

Serum Cystatin C Levels in Children with Nephrosis or Diabetes: A Pilot Study

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ABSTRACT

Background: The glomerular filtration rate is the best overall index of renal function in health and disease. Recently, cystatin C, a low molecular weight protein, freely filtered through the glomerulus and almost completely reabsorbed and catabolized by tubular cells, has been proposed as a new and very sensitive serum marker of change in glomerular filtration rate.

Methods: This study investigated the relationship between cystatin C and creatinine in nephrotic and diabetic children. Serum cystatin C was determined by particle-enhanced immunoturbidimetry using the cystatin PET-kit. Serum creatinine, urea, and albumin were determined by auto-analyzer.

Results: Sensitivity and specificity for

the diagnosis of renal impairment (defined as $GFR < 35.85$ mL/min) calculated by a receiver operating characteristic (ROC) curve for serum cystatin C and serum creatinine in all groups. With a cutoff value of 35.85 mL/min, the area under the ROC curve (AUC) was 0.75, 0.76 (respectively) for creatinine, and 0.68, 0.62 (respectively) for cystatin C in nephritic and diabetic group. The AUC was statistically significant between serum cystatin and creatinine. The ROC plot indicates that cystatin c is a better marker than serum creatinine for detecting impaired GFR. There was no correlation between cystatin C and creatinine levels in all groups.

Conclusion: Serum cystatin C measurement appears to be equivalent to creatinine as a marker for estimating the glomerular filtration rate in children with nephritis and diabetes.

INTRODUCTION

The glomerular filtration rate (GFR) is an important index of renal function in health and disease.¹⁻³ It is defined as the

Table 1. Clinical Characteristics of The Patients and Control Group (Value represent the mean \pm SD).

Variables	Control (n=25)	Diabetic (n=21)	Nephrotic (n=22)	Two tailed F [*]	Significance P
Age (Year)	8.0 \pm 1.9	8.9 \pm 2.0	7.64 \pm 2.1	2.25	0.114
Weight (Kg)	25.4 \pm 7.7	28.4 \pm 7.5	28.8 \pm 8.6	1.35	0.267
Height (Cm)	124.0 \pm 13.2	131 \pm 12.7	119.2 \pm 14.4 [†]	4.20	0.019
BMI (kg/m ²)	16.2 \pm 1.8	16.3 \pm 1.4	19.9 \pm 2.2 [‡]	30.70	0.000
Urea (mmol/L)	5.4 \pm 1.0	5.65 \pm 1.6	7.43 \pm 6.63	1.80	0.166
Glucose (μ mol/L)	5.5 \pm 0.5	16.5 \pm 0.5 [‡]	5.7 \pm 0.5	368.08	0.000
Creatinine (mmol/L)	36.2 \pm 9.0	47.03 \pm 13.4	49.50 \pm 31.4	2.95	0.059
Cystatin C (mg/L)	0.70 \pm 0.2	0.8 \pm 0.2	1.0 \pm 0.5 [†]	4.32	0.017
Albumin (g/L)	45.1 \pm 5.0	45.1 \pm 5.10	18.9 \pm 4.8 [†]	205.54	0.000

*One way ANOVA test
[†]P<0.05
[‡]P<0.0001

clearance of substance carried in the plasma that is not metabolized outside the kidney and that is filtered freely across the glomerular membrane.^{4,5} Although serum creatinine concentration is an indicator of the most commonly used measures of GFR, it is not accurate marker as an index of GFR. Glomerular hyperfiltration has been reported in children with diabetes after a short duration of the disease and is proposed to be a diabetic nephropathy.⁶⁻⁹ There are a number of problems with its use, particularly in children. Creatinine production is directly related to muscle mass,^{10,11} and the investigation of renal function in children is complicated by the superimposition of continuing renal development overlying any possible renal damage.¹¹ Serum creatinine is insensitive to small changes in renal function and is proportional to muscle mass and body weight, which increase with growth in children.^{4,11}

On the other hand, a number of reports have been shown that serum cystatin C concentration might be a relatively sensitive marker of GFR decrease.¹² Cystatin C is described as a basic protein (with low a molecular

weight odd 13.3 kDa) and as a potent inhibitor of cysteine proteinases.¹²⁻¹⁴ Cystatin C is generated at a constant rate by all nuclear cells, and its production is unaltered in inflammatory diseases.^{5,15,16} Cystatin C freely crosses the glomerular membrane as it is reabsorbed and metabolized in the tubules.^{5,12} The plasma concentration of cystatin C has been shown to be a significantly better marker for GFR than the plasma creatinine concentration both in adults^{16,17} and in children.¹⁸⁻²¹

In this study, we investigated the relationship between serum cystatin C and creatinine in children with nephrosis or diabetes.

