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Original paper

Elevated cystatin C: is it a reflection for kidney or liver impairment in hepatic children?

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Abstract

Aim of the study: To assess if elevated serum cystatin C (Cyst-C) is an indicator for renal or hepatic dysfunction in presence of liver fibrosis.

Material and methods: Data of 50 children with chronic liver diseases (CLDs), out of which 25 were without renal impairment, and 25 with renal impairment were analyzed. Twenty healthy children served as a healthy control group. Routine investigations, creatinine clearance, hepatitis viral markers, abdominal ultrasonography, and liver biopsy were performed for patients with CLDs. Measurement of serum Cyst-C concentration by particle induced immunonephelometry were completed for both patients and control group.

Results: Results showed that serum Cyst-C is not correlated with the degree of hepatic impairment ($p > 0.05$). Cyst-C levels were significantly higher in patients with renal impairment (3.66 ± 0.85) than those without (0.71 ± 0.12), and healthy control group (0.63 ± 0.85). Cystatin-C showed significant elevation in patients with severe fibrosis with renal impairment (3.66 ± 0.85) than those without (0.76 ± 0.04) ($p < 0.0001$). Cyst-C at cutoff levels of 1.65 mg/l showed 100% accuracy in discrimination between those with and those without renal impairment. Cyst-C > 2.34 mg/l predicting GFR < 40 ml/min with accuracy of 90%. Cyst-C > 2.73 mg/l predicting GFR < 20 ml/min with accuracy of 81.5%.

Conclusions: Serum Cyst-C is a promising marker to estimate renal impairment in children with CLDs. Further studies are needed to estimate the accuracy of serum Cyst-C for early detection of renal impairment and close monitoring of the hepatic children.

Key words: cystatin C, chronic liver disease, renal impairment.

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Introduction

Chronic liver diseases (CLDs) are an important health issue since severe hepatic dysfunction could lead to persistent inflammation, necrosis, liver cirrhosis, and even hepatocellular carcinoma [1]. Hepatorenal syndrome (HRS) is a reversible functional renal impairment that occurs in patients with advanced liver cirrhosis or those with fulminant hepatic failure. It is characterized by marked reduction in glomerular filtration rate (GFR) and renal plasma flow (RPF) in the absence of other causes of renal failure; tubular function is preserved with the absence of proteinuria or histological changes in the kidney [2].

Demonstration of renal dysfunction by traditional methods has many difficulties. Serum creatinine (Cr) or calculated Cr clearance are the most convenient estimates of GFR, requiring only a single blood sample. However, serum Cr measurements in cirrhosis are affected by different factors such as loss of muscle mass, reduced dietary protein intake, sex, and ethnic origin as well as by interference with the standard Jaffe colo-

rimetric method of Cr determination by bilirubin and other compounds, which accumulate in liver failure. On the other hand, Cr is an imperfect filtration marker, because it is secreted by the tubular cells into the tubular lumen, especially if renal function is impaired. When the GFR is low, the serum Cr and Cr clearance overestimate the true GFR. Some drugs (such as cimetidine or trimethoprim) have the effect of reducing tubular secretion of Cr. Similarly, urea is synthesized by the liver and may be reduced as a consequence of hepatic insufficiency [3].

Creatinine clearance needs 24-hours urine collection, therefore it's unreliable especially in out-patients, as the collection may be incomplete. This results in an underestimation of renal function [4]. Inulin clearance cost and technical difficulties preclude its routine use [3]. β_2 microglobulin is affected by presence of infections or malignancies. Chromium-^{51} labeled ethylenediaminetetraacetic acid (^{51}Cr-EDTA) is costly, invasive, time consuming, and stressful for children and parents [5].

Cystatin C (Cyst-C), a low molecular weight protein produced at a constant rate by all nucleated cells, freely filtered across the glomerular membrane and neither secreted or absorbed, and completely catabolized in the proximal tubule, independent of muscle mass, sex and age [3]. Plasma cyst-C is a new marker of GFR [6], which provides accurate data for calculating GFR independent of the drug doses [7].

