Cystatin C as a Marker of Glomerular Filtration Rate in Voluntary Kidney Donors

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Abstract

Objectives: **Cystatin C is emerging as an endogenous marker of glomerular filtration rate. This study sought to assess the usefulness of serum cystatin C as a marker of glomerular filtration rate in comparison with serum creatinine and serum creatinine-based glomerular filtration rate estimations in voluntary kidney donors.**

Materials and Methods: **Serum cystatin C and serum creatinine were estimated in 35 voluntary kidney donors. Glomerular filtration rate was estimated using: (1) Cockcroft-Gault method normalized to 1.73 m^2 of body surface area, (2) 4-variable Modification of Diet in Renal Diseases formulae, and (3) ^{99m}Tc-DTPA double plasma sampling method. Glomerular filtration rate-double plasma sampling method was used as a reference value. Results were expressed as means ± SD.**

Results: **The mean age of the participants was 44.23 ± 8.61 years old (19 women, 16 men). The mean serum creatinine was 0.83 ± 0.14 mg/dL, and the mean serum cystatin C was 0.71 ± 0.12 mg/L. Serum cystatin C showed significant correlation with serum creatinine (r = 0.864; *P* < .001). Glomerular filtration rate-MDRD showed the strongest correlation with glomerular filtration rate-double plasma sampling method (r = 0.93; *P* < .001), followed by glomerular filtration rate-Cockcroft-Gault (r = 0.76; *P* < .001), serum creatinine (r = - 0.68; *P* < .001), and serum cystatin C (r = - 0.59; *P* < .001). The mean serum cystatin C values were 22.6%**

higher in men than in women. There was a significant correlation of serum cystatin C with glomerular filtration rate-Cockcroft-Gault (r = - 0.50; *P* = .002), glomerular filtration rate-MDRD (r = - 0.59; *P* < .001), and glomerular filtration rate-double plasma sampling method (r = - 0.59; *P* < .001).

Conclusions: **Serum cystatin C is an optimal marker of glomerular filtration rate in voluntary kidney donors.**

Key words: *Cockcroft-Gault formula, MDRD formula, ^{99m}Tc-DTPA double plasma sampling method*

Introduction

Accurate estimation of glomerular filtration rate (GFR) is essential for appropriate donor selection in live-donor transplant. Inulin clearance and radioisotope renograms are ideal methods but are time-consuming and cumbersome. There is a need for a noninvasive marker that can avoid these difficulties and serve as a rapid screening tool in donor evaluation. Serum creatinine (SCr)-based GFR estimates vary depending on the individual's age, race, muscle mass, and sex. Although serum cystatin C (SCysC) has been identified as an improved endogenous marker of GFR,[1] its clinical use has not been established fully. We studied the role of cystatin C as a noninvasive marker of GFR in voluntary kidney donors.

Materials and Methods

This is a single-center study involving 35 voluntary kidney donors selected after initial clinical and biochemical evaluation. Exclusion criteria were age ≥ 60 years, measured with ^{99m}Tc-DTPA (double plasma sampling method) glomerular filtration rate

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< 80 mL/min/1.73 m^2, serum creatinine ≥ 1.5 mg/dL, impaired fasting glucose and/or impaired glucose tolerance, history of thyroid illness and/or deranged thyroid function tests, and medication use in the past month and/or during the time of enrollment. Informed consent was obtained from all participants. The study was approved by the institute review committee and conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Study samples for SCysC and SCr were obtained on the day of GFR estimation before the procedure. Serum cystatin C samples were either immediately analyzed or stored at 2°C to 4°C for a maximum of 21 days. Serum creatinine was measured immediately by Jaffe's kinetic method using the Transasia EM200 Autoanalyzer (Transasia Bio Medicals, LTD., Mumbai, India), and GFR was calculated using the Cockcroft-Gault (GFR-CG) method[2] normalized to 1.73 m^2 of body surface area, and 4-variable MDRD (GFR-MDRD)[3] formulae: GFR-MDRD = 186 × creatinine$^{-1.154}$ × age$^{-0.203}$ × (0.742, if female) (age in years and serum creatinine in mg/dL). Body surface area (BSA) was calculated using the Mostellar formula: BSA (M^2) = ([height (cm) × weight (kg)] ÷ 3600)½. Serum cystatin C was measured by particle-based immunoturbidimetric assay (AutoPure EU-FLO reagent kit, Accurex Biomedical Pvt. Ltd, Mumbai, India) on an Transasia EM200 Autoanalyzer.

