

SERUM CYSTATIN C AS A NEW MARKER OF GLOMERULAR FILTRATION RATE (GFR)

H. VAHHAB AGHAI, P. PASALAR,*
I. NAJAFI,** AND M. KADKHODAEI

*From the *Department of Physiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, the *Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, and the **Department of Nephrology of Dr. Shariati Hospital, Tehran University of Medical Sciences, Tehran, I.R. Iran.*

ABSTRACT

Cystatin C is a 13 KD basic protein that is a member of the cystatin super-family of cysteine protease inhibitors. The cystatin C gene seems to be a house keeping gene, which is compatible with a stable production rate of cystatin C by most cells. This protein is freely filtered through the glomerulus and almost completely reabsorbed and catabolized by proximal tubular cells.

Because of these characteristics cystatin C is assumed to be a better marker of glomerular filtration rate than other markers.

115 new cases of renal disease aged between 14 and 88 years and 121 healthy subjects, aged between 11 and 78 years were studied.

In all of the subjects serum cystatin C and creatinine were determined and creatinine clearance was determined only in patients. Cystatin C was determined by a particle-enhanced turbidimetric assay and creatinine was measured by Jaffe's method.

In addition, to assess the diagnostic efficiency of serum cystatin C in comparison to that of serum creatinine and creatinine clearance in predicting changes in GFR, we performed Tc99m - DTPA clearance on 53 subjects including controls and patients.

A linear relationship was found between Tc99m - DTPA clearance and 1/serum cystatin C ($r=0.712$, p -value <0.001), 1/serum creatinine ($r=0.709$, p -value <0.001) and creatinine clearance ($r=0.777$, p -value <0.001).

Diagnostic accuracy in the identification of reduced GFR measured as area under the receiver-operating characteristic plot was 0.878 ± 0.050 (Mean \pm SE) for cystatin C, 0.866 ± 0.051 for creatinine and 0.866 ± 0.051 for creatinine clearance. The serum cystatin C reference values (mean \pm 1.96 SD) determined was 0.83 - 0.88 mg/L.

A cutoff cystatin C concentration of 0.82 mg/L had 92% sensitivity and 79% specificity for detecting abnormal GFR.

There was no significant correlation between cystatin C and age (p -value <0.219) and weight (p -value <0.193).

This study demonstrates that serum cystatin C has an increased diagnostic accuracy for reduced GFR when compared with serum creatinine and creatinine clearance. Hence, cystatin C seems to be an alternative for the estimation of GFR.

INTRODUCTION

The estimation of glomerular filtration rate (GFR) is commonly performed by measuring renal clearance of exogenous or endogenous substances and by determining endogenous serum markers. Unfortunately, the ideal marker for measuring GFR has yet to be discovered. Although inulin clearance is considered the gold standard test, it is expensive and requires specialized technical personnel over a period of several hours.¹

Serum creatinine and creatinine clearance are the most widely used methods for the noninvasive estimation of GFR in clinical practice. Serum creatinine is considered relatively specific, but not very sensitive since its levels only increase significantly when more than 50% of the GFR is reduced. In the past, it was postulated that serum levels of low mo-

lecular mass proteins² such as β_2 -microglobulin,³ α_1 -microglobulin,⁴ retinol binding protein,⁵ etc. might reflect changes in GFR, because these proteins are freely filtered by the glomerulus and then almost completely reabsorbed and catabolized by proximal tubular cells.⁶

However, no protein marker has been effectively introduced into clinical practice, perhaps because they are significantly influenced by several extra-renal factors. Since 1985 cystatin C has been proposed as a new sensitive and accurate endogenous marker of changes in GFR.^{7,8}

Cystatin C is a nonglycosylated basic protein of the cystatin superfamily of cysteine proteinase inhibitors.⁹⁻¹² It has a relative molecular mass of 13359 and consists of 120 amino acid residues.¹³

Cystatin C is produced by all investigated nucleated cells, and the production rate is unaltered in inflammatory condi-