The value of serum Cyst-C as a marker for liver fibrosis and renal dysfunction in children with CLDs will be evaluated in our study.

Material and methods

Data of fifty children with CLDs with different etiologies (Table 1) from the Pediatric Hepatology Department, National Liver Institute, Menofia University, between May 2011 and May 2015 were analyzed. Twenty-five of them were without renal impairment (group 1), and 25 children with renal impairment (group 2), which was evaluated on the base of blood urea, serum Cr, and Cr clearance. Twenty healthy children of matched age and sex serves as a healthy control group (group 3). All children were provided with a written informed consent that was signed by the parents of each child in order to participate in the study, and for collection and storage of serum. The study was approved by the Research Ethics Committee of the National Liver Institute, Menofia University, and conforms to the 1975 Declaration of Helsinki.

Diagnosis of CLDs were based on clinical, biochemical, serological, and histopathological investigations. All groups included in this study were investigated on the base of full medical history and through clinical examinations. Complete blood count (CBC), liver function tests (LFTs), complete urinalysis, blood urea, serum Cr, and Cr clearance, hepatitis markers, especially hepatitis B (HB) surface antigen, anti-HB core antibody, and hepatitis C virus (HCV) antibody were performed for all patients. Measurement of serum Cyst-C concentration by particle induced immunonephelometry were performed for all patients and the control group. Abdominal ultrasonographic examination was completed to define the status of the liver, spleen, kidney, and other abdominal findings. Ultrasound-guided liver biopsy specimens were performed for all the patients. Serum samples were obtained, and renal functions were assessed by serum Cr, calculated GFR (CGFR) and serum Cyst-C measurement. Serum Cr was measured by Jaffe reaction using Bayer Dax 48 and expressed in micromole per liter. CGFR was obtained by applying the Schwartz formula:

$$\text{CGFR} = \frac{\text{Height (cm)} \times 0.413}{\text{Cr (mm/l)}}$$

Table 1. The etiology of chronic liver diseases with and without renal impairment

Group 1 (*n* = 25)	%	Cystatin-C level	Group 2 (*n* = 25)	%	Cystatin-C level
Cholestasis	36	0.70 ± 0.14	Viral hepatitis (B & C)	52	3.43 ± 0.8
Wilson's disease	16	0.79 ± 0.01	Budd-Chiari syndrome	20	4.05 ± 1.4
Viral hepatitis	12	0.75 ± 0.04	Congenital hepatic fibrosis	16	3.98 ± 0.14
Naïve autoimmune hepatitis	12	0.63 ± 0.34	Naïve autoimmune hepatitis	12	3.55 ± 0.1
Metabolic disease	8	0.67 ± 0.14			
Congenital hepatic fibrosis	8	0.72 ± 0.13			
Malignancy (hepatoblastoma)	4	0.71 ± 0.15			
Budd-Chiari syndrome	4	0.71 ± 0.17			
p-value		0.789			0.497

Serum Cyst-C was measured with N Latex Cyst-C kit on the Dad Behring Nephelometer BN2, using the particle enhanced nephelometric immunoassay (PENIA) methods. Results are expressed in mg/l.

Impaired GFR was defined as: GFR > 80 ml/min/1.73 m^2 [8].

Statistical analysis was performed by SPSS (Statistical Package for Social Science) program version 13 for Windows. Correlation between quantitative variables were assessed with the Spearman correlation coefficient (*r*). Comparisons of quantitative data between each 2 groups were done by Mann-Whitney U-test. χ^2 test was used to compare the qualitative data. The diagnostic validity of Cyst-C to detect reduced GFR was evaluated by receiver operative characteristic curve (ROC) analysis. SPSS was used for calculation of the area under the ROC curve, and the sensitivity/specificity data at certain cutoffs.