Glomerular filtration rate FR (GFR - DTPA) was measured using the ^{99m}Tc-DTPA (diethylenetriamine pentaacetic acid) 2-plasma sample method of Russell. After a 12-hour overnight fast, 1 MCU of ^{99m}Tc-DTPA was administered intravenously and plasma samples were obtained at 60 minutes and 180 minutes postinjection. Glomerular filtration rate was measured by slope intercept method using Russell's algorithm.[4]

Results are expressed as means ± SD. A *P* value of < .05 was considered significant. Correlation between variables was studied using Pearson product moment correlation for parametric variables. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 17.0, IBM Corporation, Armonk, New York, USA).

Results

Baseline characteristics of the 35 participants are shown in Table 1. The mean age of participants was 44.23 ± 8.61 years and mean BSA was 1.70 ± 0.11. The GFR-CG was significantly higher compared with GFR-MDRD (*P* = .04; 95% CI). However, there was no significant difference between GFR-DTPA with GFR-CG or GFR-MDRD. The mean SCr and SCysC values of male participants were 18.5% and 22.6% higher than female participants. When distribution of serum cystatin C was stratified by participant sex, overall distribution was shifted to higher values in men as compared with women, as is seen with serum creatinine (Figure 1).

SCysC showed significant correlation with SCr (r = 0.864; *P* < .001). There was high correlation between SCr and GFR-CG (r = - 0.67; *P* < .001), SCr and GFR-MDRD (r = -0.64; *P* < .001), and between SCr and GFR-DTPA (r = - 0.68; *P* < .001). Serum cystatin C showed significant but weaker correlation with GFR-CG (r = - 0.50; *P* = .002), GFR-MDRD (r = -0.59; *P* < .001), and GFR-DTPA (r = - 0.59; *P* < .001). The correlation coefficient of GFR-MDRD and GFR-CG with GFR-DTPA was 0.93 (*P* < .001) and 0.76 (*P* < .001).

After stratification by sex, correlation of SCysC with SCr was higher in women compared to men (r = 0.92; *P* < .001 and r = 0.64; *P* = .007). Among men, correlation of SCr with GFR-CG (r = - 0.73; *P* = .001), GFR-MDRD (r = -0.93; *P* < .001), and GFR-DTPA (r = - 0.87; *P* < .001) was higher as compared to SCysC, which did not have significant correlation with GFR-CG (*P* = NS), but had weaker yet

Table 1. Baseline parameters of the study population.

Parameter	Total population (n=35)	Men (n=16)	Women (n=19)	*P* value (male vs female)
Weight (kg)	66.14 ± 6.64	70 ± 4.1	62 ± 5.6	*P* < .001
Age (y)	44.23 ± 8.61	46 ± 8.8	42 ± 7.9	NS
Serum creatinine (mg/dL)	0.83 ± 0.14	0.92 ± 0.09	0.75 ± 0.11	*P* < .001
Serum cystatin C (mg/L)	0.71 ± 0.12	0.77 ± 0.09	0.65 ± 0.11	*P* = 003
GFR - CG (mL/min/1.73 m^2)	98.70 ± 8.87	96.06 ± 10.50	100.93 ± 6.73	NS
GFR - MDRD (mL/min/1.73 m^2)	93.37 ± 12.72	95.08 ± 12.20	91.93 ± 13.30	NS
GFR - DTPA (mL/min/1.73 m^2)	98.22 ± 10.71	97.87 ± 9.19	98.52 ± 12.08	NS

Abbreviations: GFR-CG, glomerular filtration rate calculated using Cockroft-Gault; GFR-DTPA, glomerular filtration rate calculated using ^{99m}Tc-DTPA; GFR-MDRD, glomerular filtration rate calculated using 4-variable MDRD formulae
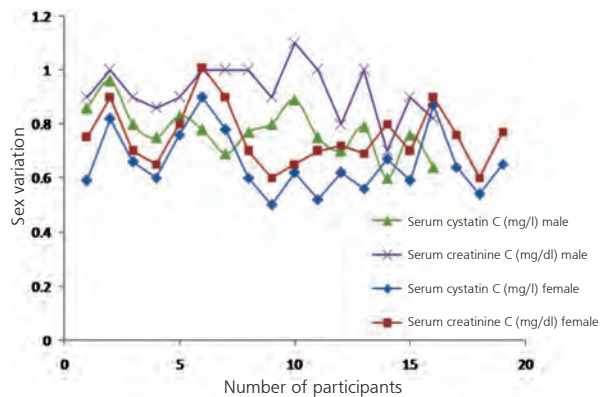
Figure 1. Sex variation of serum cystatin C and serum creatinine

significant correlation with GFR-MDRD (r = - 0.55; *P* < .02) and GFR-DTPA (r = - 0.56; *P* < .02). Men had a stronger correlation of GFR-MDRD (r = - 0.956; *P* < .001) than GFR-CG (r = 0.852; *P* < .001) with GFR-DTPA. In women, correlation values of SCr with GFR-CG (r = - 0.63; *P* = .04), GFR-MDRD (r = - 0.96; *P* < 0.001), and GFR -DTPA (r = - 0.85; *P* < .001) were comparable to the correlation of SCysC with GFR-CG (r = - 0.62; *P* = .004), GFR-MDRD (r = - 0.89; *P* < .001), and GFR-DTPA (r = - 0.72; P < .001). Among women, the correlation of GFR-MDRD (r = - 0.95; *P* < .001) was higher than of GFR-CG (r = 0.828; *P* < .001) with GFR -DTPA.

