Diagnostic Value of Serum Cystatin C as an Early Indicator of Renal Impairment in Chronic HCV Egyptian Patients with Liver Cirrhosis

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Abstract: Background and aim: Diagnosis of moderately impaired renal function is of great importance in patients with liver cirrhosis. Patients with a markedly impaired glomerular filtration rate can be diagnosed easily by elevated serum creatinine concentrations but, moderately reduced renal function may be missed. Cystatin C (CysC) has been suggested as a sensitive marker of renal function, independent of sex or muscle mass. Therefore, the aim of this study was to investigate the value of serum cystatin C concentrations for the detection of moderately impaired renal function in chronic HCV Egyptian patients with liver cirrhosis as well as its correlation with Child-Pugh score and renal resistive index (RRI). Patients and Methods: This study was conducted on seventy subjects; group I fifty non azotemic chronic HCV patients with liver cirrhosis (furtherly subdivided according to the Child-Pugh score into group I^a, I^b, I^c) and group II twenty healthy subjects with matching age and sex as control group. Liver function tests, renal function tests, CysC levels and RRI were measured on the same day for all patients. CysC levels were measured using the automated latex-enhanced immunonephelometric method. Results: Mean serum levels of serum Cystatin C were 0.66±0.05, 1.02±0.28, 1.17±0.32 and 0.65±0.10 mg/dl in groups I^a, I^b, I^c and II respectively. Serum cystatin C was significantly higher in cirrhotic patients than in controls. Moreover, It was significantly higher in Child C cirrhotic patients than in those with Child B and A (F=19.14 and P=0.001). Significant positive correlations were found between serum cystatin C and each of blood urea (BU), serum creatinine, RRI and Child-Pugh score in patients with HCV induced liver cirrhosis.(r=0.454,0.781,0.508 and 0.412respectively) ($p \leq 0.01$). On the other hand, significant negative correlation was found between serum cystatin C and creatinine clearance. (r= -0.746 and $p \leq 0.01$). Conclusion: Determination of serum cystatin C is advantagous over serum creatinine particularly in early detection of mild renal impairment in patients with liver cirrhosis.

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1. Introduction:

Impairment of renal function in patients with liver cirrhosis usually progresses in parallel with severity of liver cirrhosis and portal hypertension (1). Diuretic abuse, gastrointestinal bleeding and infection are common predisposing factors of renal dysfunction(2).

Patients with liver cirrhosis and functional renal failure are particularly sensitive to decreased plasma volume. (3) Therefore, parameters of moderately impaired renal function are valuable and close monitoring of renal function is of great clinical importance (1).

In patients with liver cirrhosis, malnutrition and reduced muscle mass can accentuate the difference between serum creatinine level and actual renal performance. Moreover, measurement of Creatinine might be influenced by high serum bilirubin. Although, inulin clearance is considered the gold standard for measurement of glomerular filtration rate (GFR), its cost makes it difficult to use in clinical practice. (4)

Cystatin C is a non-glycosylated low molecular weight protease inhibitor produced by nucleated cells. It is considered as a sensitive indicator of early renal impairment and glomerular filtration rate over serum creatinine. (5) Furthermore, its value is not affected by sex, muscle mass, hyperbilirubinaemia or haemolysis. (5,6) The rate of production of cystatin C is stable regardless of age or any inflammatory process. (6)

Aim of the work

The aim of the present work was to verify the value of serum cystatin C concentrations in chronic HCV Egyptian patients with liver cirrhosis as well as its correlation with Child-Pugh score and renal resistive index (RRI).

2. Patients and Methods:

Patients

This study was conducted on seventy subjects; fifty non azotemic chronic HCV patients with liver cirrhosis (further subdivided according to the Child-Pugh score) and twenty healthy subjects with matching age and sex as control group. Diabetic, renal and hypertensive patients were excluded from the study. All patients were chosen from Tropical

Medicine Department, Alexandria University. Patients gave their informed consent to participate. The protocol was approved by the committee of ethics medical research, Faculty of Medicine, University of Alexandria .Proper and detailed history taking and thorough clinical examination were done.