Results

In total, 50 children with CLDs were enrolled in the study; 25 of them without renal impairment (group 1) and 25 with renal impairment (group 2). Twenty healthy children (group 3) represented healthy control group. Group 1, group 2, and group 3 mean age were 8.9 ± 2.8, 10.2 ± 3.1, and 9.6 ± 4.6 years, respectively ($p = 0.183$). Males represented 64%, 64%, and 60% in group 1, group 2, and group 3, respectively ($p = 0.916$). On comparing, the levels of cystatin-C between different etiologies in each group were found of no significant difference (Table 1).

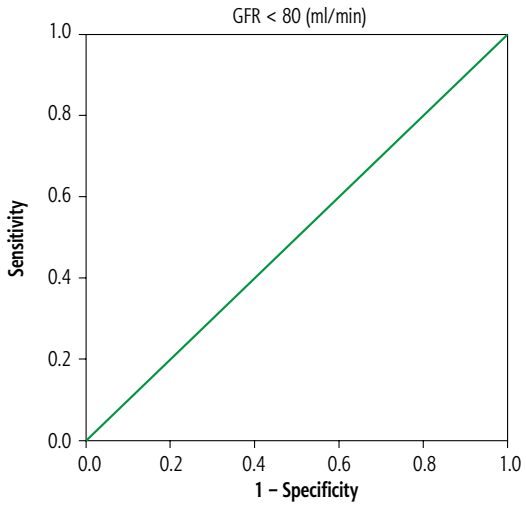
No statistical significant difference between group 1 and 2, with regards to CBC parameters and LFTs were found. Urea, Cr levels, and Cyst-C levels were significantly higher in group 2 than in group 1 and 3. Cr clearance was significantly impaired in group 2, while no significant difference was found between group 1 and 3 (Table 2). Cyst-C, at a cutoff level of 1.65 mg/l had sensitivity, specificity, and positive predictive value (PPV). Negative predictive value (NPV) and accuracy of 100% in discrimination between those with renal impairment and those without (Fig. 1) were found. Cyst-C > 2.34 mg/l predicting GFR < 40 ml/min with accuracy of 90%. Cyst-C > 2.73 mg/l predicting GFR < 20 ml/min with accuracy of 81.5%. Significant correlation between Cyst-C and AST, ALT, and GGT was found in group 1, while we did not found similar results in group 2 (Table 3). Cyst-C was not correlated with the true function of the liver (albumin and prothrombin) in any of both groups (Table 3).

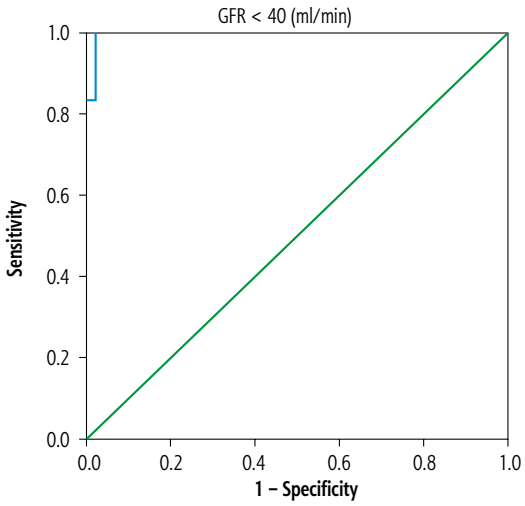
On assessing the mean level of cystatin-C in different grades of fibrosis, we found significant elevation in cystatin-C level in kidney impaired patients with severe fibrosis (3.66 ± 0.85) than patients with severe fibrosis but with preserved kidney function (0.76 ±