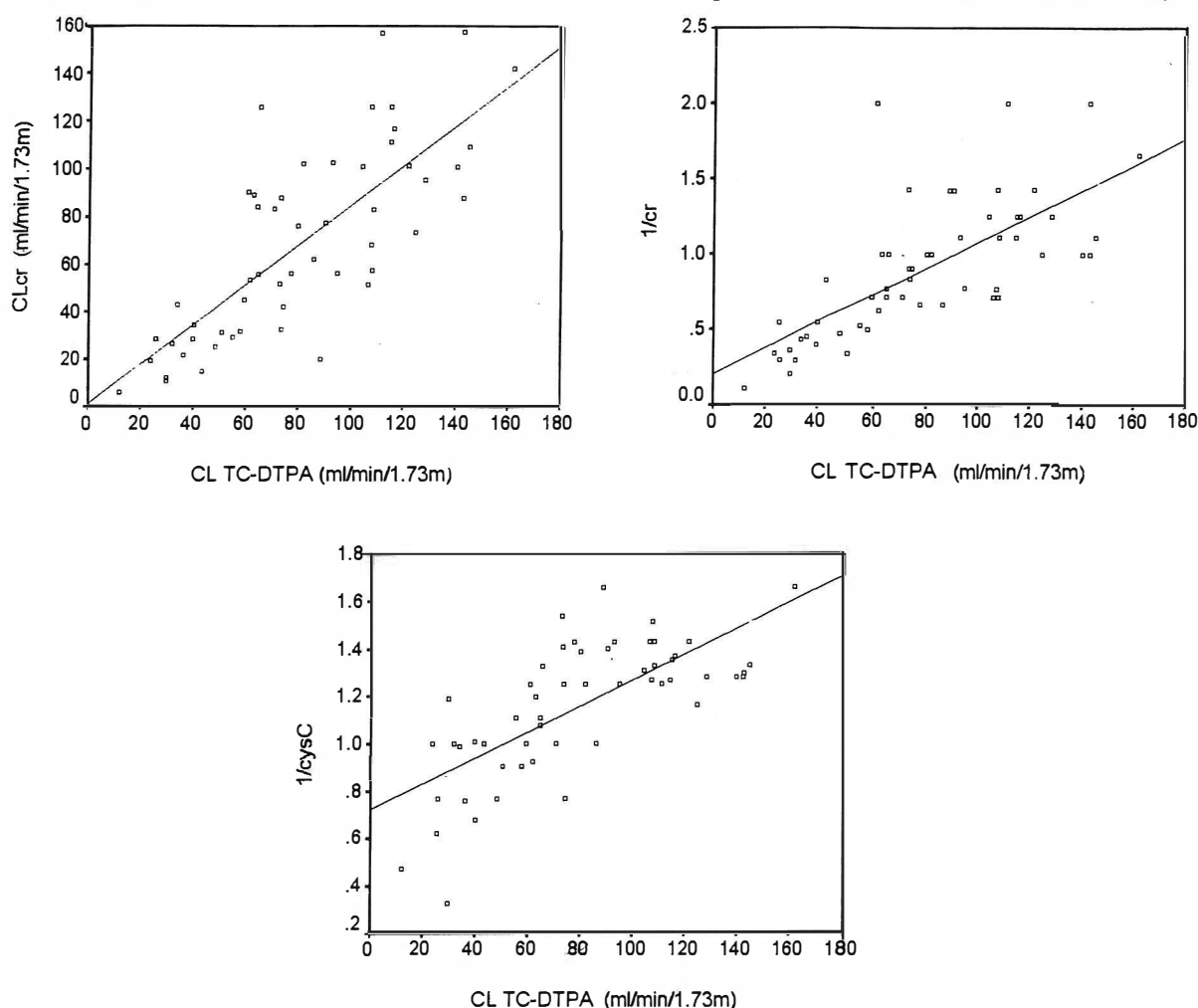


Fig. 1-A,B,C: Bivariate plots of Tc_{99m} -DTPA versus $1/cys\ C$, $1/Cr$ and $CLCr$ in 53 subjects. Linear regression analysis gave $1/cys\ C = 0.722 + 0.0055 \cdot CLTc_{99m}\text{-DTPA}$ ($r = 0.712$, $p < 0.001$), $1/Cr = 0.210 + 0.0087 \cdot CLTc_{99m}\text{-DTPA}$ ($r = 0.709$, $p < 0.001$) and $CLCr = 1.348 + 0.838 \cdot CLTc_{99m}\text{-DTPA}$ ($r = 0.777$, $p < 0.001$) indicated by bold lines.