Laboratory analysis

Routine laboratory investigations including; complete blood picture, liver function tests, renal function tests, fasting and 2hr-postprandial blood sugar and lipid profile (triglycerides, cholesterol, VLDL, LDL, HDL) and complete urine analysis.

Serum samples were obtained on the day of urine collection for creatinine clearance and measurement of creatinine, urea, and cystatin C concentrations. Creatinine was analyzed by a rate blanked modified Jaffé method. (7) Urea was determined using a kinetic urease method followed by a GLDH-UV test where the decrease in NADH adsorbance is determined photometrically. (8) Both assays (Roche Diagnostic Systems, Mannheim, Germany) were implemented on an Hitachi multianalyzer system. The between run coefficient of variance of these methods was constantly below 3%. Cystatin was determined with the Dade Behring N Latex Cystatin C assay (Dade Behring Diagnostics, Marburg, Germany), (9) a particle enhanced nephelometric immunoassay implemented on the Dade Behring Nephelometer II. Intra and interassay coefficients of variation were always below 5% in accordance with earlier reports. (10) Creatinine clearance (CrCl) was calculated as a product of urinary Cr and 24-h urine volume divided by serum Cr (mg/dl) and multiplied by 1440.

Renal resistive index

Each individual was assessed using renal Doppler ultrasonography. The radiologist used the same ultrasound machine (Acuson X300, Siemens, Germany) and a convex transducer with a 3.5 – 5 MHz probe to assess renal hemodynamics in all subjects. To avoid confounding effects on renal hemodynamics caused by food ingestion, all examinations were performed in the morning after overnight fasting. The intrarenal arteries were evaluated bilaterally using color Doppler images of the distal arcuate branches. At least three peak systolic flow rates and three peak diastolic flow rates were noted for each individual and average values were recorded. RRI was then calculated using the formula: peak systolic flow – end-diastolic flow/peak systolic flow.

Statistical analysis:

Differences between groups were analyzed with the unpaired *t* test or the Mann-Whitney U test, where appropriate. Data are presented as mean (SD).Sensitivity, specificity, and diagnostic efficiency were calculated for each value of cystatin C, creatinine, and RRI (11). The cut off value was then determined at the maximum efficiency derived from analyzing all patients. The significance of differences in sensitivity, specificity, or efficiency between the parameters was evaluated by the McNemar test with a two tailed probability. A p value <0.05 was considered statistically significant for all tests applied.

Receiver-operator characteristic (ROC) curves were calculated by standard procedures. (12) The area under the curve (AUC) and 95% confidence interval (CI) were calculated for each plot. The statistical significance of differences between AUC values was determined as proposed recently. (13) Accordingly, a *z* value was calculated for each comparison and values of *z* above 1.96 were taken as evidence that ROC areas were different, assuming a two tailed probability.

3. Results

The characteristics of the 50 enrolled patients and 20 controls are presented in Table I.

The mean value of serum cystatin C was significantly higher in patients with liver cirrhosis than controls (1.05± 0.32 and 0.65± 0.10mg/dl respectively) (t=5.29, P= 0.001). Moreover, serum cystatin C was significantly higher in Child C cirrhotic patients than in those with Child B and A (F=19.14 and P=0.00)

Significant positive correlations were found between serum cystatin C and each of BUN, serum creatinine, RRI and Child-Pugh score in patients with HCV induced liver cirrhosis.(r=0**.454,0.781,0.508** and 0**.412**respectively) ($p \leq 0.01$) (Table II). On the other hand, significant negative correlation was found between serum cystatin C and creatinine clearance. (r= **-0.746 and** $p \leq 0.01$) (Table II).

In patients with liver cirrhosis Receiver-operator characteristic (ROC) (Figure 1) curve analysis showed that the diagnostic potential of cystatin C and RRI to detect patients with a creatinine clearance of less than 80 ml/min was superior to that of creatinine. Cys.C at a cut off value of 1.14mg/dl had 85% specificity and 66% sensitivity. RRI at a cut off value of 0.665sec had 74% specificity and 79% sensitivity. Serum creatinine at a cut off value of 1.0500mg/dl had 70%specificity and 62% sensitivity to detect patients with a creatinine clearance of less than 80 ml/min.

Table (III) shows positive predictive value, negative predictive value and efficacy of serum Cystatin C, RRI and serum creatinine in the prediction of decreased creatinine clearance of less than 80 ml/min.