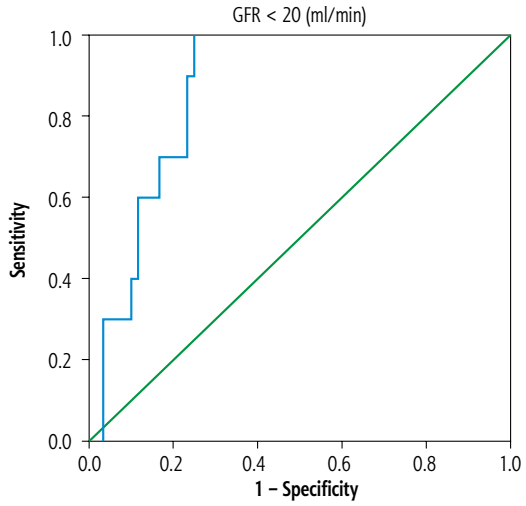
Table 2. Disease duration and laboratory parameters in all studied groups

Parameters	Group 1 (*n* = 25)	Group 2 (*n* = 25)	Group 3 (*n* = 20)	P1	P2	P3
Disease duration (years)	3.8 ± 1.9	5.5 ± 2.7		0.027		
Hb (gm/dl)	10.25 ± 1.8	9.29 ± 1.1	11.91 ± 0.87	0.077	< 0.0001	< 0.0001
WBC × 10^3/ml	10.66 ± 9.2	11.68 ± 6.9	7.68 ± 2.04	0.177	0.472	0.011
Platelets × 10^3/ml	178.8 ± 137.7	133.84 ± 100.4	243.2 ± 73.2	0.268	0.018	< 0.0001
TB (mg/dl)	10.6 ± 14.2	8.60 ± 10.5	0.86 ± 0.17	0.961	0.004	< 0.0001
DB (mg/dl)	6.18 ± 8.1	6.22 ± 8.2	0.15 ± 0.39	0.846	< 0.0001	< 0.0001
ALT (IU/l)	127.30 ± 106	89.72 ± 65.4	23.2 ± 7.1	0.177	< 0.0001	< 0.0001
AST (IU/l)	183.24 ± 204	111.64 ± 67.4	20.2 ± 6.6	0.574	< 0.0001	< 0.0001
GGT (IU/l)	100 ± 101.9	107.6 ± 102.4	25.2 ± 7.58	0.938	< 0.0001	< 0.0001
TP (gm/dl)	6.06 ± 0.79	6.17 ± 1.1	7.06 ± 0.27	0.892	< 0.0001	0.011
Alb (gm/dl)	2.88 ± 0.87	2.65 ± 1.05	4.18 ± 0.32	0.214	< 0.0001	< 0.0001
PC %	52.6 ± 26.7	52.1 ± 19.2	94.4 ± 4.35	0.648	< 0.0001	< 0.0001
UREA (mg/dl)	32.83 ± 27.9	192.44 ± 74.7	26.7 ± 7.62	< 0.0001	0.314	< 0.0001
Cr (mg/dl)	0.47 ± 0.24	3.61 ± 1.5	0.66 ± 0.27	< 0.0001	0.127	< 0.0001
Cr clearance (ml/min)	121.8 ± 11.1	23.96 ± 8.2	124.8 ± 13.7	< 0.0001	0.464	< 0.0001
Cyst-C (mg/l)	0.71 ± 0.12	3.66 ± 0.85	0.63 ± 0.85	< 0.0001	0.08	< 0.0001

P1 – probability of significance between group 1 and group 2; P2 – probability of significance between group 1 and group 3; P3 – probability of significance between group 2 and group 3







	GFR < 80	GFR < 40	GFR < 20
AUROC	1	0.996	0.868
p-value	< 0.0001	< 0.0001	< 0.0001
Cutoff	> 1.65	> 2.34	> 2.73
Sensitivity	100%	100%	90%
Specificity	100%	97.8%	76.7%
PPV	100%	100%	80%
NPV	100%	100%	95.8%
Accuracy	100%	99%	81.5%

AUROC – area under the receiver operative characteristic curve, PPV – positive predictive value, NPV – negative predictive value

Fig. 1. ROC curve showing performance of Cyst-C in discrimination between different grades of renal impairment

0.04), and those with moderate (0.63 ± 0.12) and mild fibrosis (0.73 ± 0.14) ($p < 0.0001$).