tions.¹⁴ Determination of the structure of the cystatin C gene and its promotor has shown that the gene is of the house-keeping type which is compatible with a stable production rate by most cell types.¹⁵ Cystatin C is present in all investigated body fluids. Normal levels of serum cystatin C have been reported to be from 0.61-2.5 mg/L.¹³

Cystatin C is mainly catabolized by renal glomerular filtration followed by tubular reabsorption and degradation of the protein.¹⁶ The low molecular mass of cystatin C, in combination with its stable production rate, indicates that the plasma concentration of cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), making cystatin C a better indicator of GFR.^{17,18}

MATERIAL AND METHODS

We collected data from 121 healthy subjects (68 male, 53 female) aged between 11 to 78 years and 115 new cases of renal diseases (56 male and 59 female, serum creatinine <3.5 mg/dL) ranging in age from 14 to 88 years old.

Serum samples were obtained from healthy subjects and all patients to measure cystatin C and creatinine levels.

Urine samples were collected from patients to determine creatinine clearance. In addition to assess the diagnostic efficiency of serum cystatin C in comparison to that of serum creatinine and creatinine clearance in predicting changes in GFR, we performed Tc_{99m} -DTPA clearance on 53 subjects including healthy persons and patients.¹⁹

Serum cystatin C was determined using a commercially available kit (Dako Inc., Copenhagen, Denmark) for a rapid automated particle-enhanced turbidimetric method,^{20,21} which was applied on a Merck autoanalyzer.

Creatinine was determined by Jaffe's method with a Hitachi autoanalyzer.

For determination of Tc_{99m} -DTPA clearance, we used 2 venous plasma samples drawn between 2 and 4 hours after a single injection of 0.5 mCi Tc_{99m} -DTPA to define the elimination phase from plasma.

Statistical analysis was performed using EXCLE 97 and SPSS 9.1 statistical software for windows.

P-values below 0.001 were considered statistically significant. The correlation between the three endogenous markers and Tc_{99m} -DTPA was studied by linear regression analysis. In the evaluation of the results Pearson correlation coefficients were calculated. As the serum concentration of a substance is inversely related to its clearance, reciprocals of cystatin C and creatinine were calculated for comparison with CL Tc_{99m} -DTPA.²² Diagnostic sensitivity and specificity for the identification of abnormal renal function (CL Tc_{99m} -DTPA <80 mL/min/1.73m²) were studied by receiver-operating characteristic (ROC) plots.

RESULTS

In 121 healthy subjects, average age was 51±18 years, average weight was 65±14 kg and cystatin C values were normally distributed with a mean of 0.86±0.18 mg/L and the mean serum creatinine concentration was 0.73±0.24 mg/dL.

In 115 patients, average age was 54±16 years, average weight was 60±11.9 kg and mean serum cystatin C was 1.14±0.42 mg/L where the mean serum creatinine concentration was 1.63±0.84 mg/dL. By determination of creatinine clearance in this group, its mean was 26.2±12.3 mL/min/1.73m².

The mean GFR by determination of Tc_{99m} -DTPA clearance in patients was 57.3±26.7 mL/min/1.73m² and in 24 control subjects was 106.5±29 mL/min/1.73m².

In this study a linear relationship was found between Tc_{99m} -DTPA clearance and 1/serum cystatin C ($r=0.712$, p -value <0.001), 1/serum creatinine ($r=0.709$, p -value <0.001) and creatinine clearance ($r=0.777$, p -value <0.001).

Reciprocals of cystatin C and creatinine concentration and creatinine clearance were plotted against Tc_{99m} -DTPA clearance (Fig. 1-A, B, C).

No significant correlation was found between cystatin C level and age ($p<0.219$) and weight ($p<0.193$), when data for the 121 persons with normal GFR were used.