Discussion

The purpose of this study was to identify the clinical use of SCysC in living donor evaluation programs. The mean measured GFR of 98.22 ± 10.71 mL/min/1.73 m^2 in our donor population is less than the normal value of GFR in Western population. Previous studies from our center and other Indian studies have established a physiological lower basal GFR in healthy a Indian adult population with preserved response to protein-induced hyperfiltration.[5-7]

Cystatin C is a 13-Kda basic protein produced at a constant rate by all nucleated cells, filtered by the glomeruli, and entirely catabolized by the tubules.[1] Earlier literature indicates SCysC to be independent of muscle mass, age, sex, and race, making it a "near-ideal" marker of GFR. Use of SCysC as a screening tool is constrained owing to its cost, its lack of recognition as a standard universally accepted assay, interference by steroids, inflammation, thyroid disorders, and few studies in healthy volunteers.

As expected, we found a significant correlation between SCr and SCysC, thus supporting the validity of its use. Various studies have validated SCysC as a marker of a fall in GFR in acute kidney injury, chronic kidney disease, and renal transplant recipients[8-10]; however, literature is scarce regarding its role as a screening tool in healthy individuals. The SCysC values in our population ranged from 0.5 to 0.96 mg/L (0.6 to 0.96 mg/L in men, 0.5 to 0.90 mg/L in women). This is similar to the normal values described in other populations using the automated particle-enhanced turbidimetric assay.[11-13]

We found a significant sex difference in the estimated SCysC levels with lower levels in women similar to SCr. This could be due to the lower mean GFR in women compared to men; however, difference in GFR according to sex was not significant by any of the methods of estimation we used. Literature on sex difference of SCysC is polarized, with most investigators not finding any significant sex difference.[14, 15] Evangelopoulos and associates[16] studied 490 healthy adults and observed higher levels of SCysC in men as compared to women across all age groups. Groesbeck and associates[17] observed 12.7% higher values of SCysC in males than those for females among 719 US adolescents between 12 and 19 years of age. Similar results were obtained by Köttgen and associates in their study of 7596 participants in the NHANES-III survey, and Ichihara and associates in 596 healthy Japanese subjects.[18, 19] In contrast, Al Wakeel and associates[20] analyzed 300 healthy adults and found mean SCysC levels in women (0.778 ± 0.118 mg/L) to be significantly higher than in men (0.726 ± 0.095 mg/L). These contrasting data suggest growing evidence regarding the impact of sex as well as age on the SCysC levels.

There were no significant differences between the SCr-based GFR estimates (GFR-CG or GFR-MDRD) and the measured GFR-DTPA. This implies the validity of SCr-based estimates in predicting actual (measured) GFR. Although this conclusion could be limited by the fact that MDRD-equation is not validated in healthy Indian individuals and that we selected participants with measured GFR $\geq$ 80 mL/min/1.73 m^2, it still adds evidence to the accuracy of the Crockroft-Gault and the 4-variable MDRD formulae in GFR calculation.

The GFR-MDRD showed the strongest correlation with GFR-DTPA (r = 0.93; *P* < .001), followed by

GFR-CG (0.76; P < .001), SCr (r = - 0.68; P < .001), and SCysC (r = - 0.59; P < .001). As compared to SCr, we found a significant but weaker correlation between SCysC and the 3 GFR estimates used. After stratification by sex, SCysC performed poorly in men compared to women. Literature comparing SCysC and SCr with measured GFR presents contradictory conclusions. Khyse-Andersen and associates[21] studied 27 healthy volunteers and found the correlation of SCysC to clearance of iohexol to be significantly greater than that of SCr (r = 0.87 vs r = 0.71; P < .05). Hojs and associates[22] did not find a significant difference in the diagnostic accuracy of SCysC over creatinine clearance calculated from the MDRD formula. Recently, Erikson and associates[23] observed that SCysC is not a better marker of GFR than plasma creatinine in the general population. Our study does not help to substitute SCysC as an independent and better predictor of GFR than serum creatinine-based estimates; rather, it adds evidence to its role as a viable alternative marker of renal function in healthy individuals.

In conclusion, SCysC levels exhibit significant correlation with GFR as measured by ^{99m}Tc-DTPA double plasma sampling method. Further large scale, longitudinal studies are required to establish its role as a screening tool in healthy individuals.

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