Discussions

Attempts have been made to use non-invasive techniques for assessment of the degree of liver fibrosis and renal impairment in hepatic children.

The current study showed that serum Cyst-C levels is affected by the degree of kidney impairment (group 2). Serum Cyst-C levels were closely correlated with the levels of urea, Cr, and Cr-GFR. In agreement with that of Park *et al.*, an increased serum Cyst-C levels were found to be related to decreased renal function [9]. Moreover, several reports have suggested that Cyst-C is more sensitive than Cr in detecting reduced renal function, and its levels are a good marker for predicting HRS and survival in patients with cirrhotic ascites and normal Cr levels [10]. Gerbes *et al.* reported that serum Cyst-C proved to be a valuable tool for early diagnosis of moderately impaired renal function in adult patient with cirrhosis [11].

The absence of correlation between serum Cyst-C and true functions of the liver (albumin and prothrombin) is associated with significant elevation of Cyst-C level in patients with severe fibrosis with renal impairment than in those without renal impairment. The nearer the levels of Cyst-C in control group and in patients without renal impairment, and absence of association between Cyst-C and the etiology of liver disease, reflects that the variations of Cyst-C levels occurred most likely according to the severity of renal

impairment, rather than severity of the hepatic condition. This was in accordance with a study done by Buyukberber *et al*., which may add to the accuracy of Cyst-C in detecting kidney function [12]. In agreement with that, Orlando *et al*. reported that plasma Cyst-C is a more accurate GFR marker in cirrhotic adult patients than plasma Cr and Cr-GFR [3].

We had calculated a Cyst-C cutoff point, validity in detecting cases of CLDs with renal impairment at 1.65 mg/l with 100% sensitivity and 100% specificity. Cyst-C > 2.34 mg/l predicting GFR < 40 ml/min with accuracy of 90%. Cyst-C > 2.73 mg/l predicting GFR < 20 ml/min with accuracy of 81.5%. Similar results were reported by Samyn *et al*.; they calculated a Cyst-C cutoff point at 1.06 mg/l with 91% sensitivity and 81% specificity [5]. Also, Kim *et al*. presented that the cutoff value of 1.1 mg/l could be an adequate reference levels for detecting early renal dysfunction in these patients [13]. Omar *et al*. found that serum CysC and CysC-based formulae were not only the best measures that reflected the actual renal performance in cirrhotic patients, but also the most accurate in detecting early stages of renal impairment in these patients [14].

Table 3. Correlation between Cyst-C in CLDs with and without renal impairment regarding to age, disease duration, and liver function

Parameter	Group 1 (*n* = 25)		Group 2 (*n* = 25)	
	r	*p*-value	*r*	*p*-value
Age (year)	0.184	0.377	–0.069	0.742
Disease duration (year)	0.163	0.437	–0.178	0.396
T. bilirubin (mg/dl)	0.151	0.471	–0.246	0.237
D. bilirubin (mg/dl)	0.150	0.473	–0.307	0.136
ALT (IU/l)	0.441	0.027	0.021	0.920
AST (IU/l)	0.667	< 0.0001	–0.085	0.687
GGT (IU/l)	–0.462	0.020	–0.140	0.504
TP (gm/dl)	–0.094	0.656	0.210	0.313
Alb (gm/dl)	0.035	0.870	0.082	0.698
PC (%)	0.201	0.335	0.048	0.821
Cr (mg/dl)	0.433	0.023	0.855	< 0.0001
Cr clearance (ml/min)	–0.951	< 0.0001	–0.977	< 0.0001

Conclusions

Serum Cyst-C is a promising marker to estimate renal impairment in children with CLDs. Further studies are needed to estimate the accuracy of serum Cyst-C for early detection of renal impairment and close monitoring of the hepatic children.

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Disclosure

Authors report no conflict of interest.

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