Table I: The characteristics in the four studied groups.

	Group I[a] Child A (n=7)	GroupI[b] Child B (n=20)	GroupI[c] Child C (n=23)	Group II Controls (n=20)	F	Significance between Groups
Sex, M/F	2/5	13/7	13/10	11/9	-	-
Age, years	43.5±1.61	42.±7.73	44.5±4.68	44±2.17	0.822	-
Bilirubin (up to 1.00 mg/dl)	0.54±0.05	1.05±0.58	4.27±1.50	0.29±0.11	50.999	I^a -I^c, I^b-I^c, I^b- II, I^c- II
Prothrombin time (11-15 sec)	14.71±0.26	15.34±2.05	20.89±2.57	11.70±1.41	37.933	I^a -I^c, I^a - II, I^b-I^c, I^b-IV, I^c- II
Albumin (3.4-5 g/dl)	3.05±0.05	2.42±0.52	1.91±0.22	4.17±0.17	24.898	I^a -I^b, I^a -I^c, I^a - IV, I^b-I^c, I^b- II, I^c-IV
BU(7-18 mg/dl	9.57±0.53	14.25±12.94	15.86±6.41	11.50±5.68	1.450	-
Creatinine (0.6-1.3 mg/dl)	0.71±0.01	1.03±0.33	1.10±0.23	0.62±0.07	4.966	I^a -I^b, I^a -I^c, I^b- II, I^c- II
Creatinine Clearance (80-130ml/min	121.14±1.06	81.94±14.98	79.63±33.06	151.75±16.11	6.448	I^a -I^b, I^a -I^c, I^a – II, I^b- II, I^c- II
Cystatin C (mg/dl)	0.66±0.05	1.02±0.28	1.17±0.32	0.65±0.10	7.857	I^a -I^b, I^a -I^c, I^b-I^c, I^b- II, I^c- II
RRI (sec)	0.57±0.01	0.63±0.03	0.73±0.08	0.53±0.02	22.892	I^a - I^c, I^b-I^c, I^b- II, I^c- II
Child Pugh score	6±0.00	8.05±0.68	12.26±1.78		-	-

* Significant at $P \leq 0.05$

Data are given as mean±SD

Table II: Correlations between serum Cystatin C and studied parameters

	Serum cystatin C
BUN	0.454**
Cr	0.781**
CrCl	-0.746**
RRI	0.508**
Child-Pugh score	0.412**

**. Correlation is significant at the 0.01 level (2-tailed).

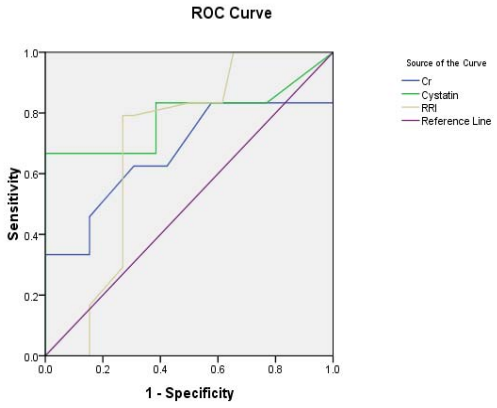

Figure 1: Receiver-operator characteristic curves for detection of patients with a creatinine clearance less than 80 ml/min. Sensitivity and specificity are displayed at various discrimination levels for serum concentration of cystatin C, RRI and serum creatinine.

Table (III): Positive predictive value, negative predictive value and efficacy of serum Cystatin C, RRI and serum creatinine

	Positive predictive value	Negative predictive value	Efficacy
Cystatin C	80%	73%	76%
RRI	70%	78%	74%
Serum creatinine	60%	66%	64%

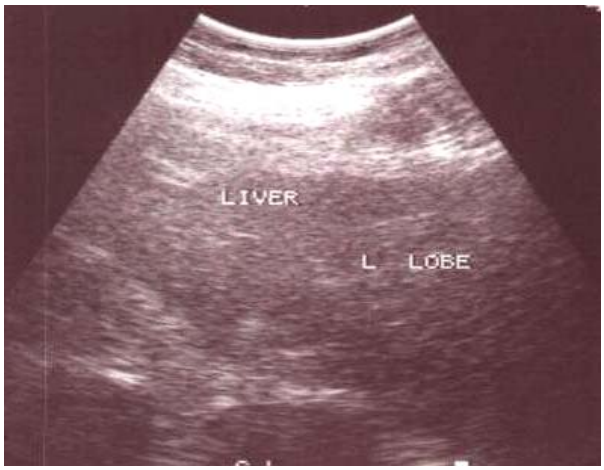

Figure 2: US picture showing cirrhotic shrunken liver with caudate lobe hypertrophy.

