	-132
Child-Pugh score	10.3	±1.6	7.0	-13.0
Serum sodium (mmol/L)	129.3	±5.9	116.0	-146.0
Serum potassium (mmol/L)	4.1	±0.7	2.7	-6.0
Ascitic fluid	-	-	-	-
TC (cumm)	576.7	±1583.8	20.0	-9000.0
Neutrophil (%)	29.9	±25.0	4.0	-90.0
Lymphocyte (%)	70.1	±25.0	10.0	-96.0
Total protein (gm/dl)	1.1	±0.5	0.1	-2.6
SAAG (gm/dl)	1.8	±0.4	1.0	-3.0

Table 2: Mean creatinine, cystatin C, GFR by creatinine, GFR by cystatin C and GFR by (cre-cys) of the study population.

	Case (n=25)		Control (n=25)		P value
	Mean	±SD	Mean	±SD	
Creatinine (mg/dl)	2.97	±3.05	0.96	±0.25	0.013[s]
Cystatin C (mg/L)	2.4	±1.04	1.08	±0.35	0.021[s]
GFR by creatinine (ml/min)	34.36	±10.43	96.76	±34.78	0.001[s]
GFR by cystatin C (ml/min)	30.6	±10.71	82.73	±28.49	0.001[s]

GFR by cre-cys (ml/min)	30.53	±9.4	86.9	±27.46	0.001[s]

s=significant

P value reached from unpaired t-test

Table 3: Association between creatinine, cystatin C, GFR by creatinine, GFR by cystatin C and GFR by (cre - cys) with Child Pugh.

Child Pugh	**Case**	**Control**	**P value**
	Mean±SD	**Mean±SD**	
B score (n=16)	**(n=6)**	**(n=10)**	
Creatinine (mg/dl)	2.29±0.76	0.95±0.23	0.001[s]
Cystatin C (mg/L)	2.41±0.94	1.07±0.35	0.001[s]
GFR by creatinine (ml/min)	34.57±10.1	95.08±35.75	0.001[s]
GFR by cystatin C (ml/min)	28.57±9.74	82.17±30.27	0.001[s]
GFR by cre-cys (ml/min)	30.0±9.38	87.0±28.15	0.001[s]
C score (n=34)	**(n=20)**	**(n=14)**	
Creatinine (mg/dl)	3.18±3.45	0.98±0.28	0.011[s]
Cystatin C (mg/L)	2.41±1.09	1.1±0.37	0.001[s]
GFR by creatinine (ml/min)	34.3±10.76	97.89±35.12	0.001[s]
GFR by cystatin C (ml/min)	31.22±11.12	83.11±28.14	0.001[s]
GFR by cre-cys (ml/min)	30.7±9.61	86.83±27.82	0.001[s]

s= significant

P value reached from unpaired t-test

Table 4: Receiver-operator characteristic curves of creatinine, cystatin C, GFR by cystatin C, GFR by creatinine and GFR by (cre - cys)for prediction of HRS.

	Cut of value	Sensitivity	Specificity	Area under the ROC curve	95% CI	
					Lower bound	Upper bound
Creatinine	1.38	100.0	96.7	1.000	1.000	1.000
Cystatin C	1.29	96.7	76.7	0.974	0.943	1.000
GFR by cystatin C	32.5	46.7	3.3	0.026	0.000	0.063
GFR by creatinine	42.5	26.7	3.3	0.013	0.000	0.034
GFR by cre-cys	38.5	10.0	0.0	0.004	0.000	0.013

Based on ROC curves serum creatinine at cut off value of 1.38 mg/dl had sensitivity 100.0% and specificity 96.7% for diagnosis of HRS, Serum cystatin C at cut-off value of 1.29 mg/ml had sensitivity 96.7% and specificity 76.7% for HRS. At cut-off value of 32.5 ml/min of GFR by cystatin C had sensitivity 46.7% and specificity 3.3%, 42.5ml/min of GFR by creatinine had sensitivity 26.7% and specificity 3.3% and 38.5ml/min of GFR by cre-cys had sensitivity 10.0% and specificity 0.0% for HRS (Table 4).