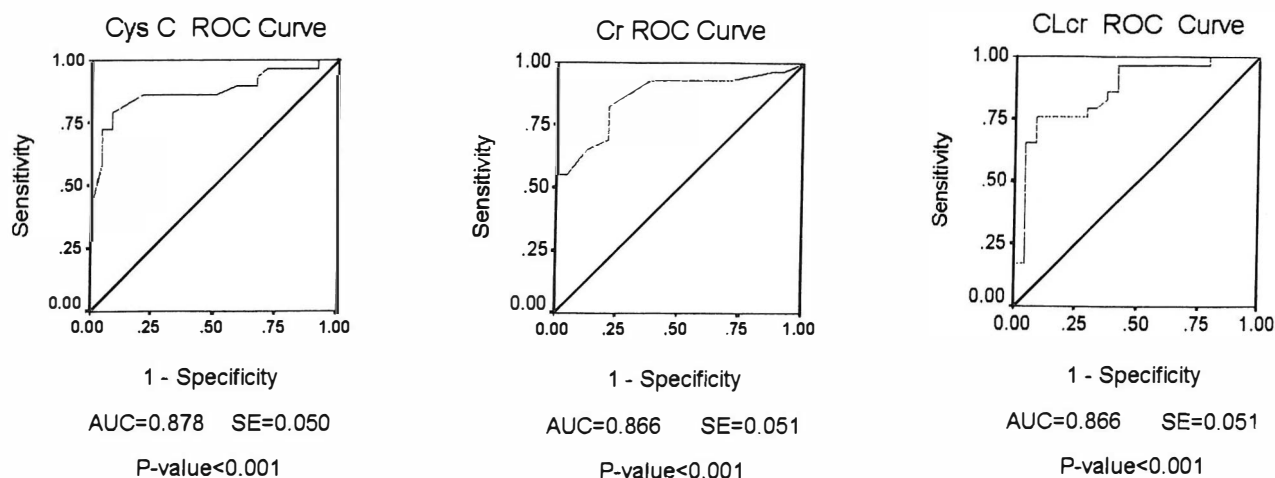


Fig. 2-A,B,C: ROC curves for serum cystatin C, creatinine and creatinine clearance in 53 cases and controls.

Figure 2 (A, B and C) shows the ROC plots for cystatin C, creatinine and creatinine clearance for all 53 cases and controls. The area under the cystatin C ROC curve ($AUC=0.878$, $SE=0.050$) was slightly (p -value <0.001) larger than that under the creatinine ($AUC=0.866$, $SE=0.051$) and creatinine clearance ($AUC=0.866$, $SE=0.051$) ROC curves demonstrating that the diagnostic accuracy of the serum level of cystatin C is superior to that of creatinine and creatinine clearance in identifying individuals with reduced GFR in the population studied. When the serum cystatin C concentration for all 121 persons with normal GFR was used to define the 95% confidence interval, this was found to be 0.83-0.88 mg/L.

From these data a cystatin C cutoff concentration of 0.82 mg/L detected impaired renal function with a specificity of 79% and sensitivity of 92%.

DISCUSSION

Cystatin C has been recognized as a potential marker of GFR since 1985. To test the hypothesis that cystatin C is a suitable marker of GFR, the present study set out to compare this parameter with the two parameters most commonly used in clinical practice, the serum creatinine concentration and creatinine clearance. All parameters were tested against the results of a Tc_{99m} - DTPA clearance serving as the gold standard.

This preliminary study on cystatin C in renal patients seems to indicate that this serum marker is not significantly influenced by extra-renal factors such as age and weight.

Our results show that cystatin C is closely related to GFR, significantly increasing when Tc_{99m} - DTPA clearance values decrease. The overall correlation between serum cystatin C and GFR was stronger than that between serum creatinine and GFR.

The diagnostic accuracy of cystatin C assessed by ROC analysis was greater than that of creatinine or creatinine clearance in discriminating between patients with normal GFR and those with reduced GFR. Thus when the objective is to identify subjects with GFR impairment (sensitivity), serum cystatin C may be clinically utilized as a more efficient diagnostic tool than serum creatinine clearance.

We should add here that the range of confidence interval and the cutoff limit point which we obtained from our data were lower than that announced in previous studies, which may have a genetic basis.

However, further studies are required in order to confirm these findings on more representative numbers of patients.

ACKNOWLEDGEMENTS

This study was supported by grant number 775 of Tehran Medical Sciences University.