MATERIALS AND METHODS

Patients and Controls

We investigated 22 children with nephrotic syndrome (10 female, 12 male; age, 7.64 \pm 2.13 years), 21 children with diabetes (8 female, 13 male; age, 8.9 \pm 2.00 years), and 25 healthy children (14 female, 11 male; age, 8.0 \pm 1.94 years).

Methods

Blood was collected after overnight fasting from all patients and control groups

Table 2. Diagnosis Accuracy of Reduced GFR from Serum Creatinine and Cystatin C in Nephrotic and Control Groups

	Threshold value	Area Under the ROC Curve Mean ± SE	Sensitivity	95% CI	Specificity	95% CI
Creatinine	35.85 [*]	0.75±0.07	0.68	0.45-0.86	0.56	0.34-0.75
	31.50 [†]		0.95	0.77-0.99	0.32	0.14-0.53
	42.5 [‡]		0.45	0.24-0.67	0.80	0.59-0.93
Cystatin C	0.73 [*]	0.68±0.08	0.73	0.49-0.89	0.60	0.38-0.78
	0.56 [§]		0.86	0.65-0.97	0.28	0.12-0.49
	0.88 [‡]		0.45	0.24-0.67	0.80	0.59-0.93

^{*}Optimum Cut –off value result from ROC curve
[†]Threshold with diagnostic sensitivity of 95%
[‡]Threshold with diagnostic sensitivity of 80%
[§]Threshold with diagnostic sensitivity of 86%

between 9 AM and 11 AM during regular outpatient visits. Venous blood samples were immediately centrifuged (1500xg for 10 minutes at +4°C) and serum samples were used for cystatin C, creatinine, urea, and albumin assays.

Serum cystatin C was determined by particle-enhanced immunoturbidimetry^{5,9} using the Cystatin PET-kit (DAKO, Hamburg, Germany) by auto-analyzer (Cobas-Mira Plus, Roche, Basel, Switzerland). The results could be obtained within 1 hour. Cuvettes were washed before performing the cyst C assay, as recommended. Serum CR, BUN, and albumin were determined by auto-analyzer (DPP Modular System, Roche, Switzerland).

The study protocol was approved by the Ethics Committee of Istanbul Faculty of Medicine in accordance with the ethical standards of the Helsinki declaration of 1975, and the parent’s oral consent was obtained in each case.

Statistical Analysis

One –Way Anova test was used for statistical analysis and multiple comparisons among groups were evaluated by Tukey test. Receiver Operating Characteristic

Curve (ROC) analysis was studied among groups as described by Zweig and Campbell⁽²²⁾. Among groups occurring by change and statistical significance was assigned as *P*<0.05.

RESULTS

Clinical characteristics of the diabetic, nephrotic, and control groups are shown in Table 1. Serum cystatin C and serum creatinine levels were significantly increased in the nephrotic group, whereas serum albumin significantly decreased when compared to the control and diabetic groups (*P*<0.05, *P*<0.0001 respectively). Serum glucose levels in the diabetic group were significantly increased when compared to other groups (*P*<0.0001). There was no correlation between cystatin C and creatinine in all groups.

Sensitivity and Specificity (Application Group)

Comparisons of ROC plots for cystatin C and creatinine are shown in nephrotic and diabetic groups (Figures 1 and 2, Table 1). The area under ROC plot for cystatin C was 0.685 ± 0.080 (nephritic

Table 3. Diagnosis accuracy of reduced GFR from serum creatinine and cystatin C in Diabetic and Control Groups

	Threshold value	Area Under the ROC Curve Mean ± SE	Sensitivity	95% CI	Specificity	95% CI
Creatinine	37.60 [*]	0.76±0.07	0.76	0.52-0.91	0.72	0.50-0.87
	28.75 [†]		0.90	0.76-0.99	0.16	0.04-0.36
	45.05 [‡]		0.52	0.29-0.74	0.84	0.63-0.95
Cystatin	0.70 [*]	0.62±0.08	0.71	0.47-0.88	0.56	0.34-0.75
	0.47 [†]		0.90	0.69-0.98	0.24	0.09-0.45
	0.90 [§]		0.47	0.21-0.65	0.80	0.59-0.80

^{*}Optimum cutoff value result from ROC curve.
[†]Threshold with diagnostic sensitivity of 90%
[‡]Threshold with diagnostic sensitivity of 84%
[§]Threshold with diagnostic sensitivity of 80%

group) and 0.621 ± 0.084 (diabetic group) when compared with control group (Tables 2 and 3) In both groups, the area under ROC plot for creatinine was 0.705 ± 0.075 and 0.756 ± 0.073 (Tables 2 and 3). Although there was a significant difference in area under curve (AUC) between creatinine and cystatin C in children with nephrosis, there was a significant difference between AUC and cystatin C in children with diabetes.

