Serum Cystatin C Levels in Renal Transplant Recipients

N. Krishnamurthy · K. Arumugasamy ·
Usha Anand · C. V. Anand · V. Aruna ·
G. Venu

Received: 26 March 2010 / Accepted: 24 September 2010 / Published online: 19 November 2010
© Association of Clinical Biochemists of India 2010

Abstract Cystatin C is an emerging parameter for the assessment of renal allograft function. The objective of the study was to compare the efficacy of serum cystatin C (SCys) with the established parameter serum creatinine (SCr) in the assessment of renal function in renal transplant recipients (RTR). The glomerular filtration rate (GFR) of 30 renal transplant patients and 29 control subjects was determined using ^{99m}Tc Diethylene-triamine-penta-acetate (DTPA) method. SCr was measured using an automated Jaffe's assay and SCys was measured using latex particle enhanced turbidimetric immuno assay (PETIA). The modification of diet in renal disease (MDRD) formula was used to calculate GFR from SCr, while the Le Bricon formula was used to derive GFR based on SCys. Statistical analysis was performed using MedCalc software. SCr and SCys levels were significantly higher, while DTPA clearance was significantly lower in RTR ($P < 0.0001$) when compared with controls. The correlation coefficient (r value) between calculated GFR based on MDRD method and DTPA

clearance was 0.343 ($P = 0.06$) while the calculated GFR based on Le Bricon formula was 0.694 ($P < 0.001$). The results have shown that SCys is a better parameter than SCr in assessing renal function in RTR. The inclusion of SCys as an additional parameter would certainly help in detection of even a marginal decline in renal function and also in adjusting the dosage of immunosuppressive drugs.

Keywords Cystatin C · Creatinine · DTPA · Glomerular filtration rate

Introduction

Laboratory evaluation of renal allograft function is an essential tool in assessing the progression of renal transplant recipients (RTR). Timely detection of even a marginal decline in renal allograft function is critical in the management of patients after transplantation. Determination of glomerular filtration rate (GFR) is considered to be the best parameter for assessing the functioning of the grafted kidney. The so called “gold standard” methods are based upon the measurement of the clearance of radio contrast agents such as ^{51}Cr EDTA, and iohexol. However these methods are laborious, complex and expensive and might even be hazardous in a patient who has undergone renal transplantation. Till date, the assessment of renal function adequacy in RTR is based solely upon the estimation of SCr. Adjustments of the dosage of immunosuppressive drugs are based upon the value of SCr [1]. A clinically significant increase in SCr may not be detected until the GFR has declined by 25% or more [2]. Also SCr is affected by several non-renal factors such as age, gender, race, muscle mass, diet and catabolic state. Numerous commonly used drugs or endogenous substances interfere

A part of this work was presented at the Annual Conference of the Association of Clinical Biochemists of India, held at Kochi, India, in 2009.

N. Krishnamurthy · K. Arumugasamy
Department of Biotechnology, Sathyabama University, Chennai,
Tamil Nadu, India

U. Anand · C. V. Anand (✉) · V. Aruna
Department of Biochemistry, PSG Institute of Medical Sciences
and Research, Peelamedu, Coimbatore 641004,
Tamil Nadu, India
e-mail: anandvasudevarao@gmail.com;
anand_vasudevarao@yahoo.com

G. Venu
Department of Nephrology, PSG Institute of Medical Sciences
and Research, Coimbatore, Tamil Nadu, India

with the measurement of SCr leading to falsely high or low creatinine values [3]. Secretion and reabsorption of creatinine by the renal tubule could lead to under or over estimation of GFR [4].

SCys has been proposed as an alternative marker of GFR. Cystatin C is a 122 amino acid, 13 kDa protein and a cysteine protease inhibitor. It is the product of a “house-keeping” gene expressed in all nucleated cells and is produced at a constant rate. Cystatin C is freely filtered by the glomerulus. It is not secreted, but is reabsorbed by the proximal tubular cells and subsequently catabolized so that it does not return to the circulation [5]. Cystatin C is independent of muscle mass, sex and age [6].