Table 5: Multivariable regression analysis for association between GFR by creatinine, GFR by cystatin C and GFR by (cre-cys)

	Coefficients	**P value**	**95% CI**	
			Lower	**Upper**
GFR by creatinine	-0.01	0.13^{ns}	-0.01	0.00
GFR by cystatin C	-0.02	0.02^{s}	-0.03	0.00
GFR by cre-cys	0.04	0.001^{s}	0.01	0.06

s=significant, ns= not significant

Multivariable logistic regression analysis was performed

Coefficient of GFR by creatinine was -0.01 (CI -0.01 to 0.00) which was not statistically significant. Coefficient of GFR by cystatin C was -0.02 (CI -0.03 to 0.00) and GFR by (cre - cys) was 0.04 (CI 0.01 to 0.06) which were statistically significant (Table 5).

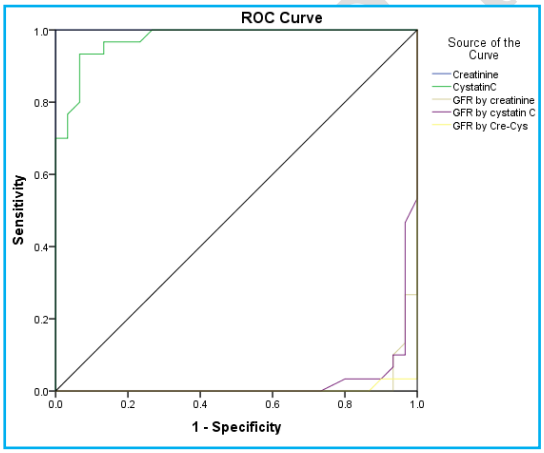

Figure 1: Receiver-operator characteristic curves of creatinine, cystatin C, GFR by cystatin C, GFR by creatinine and GFR by cre-cys.

Discussion

This observational cross sectional study was conducted in the Department of Hepatology, BSMMU. During the study period from July 2014 to June 2017, total 50 patients were admitted at inpatient and visited outpatient departmentwere enrolled. Thirty patients were cirrhosis with HRS considered as cases and 30 patients were cirrhosis without HRS as control. In this present study showed in case group 52% patients were belonged to age 51-60 years and in control group 46% were in 41-50 years. The mean age difference was statistically significant ($p<0.05$) between two groups. Murty et al. [14]foundsimilar relationship. In our study majority patients were male, 96.7% in case group and 80.0% in control group. The median serum creatinine level was significantly lower in women than men (0.7 vs. 0.9 mg/dl, P=0.015), but there was no significant difference in median cystatin C levels (1.0 vs. 1.1 mg/L, P=0.545) and mGFR (74.5 vs. 77.9 ml/min/1.73 m^2, P=0.973) between women and men. Age, female sex were an independent predictor of serum creatinine level ($\beta=-0.205$, P=0.007), but not of serum cystatin C level ($\beta=-0.055$, P=0.526). In this study the mean creatinine, cystatin C,GFR by creatinine, GFR by cystatin C andGFR by cre-cyswere statistically significant ($p<0.05$) between case and control groups.Murty et al.observed [14]the mean serum creatinine was high in AKI group than healthy group. Culafic et al. [15]measuredcystatin C in patients with liver cirrhosis and they have confirmed significantly higher cystatin C in patients with cirrhosis (p= 0.036).El-Agroudy et al. [16]also showed mean differences of creatinine, cystatinC,GFR whichwere statistically significant ($p<0.05$) between case and control groups.Regarding etiology HBsAg, anti HCV and non viral causes were detected. In cases group, 60.0% were HBsAg positive, 16.7% were anti HCVpositive and 23.3% were non viral. In control group, 86.7% patients were HBsAgpositive, 13.3% were non viral and no anti HCV was detected. All enrolled patientswerein Child Pugh B and C class.We showed that 76.7% cases and 60.0% controls were in CP-C, where as 23.3% cases and 40.0% controls were in CP-B.In the study of Omar et al. [17] most patients were Child-Pugh class C (74.3%) and Child-Pugh class B (25.7%).Both in Child Pugh B and C, mean creatinine, cystatin C, GFR by creatinine, GFR bycystatin C and GFR by cre-cys were statistically significant ($p<0.05$) between case and control groups.InCulafic et al.[15]study post-hoc comparisons showed statistically significant differences in values of cystatin C between Child-Pugh A and B (P = 0.014). Woitas et al. [18]in their study found that cystatin C was significantly higher in Child-Pugh B and C patients when compared to Child-Pugh A patients. Another studyof El-Agroudyet al also showed mean creatinine 0.88+-0.31 mg/dl in Child Pugh B group and mean serum cystatin C 2.7±0.67 mg/l in Child Pugh C group.In the study of Gerbes et al. [19] the sensitivity of