DISCUSSION

The serum creatinine concentration is the test frequently used to evaluate renal function in children and adults. The secretion or reabsorption of creatinine by the renal tubule is highly unpredictable, leading to under or overestimation of GFR.⁵ In addition, it is insensitive to small changes in renal function and is proportional to muscle mass and body weight, which increase with growth. Therefore, it has been thought that serum creatinine is a poor marker of GFR.^{10,23,24} Recently, it was suggested that serum cystatin C concentration provides a better indication of changes in GFR than does serum creati-

nine.⁵

In the present study, plasma cystatin C and creatinine values were measured as markers of GFR in nephritic, diabetic, and healthy children ranging from 6 to 10 years of age. In our healthy group, a plasma cystatin C concentration was similar to the previously reported study (0.70 ± 0.10 mg/L).^{25,26} Harmoinen A et al¹⁸ determined the reference ranges for plasma cystatin C in infants and children. They showed that cystatin C concentration was a better marker than creatinine due to dependence on body mass index and age in prepubertal children.

Finney et al¹¹ demonstrated that cystatin C concentration was effectively constant from 1 year of age upwards. They suggested that cystatin C might offer a considerable advantage to pediatric nephrologists in diagnosis of GFR. In addition, Le Bricon et al²⁷ found that cystatin C was an alternative and exact marker of allograft function in adult transplant patients. Its sensitivity in detection of acute reduction in GFR provides more rapid diagnosis of acute rejection or treatment of nephrotoxicity. Poge U et al²⁸ also showed that cystatin

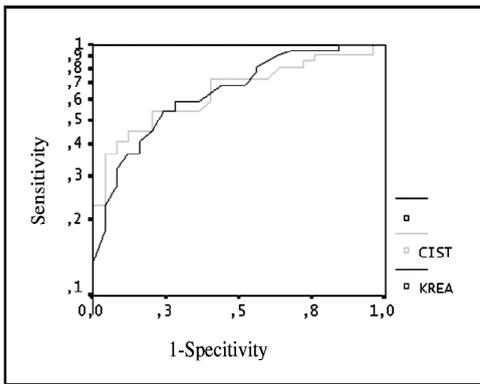


Figure 1. Receiver operating characteristic plots for creatinine and cystatin C levels in nephrotic and control groups.

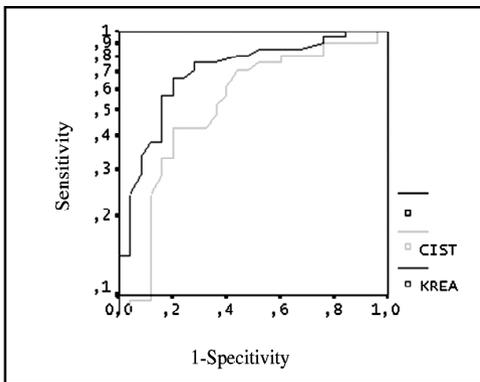


Figure 2. Receiver operating characteristic plots for creatinine and cystatin C levels in diabetic and control groups.

C was an alternative marker for assessment of GFR in renal allograft function, thus cystatin C might be preferred to creatinine. In pediatric renal transplant cases, cystatin C was not found to be effective marker for measurement of GFR.²⁹ In the present study, serum creatinine and cystatin C concentrations were more significantly increased in children with nephrotic syndrome than in children with diabetes. In addition, there was no significant correlation between cystatin C and creatinine in all groups. Recently, Holmquist and co-worker³⁰ reported that cystatin C could not be used as a useful marker to detect GFR in children with diabetes mellitus

type 2; our data are in agreement with this report.

Our results demonstrated that cyst C had higher sensitivity and met the criteria for a screening test for renal injury in nephrotic children. For this reason, serum cyst C may be a better marker than serum creatinine in children with renal injury. Serum cyst C is expected to be standardizing in children in the near future, so as to be used in clinical studies and daily practice.