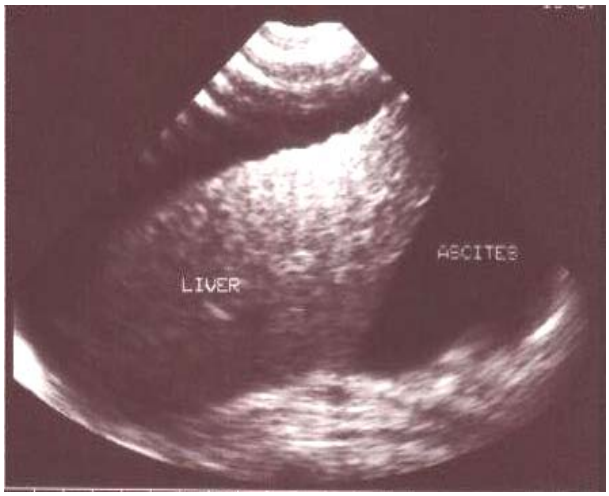

Figure 3: US picture showing cirrhotic liver and ascites.

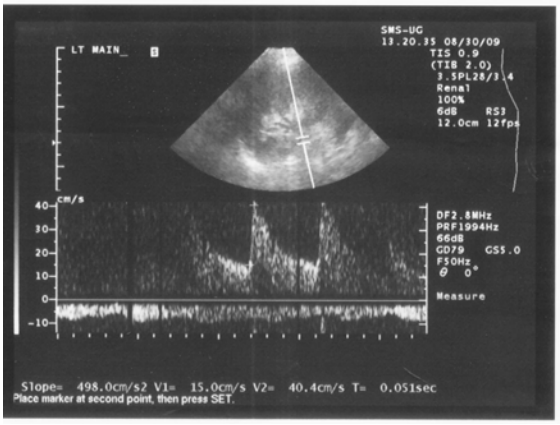

Figure 4: Renal Doppler Ultrasonography

4. Discussion:

Development of hepatorenal syndrome in patients with liver cirrhosis can be detected by measurement of serum creatinine. (14) On the other hand, serum creatinine levels can be influenced by multiple factors and it can miss cases with mild renal impairment. (15)

Therefore, the aim of this study was to investigate the value of serum cystatin C concentrations for the detection of moderately impaired renal function in chronic HCV Egyptian patients with liver cirrhosis as well as its correlation with Child-Pugh score and renal resistive index.

In the current work, Serum cystatin C was significantly higher in Child C cirrhotic patients than in those with Child B and A. Moreover, it was significantly higher in Child B cases than in Child A cases. Our results were in accordance with Randers et al. (16) and El-Agroudy et al. (17)

Gerbes et al. (18) evaluated serum cystatin C concentrations as a marker of renal function in patients with cirrhosis of the liver and found that Serum cystatin C concentrations are significantly increased in patients with cirrhosis and moderately impaired renal function. Similar results were obtained by Ustundag et al. (19)

In our study, the significant positive correlation noticed between serum cystatin C and Child Pugh score might indirectly reflect the degree of liver dysfunction which may limit the export of protein synthesized by the hepatocytes

On the other hand, Woitas et al. (20) reported that although, cystatin C was significantly higher in patients with Child B and C than in those with Child A, no differences were noted between patients with Child B and C.

In our study, 24 of 50 patients with a normal serum creatinine concentration had a decreased creatinine clearance below 80 ml/min. Furthermore, cystatin C showed higher sensitivity than creatinine

in detection of reduced creatinine clearance and hence GFR in patients with hepatitis C liver cirrhosis. ROC curve showed more diagnostic accuracy of cystatin C than serum creatinine for evaluating renal function.