cystatin C (86.7%) tended to be higher than that of creatinine (60.0%; NS) and urea (53.3%; NS) at equal specificity of 60%. The area under the receiver-operator characteristic (ROC) curves for the HRS predictors is depicted in Figure 1. Receiver-operator characteristic (ROC) were constructed using creatinine, cystatin C, GFR by cystatin C, GFR by creatinine and GFR by cre-cys of the patients with HRS, which gave a creatinine cut off value of 1.38 mg/dl with a best combination of sensitivity 100.0% and specificity 96.7% for diagnosis of HRS. At cut-off value of 1.29 mg/l the sensitivity and specificity of cystatin C in diagnosing HRS were found to be 96.7% and 76.7%, respectively. At cut off value of 32.5 ml/min the sensitivity and specificity of GFR by cystatin C were found to be 46.7% and 3.3%, respectively. At this cut-off value of 42.5 ml/min the sensitivity and specificity of GFR by creatinine were found to be 26.7% and 3.3%, respectively. At cut-off value 38.5 ml/min the sensitivity and specificity of GFR by cre-cys were 10.0% without specificity. Omar et al. [17]showed ROC curves which were coordinated to define cutoff values with acceptable sensitivity and specificity for the studied tests and formulae. One strong limitation of serum creatinine and creatinine based equations was that serum creatinine lags behind a decreasing GFR[20].So they are not accurately reflecting the present status of the renal function of the patient, a limitation that has been overcome by serum cystatin C that proved to accurately reflect the early stages of renal impairment according to the results of this study. Another study by El-Agroudy et al.[16] revealed serum cystatin C had higher sensitivity and specificity than serum creatinine in the studied subjects. At cut off value of 1.2 mg/l cystatin C had the highest sensitivity for patients group while in control group creatinine had highest sensitivity at cut off value 0.8 mg/dl. Wang D et al showed serum cystatinC and eGFR cys are superior to serum creatinine and eGFR cre in diagnosis of secondary renal impairment of hepatic cirrhosis where cystatinC at cut off value 1.24 showed sensitivity 87.6% and specificity 93.1%. At cut off value of 63.4 mi/min eGFRcys showed sensitivity 87% and specificity 94.4%.[21]Correlation coefficient of GFR by creatinine was -0.01 (CI -0.01 to 0.00) which was not statistically significant.Correlation coefficient of GFR by cystatin Cwas -0.02 (CI -0.03 to 0.00) and GFR by (cre - cys) was 0.04 (CI 0.01 to 0.06) which were statistically significant.Omar et al.[17]showed different measures and estimated the renal functions which were correlated with the isotopic GFR using Pearson's correlation. CKD-EPI (cr-cys) had the highest R^2 among all other measures.Murty et al. [14] showed multiple logistic regression applied to GFR calculated serum creatinine and serum cystatin C in AKI group.Cystatin C based GFR resulted in more negative correlation compared to creatinine based GFR in the AKI group. The pvalue was significant ($p< 0.01$) for GFR (cysC- cre).

Conclusion

For proper detection and treatment of any progressive disease a highly sensitive test is required. Renal impairment usually have a huge impact on the prognosis ofliver cirrhosis. So, establishment of a good marker for renal function is of utmost important. Our study found thatestimated GFR by using combined serumcreatinineand serum cystatinCwas the best measure that reflect actual renal performance to detect early stages of renal impairment in patients with cirrhosis of liver.

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