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REFERENCES

1. Chantler C. The measurement of renal function in children: a review. *Guy's Hospital Report*. 1973;122:25-41.
2. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem*. 1992;38:1933-1953.
3. Prevot A, Martini S, Guignard JP. Glomerular filtration markers in Pediatrics. *Rev Med Suisse Romande*. 2002;122:625-660.
4. Deinum J, Derckx FHM. Cystatin C for estimation of glomerular filtration rate? *Lancet*. 2000;356:1624-1625.
5. Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Reference values for cystatin c serum concentration in children. *Pediatric Nephrol*. 1998;12:125-129.
6. Mogensen CE, Christensen CK, Pedersen MM, et al. Renal and glycemic determinants of glomerular hyperfiltration in normoalbuminuric diabetics. *J Diabetes Complications*. 1990;4:159-165.
7. Rudberg S, Persson B, Dahlquist G. Increased glomerular filtration as a predictor of diabetes nephropathy; an 8 year prospective study. *Kidney Int*. 1992;41:822-828.
8. Chiarelli F, Verrotti A, Morgese G. Glomerular hyperfiltration increase the risk of developing microalbuminuria in diabetic children. *Pediatric Nephrol*. 1995;9:154-158.
9. Levy-Marchel C, Laborde K, Kinderman C, Dechaux M, Czernichow P. Persisting glomerular hyperfiltration in short-term dia-

- betic children without microalbuminuria. *Acta Paediatr Scand.* 1989;78:712-716.
10. Schwartz GJ, Bricon LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children and adolescents. *Pediatr Clin North Am.* 1987;34:571-590.
 11. Finney H, Newman DJ, Thakkar H, Fell JME, Price CP. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. *Arch Dis Child.* 2000;82:71-75.
 12. Schück O, Gottfridova H, Maly J, et al. Blomerular filtration assessment in individuals after orthotopic liver transplantation based on serum cystatin C levels. *Liver Trans.* 2002;8:7:594-599.
 13. Stickle D, Cole B, Hock K, Hruska AK, Scott MG. Correlation of plasma concentration of cystatin C and creatinine to insulin clearance in a pediatric population. *Clin Chem.* 1998;44:1334-1338.
 14. Abrahamson M, Olafsson I, Palsdottir A, et al. Structure and expression of human cystatin C gene. *Biochem J.* 1990;268:287-294.
 15. Schück O, Teplan V, Jabor A, Stolova M, Skibova J. Glomerular filtration rate estimation in patients with advanced chronic renal insufficiency based on serum cystatin C levels. *Nephron Clin Parct.* 2003;93:146-151.
 16. Kyshe-Anderson J, Schmidt C, Nordin G, et al. Serum cystatin C, determination by a rapid, automated particle- enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem.* 1994;40:1921-1926.
 17. Newman D, Thakkar H, Edwards R, et al. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. *Kidney Int.* 1995;47:312-318.
 18. Harmoinen A, Ylinen E, Houhala MA, Janas M, Kaila M, Kauri T. Reference intervals for cystatin C in pre- and full-term infants and children. *Pediatric Neph.* 2000;15:105-108.
 19. Helin I, Axenram M, Grubb A. Serum cystatin C as a determinant of glomerular filtration rate in children. *Clin Neph.* 1998;49:221-225.
 20. Bökenkamp A, Domanetzi M, Zinc R, Schuman G, Byrd D, Brodehl J. Cystatin C? A new marker of glomerular filtration rate in children independent of age and height. *Pediatrics.* 1998;101:875-881.
 21. Ylinen E, Ala- Houhala M, Harmoinen A, Knip M. Cystatin C as a marker for glomerular filtration rate in pediatric patients. *Pediatric Nephrol.* 1999;13:506-509.
 22. Zweig MH, Campbell G. Receiver-operating characteristics (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem.* 1993;39:561-577.
 23. Bauer JH, Brooks CS, Burch RN. Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. *Am J Kidney Dis.* 1982;2:337-346.
 24. Harmoinen AP, Kouri TT, Wirta OR, Lehtimäki TJ, et al. Evaluation of plasma cystatin C as a marker for glomerular filtration rate in patients with type 2 diabetes. *Clin Nephrol.* 1999;52:363-370.
 25. Finney H, Newman DJ, Price CD. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. *Ann Clin Biochem.* 2000;297:55-66.
 26. Keevil BG, Kilpatrick ES, Nichols SP, Maylor PW. Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. *Clin Chem.* 1998;44:1535-1539.
 27. Le Bricon T, Thervet E, Benlakehal M, Bousquet B, et al. Changes in plasma cystatin C after renal transplantation and acute rejection in adults. *Clin Chem.* 1999; 45:2243-2249.
 28. Poge U, Stoschus B, Stoffel-Wagner B, Gerhardt T, et al. Cystatin C as an endogenous marker of glomerular filtration rate in renal transplant patients. *Kidney Blood Pres Res.* 2003;26:55-60.
 29. Krieser D, Rosenberg AR, Kainer G, Naidoo D. The relationship between serum creatinine, serum cystatin C and glomerular filtration rate in pediatric renal transplant recipients: A pilot study. *Pediatric Transplantation.* 2002;6:392-395.
 30. Holmquist P, Torffvit O, Sjoblad S. Metabolic status in diabetes mellitus affects markers for glomerular filtration rate. *Pediatr Nephrol.* 2003;18:536-540.