In present study, we set out to compare the efficacy of SCr and SCys in the assessment of renal function in RTR. Furthermore the performance characteristics of the calculated GFR values based on SCr and SCys was to be compared with the GFR determined by the Diethylene-triamine-penta-acetate (DTPA) method.

Materials and Methods

Subjects

The study was conducted on two groups of human subjects; a control group consisting of 29 healthy human adults and an RTR group which consisted of 30 patients who had undergone renal transplantation. Only those with a post-transplantation period exceeding 6 months and a stable renal function were included in the RTR group. Ethical clearance for this study was obtained from the Ethical Committee of our institution.

Samples of blood were collected from all subjects when they attended the outpatient clinic for regular follow-up. Blood was drawn using vacutainers (Becton–Dickinson USA) containing a clot activator and the serum obtained was used for the estimation of SCr and SCys.

SCr was measured using an automated Jaffe’s assay on the Olympus AU 400 (Olympus Optical, Tokyo, Japan) analyzer as described previously [7]. SCys was measured using particle enhanced turbidimetric immuno assay (PETIA) method from Gentian (Gentian AS, Moss, Norway) performed on an Olympus AU 400 (Olympus Optical, Tokyo, Japan) as described previously [7].

GFR was determined by DTPA renal dynamic imaging using modified Gate’s method. The technique is based on the fact that the fractional renal uptake of intravenously administered DTPA within 2–3 min after radio tracer arrival within the kidneys is proportional to the GFR. After image acquisition, the GFR was automatically calculated by computer according to Gate’s algorithm [8].

Formulae used for calculation of GFR

- MDRD formula, $GFR = 186 \times [SCr]^{-1.154} \times [age]^{-0.203} \times [0.742 \text{ if female}] \times [1.12 \text{ if Black}]$ [9].

Several formulae have been described for the calculation of GFR based on SCys; amongst these the Le Bricon formula is specifically used for RTR.

- Le Bricon formula, $GFR = 78 \times 1/SCys + 4$ [10].

Statistical Analysis

Statistical analysis was performed using MedCalc software. The correlation between the SCr and SCys was calculated by Pearson’s correlation coefficient. Diagnostic sensitivity and specificity were studied using ROC curves. The similarities between the various GFR indices and agreement with measured DTPA clearance were assessed using Bland and Altman plots.

Results

The study has demonstrated that SCr as well as SCys levels were significantly higher in RTR ($P < 0.0001$) when compared with controls. Similarly DTPA clearance and the calculated GFR based on modification of diet in renal disease (MDRD) and Le Bricon formulae were significantly lower in RTR as compared with controls (Tables 1, 2). Both $1/SCr$ and $1/SCys$ showed a significant correlation with DTPA clearance. However the Pearson’s correlation coefficient (r value) for $1/SCys$ was 0.635 ($P < 0.0001$) while for $1/SCr$, it was 0.497 ($P = 0.005$) (Fig. 1a, b). The correlation coefficient (r value) between calculated GFR based on MDRD method and DTPA clearance was 0.343 ($P = 0.06$) while the calculated GFR based on Le Bricon formula was 0.694 ($P < 0.001$).

Table 1 Patients’ characteristics

	Controls (Mean $\pm$ SD)	RTR (Mean $\pm$ SD)	<i>P</i> value
No of subjects	29	30	–
Age (years)	32.96 $\pm$ 16.77	43.13 $\pm$ 10.00	0.001
Gender (M/F)	28/1	22/8	–
SCr (mg/dl)	1.04 $\pm$ 0.10	1.25 $\pm$ 0.31	<0.0001
SCys (mg/l)	0.89 $\pm$ 0.16	1.40 $\pm$ 0.25	<0.0001
DTPA (ml/min/1.73 m ²)	89.72 $\pm$ 14.86	58.26 $\pm$ 15.69	<0.0001
MDRD (ml/min/1.73 m ²)	86.03 $\pm$ 19.05	65.46 $\pm$ 16.37	<0.0001
Le Bricon formula (ml/min/1.73 m ²)	97.41 $\pm$ 37.83	61.59 $\pm$ 11.12	<0.0001