Gerbes et al. (18) and Coll et al. (21) found that ROC curve support an advantage of cystatin C over serum concentrations of urea and creatinine and concluded that cystatin C tended to be more sensitive and specific than creatinine throughout almost the whole range of possible cut off values.

Woitas et al, (20) showed that the sensitivity of serum cystatin C for detecting reduced GFR is higher than that of creatinine in patients with liver cirrhosis.

Randers et al, (16) studied 36 patients with liver cirrhosis with normal to severely impaired kidney function and revealed a significant difference between cystatin C and plasma levels of creatinine in detection of reduced CrCl and hence GFR .These results are similar to those of Randers et al (22) and Price et al. (23)

El-Agroudy et al, (17) found that Serum cystatin C showed higher sensitivity and specificity than serum creatinine in the studied subjects and that Cystatin C showed more significant correlation than serum creatinine with GFR by 99mTc-DTPA techniques. Moreover, he reported that a mild degree of renal dysfunction may develop unnoticed as creatinine level may remain in the normal range despite a major decline in GFR, and the use of serum creatinine may inaccurately estimate GFR due to dietary intake, tubular secretion of creatinine.

These results confirm those noticed by Coll et al. (21) They reported that serum cystatin C levels started to increase when GFR was 88 ml/min/1.73 2m, while serum creatinine level began to increase when GFR was 75ml/min 1.73 2m. These data indicate that serum cystatin C may detect mild reduction in GFR than serum creatinine. Wang et al, (24) reported close correlation between creatinine clearance and 99m Tc-DTPA.

Similarly Page et al. (25) reported that the serum creatinine level failed to rise above normal even when the GFR (Tc-DTPA) was very low (less than 25 ml/min) in their study on 23 non-azotemic cirrhotic patients whose mean GFR (inulin) was 32 ml/min,

Meanwhile, Orlando et al. (26) showed similar diagnostic accuracies in cirrhotics for serum creatinine and cystatin C. This may be attributable to methodological reasons as they measured creatinine by the enzymatic PAP method, which is far less sensitive to interferences than the routinely used Jaffe reaction.

Demirtas et al. (27) and Risch et al, (28) found that compared to creatinine-based tests, serum cystatin C is a more sensitive indicator of early decreases in GFR in kidney recipients with transplant rejection and in all cirrhosis patients with ascites, regardless of the risk level for hepatorenal syndrome.

Cystatin C is synthesized by all nucleated cells at stable rate. It is completely metabolized within the proximal renal tubules. Accordingly, its low molecular weight with its steady production may clearly reflect GFR (29,30). Furthermore, Barret et al. (29) observed that cystatin C is not an acute phase reactant and is less expensive than the GFR (Tc-DTPA) test. Thus, it seems to be a reliable, fast and easy to use marker of renal failure.

Changes in renal hemodynamics are correlated with the stage of liver failure. Doppler ultrasonography allows non-invasive evaluation of intrarenal arterial resistance, as it can easily reveal renal vasoconstriction due to substantial splanchnic vasodilatation in cirrhosis patients (31).

Sacerdoti et al. (31) suggested that renal resistive index (RRI) measurement is also a sensitive marker of intrarenal hemodynamics and it has been reported to increase even in nonazotemic patients with cirrhosis. (31,32)

In the present work RRI was significantly higher in Child C patients than those with Child B or A. Moreover, it was significantly higher in child B patients than Child A cases. Our results was similar to those of Celebi et al. (33)

Ustundag Y et al (19) observed a trend towards higher RRI in cirrhotic patients with ascites than in those without ascites; however, the difference was not statistically significant. Another study that involved 44 cirrhosis patients revealed that only the subgroup that was azotemic and decompensated had significantly higher RRI compared to non-azotemic decompensated patients. (34)

Further work is needed to identify whether the high level of serum cystatin C in cirrhotic patients results from liver fibrosis or renal dysfunction in consequence of reduced GFR. Takeuchi et al (35) suggested that cystatin C may participate in the progression of fibrosis by inactivating cathepsins.

5. Conclusion:

Determination of serum cystatin C is advantagous over serum creatinine particularly in early detection of mild renal impairment in patients with liver cirrhosis.

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