REFERENCES

1. Duarte CG: Renal function tests, 1 st ed., Boston: Little, Brown and Company, p320, 1980.
2. Bernier GM, Cohen RJ, Conrad ME: Microglobulinaemia in renal failure. *Nature* 218: 598-599, 1968.
3. Trollfors B, Norrby R: Estimation of glomerular filtration rate by serum creatinine and serum β_2 -microglobulin. *Nephron* 28: 196-199, 1985.
4. Kusano E, Suzuki M, Asano Y, Takagi K, Kawal T: Human α_1 -microglobulin and its relationship to renal function. *Nephron* 41: 320-324, 1985.
5. Bernard A, Vyskocyl AA, Mahieu P, Lauwerys R: Effect of renal insufficiency on the concentration of free retinol-binding protein in urine and serum. *Clin Chem Acta* 171: 85-94, 1988.
6. Jung K, Schulze BD, Sydlow K, Pergrande M, Precht K, Schreiber G: Diagnostic value of low-molecular mass proteins in serum for the detection of reduced glomerular filtration rate. *J Clin Chem Clin Biochem* 25: 499-503, 1987.
7. Kyhse-Andersen J, Schmidt C, Nordin O, Andersson B, Nilsson-Ehle P, Lindstrom V, Grubb A: Serum cystatin C, determined by a rapid automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem* 40: 1921-1926, 1994.
8. Newman DJ, Thakkar H, Edwards RG, Wilkie M, White T, Grubb AO, Price CP: Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. *Kidney Int* 47: 312-318, 1995.
9. Grubb A, Lofberg H: Human γ -trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis. *Proc Natl Acad Sci USA* 79: 3024-3027, 1982.
10. Barrett AJ, Davies ME, Grubb A: The place of human γ -trace (cystatin C) amongst the cysteine proteinase inhibitors. *Biochem Biophys Res Commun* 120: 631-6, 1984.
11. Gauthier F, Pagano M, Esnard F, Mouray H, Engler R: A heat stable low molecular weight inhibitor of lysosomal cysteine proteinases in human serum. *Biochem Biophys Res Commun* 110: 449-55, 1983.
12. Brzin J, Popovic T, Turk V, Borchart U, Machleidt W: Human cystatin, a new inhibitor of cysteine proteinases. *Biochem Biophys Res Commun* 118: 103-9, 1984.
13. Grubb A: Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol* 38: S20-S27, 1992.
14. Swan S: The search continues-an ideal marker of GFR. *Clin Chem* 43 (6): 913-14, 1997.
15. Abrahamson M, Olafsson L, Plasdotir A, Ulvsback M, Lundwall A, Jesson O, Grubb A: Structure and expression of the human cystatin C gene. *Biochem J* 268: 287-294, 1990.
16. Jacobsson B, Lignelid H, Bergerheim USR: Transthyretin and cystatin C are catabolized in proximal tubular epithelial cells and the proteins are not useful as markers for renal cell carcinomas. *Histopathology* 26: 559-564, 1995.
17. Newman DJ, Thakkar H, Edwards RG, Wilkie M, White T, Grubb A, Price CP: Serum cystatin C: a replacement for crea-

- tinine as a biochemical marker of GFR. *Kidney International* 46 (47): S20-S21, 1994.
18. Tian S, Kusano E, Ohara T, Tabei K, Itoh Y, Kawai T, Asano Y: Cystatin C measurement and its practical use in patients with various renal diseases. *Clin Neph* 48 (2): 104-108, 1992.
19. Randers E, Kristensen JH, Erlandsen EJ, Danielsen H: Serum cystatin C as a marker of the renal function. *Scand J Clin Lab Invest* 58 (7): 582-92, 1998.
20. Nilsson P, Grubb A: New markers for the determination of GFR: Iohexol clearance and cystatin C serum concentration. *Kidney Int* 46 (47): s-17-s-19, 1994.
21. Norlund I, Fex G, Lanke J, von Schenck H, Nilsson JE, Leksell H, Grubb A: Reference intervals for the glomerular filtration rate and cell-proliferation markers: serum cystatin C and serum β_2 -microglobulin/cystatin C-ratio. *Scand J Clin Lab Invest* 57: 463-470, 1997.
22. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J: Cystatin C-A new marker of glomerular filtration rate in children independent of age and height. *Pediatrics* 101 (5): 875-881, 1998.