Table 2 Correlation data

S No		<i>r</i> value	<i>P</i> value
1.	DTPA vs. 1/SCr	0.497	0.005
2.	DTPA vs. 1/SCys	0.635	<0.001
3.	DTPA vs. MDRD	0.343	0.006
4.	DTPA vs. Le Bricon	0.699	<0.001

r Pearson's correlation coefficients

Fig. 1 Correlation **a** between 1/SCr and DTPA clearance and **b** between 1/SCys and DTPA clearance

ROC Analysis

Figure 2a and b show the ROC curves for SCr and SCys, MDRD formula and Le Bricon formula at cut off value of 60 ml/min/1.73 m² for DTPA clearance. Table 3 shows that, the area under curve for SCr was 0.834 (*P* = 0.0001) whereas for SCys it was 0.968 (*P* = 0.0001). The area under curve for SCr based on MDRD formula was 0.820 (*P* = 0.0001) whereas Le Bricon formula was 0.968 (*P* = 0.0001). An SCr level of 1.2 mg/dl was the one having the best predictive value for assessing post transplant renal

Fig. 2 The performance characteristics of **a** SCr and Scys was analyzed using ROC curves in order to ascertain their sensitivity and specificity at the cut off value of <60 ml/min/1.73 m² DTPA and **b** MDRD and Le Bricon was analyzed using ROC curves in order to ascertain their sensitivity and specificity at the cut off value of <60 ml/min/1.73 m² DTPA clearance

Table 3 ROC analysis

	AUC	Criterion	Sn (%)	Sp (%)	<i>P</i> value
SCr	0.834	>1.2	83	54	0.044*
SCys	0.968	>1.26	95	93	
MDRD	0.82	71	83	77	0.006**
Le Bricon	0.968	62.64	95	93	

AUC Area under curve; Sn sensitivity; Sp specificity

* *P* value significant between SCr and SCys

** *P* value significantly difference between MDRD and Le Bricon formulae

function with 83% sensitivity and 54% specificity. The best predictive value for SCys was with a level of 1.26 mg/l, with 95% sensitivity and 93% specificity.

The criterion value corresponds to the calculated GFR estimate with the higher accuracy (minimal false negative and false positive results). Therefore the closer the criterion values to the cut off for DTPA clearance, the better the calculated GFR. Our results indicate much closer value 62.64 ml/min/1.73 m² for the Le Bricon formula to identify GFR $\leq$ 60 ml/min/1.73 m².

Discussion

In this study, we have compared the performance characteristics of SCr, SCys and DTPA clearance for the assessment of renal function in RTR. The mean SCr was found to be lower than the upper limit of reference range (1.5 mg/dl) while the mean SCys was found to be higher than the upper limit of reference range (1.01 mg/l) of our hospital. A comparison of the individual SCr and SCys showed that in the RTR group only five patients had values greater than 1.5 mg/dl for SCr while 28 patients had values higher than the 1.01 mg/l for SCys. This is a clear indication that dependence on SCr alone could lead to a failure to detect mild decline in renal function in RTR.

The Pearson's correlation coefficient evaluates the usefulness of SCys over the whole spectrum of renal dysfunction in order to assess whether this analyte shows a parallel rise as the GFR falls. SCys showed a higher correlation than SCr with DTPA clearance, which is commonly used in South India for the determination of GFR. Similar results have been reported by Keevil et al. [11] and Risch et al. [12] who found a better correlation between SCys and GFR than between SCr and GFR.

ROC Curves

ROC analysis is now a standard tool to assess, define, and compare the diagnostic validity of tests. This statistical parameter also showed that SCys had greater accuracy than SCr at detecting GFR $<$ 60 ml/min/1.73 m²; there was a significant difference for the area under the curves between SCys and SCr ($P = 0.044$). Our findings are in agreement with Christensson et al. [13], who found that cystatin C had a significantly higher sensitivity than the serum creatinine in the ability to detect a fall in the GFR $<$ 60 ml/min in renal transplant subjects.

The current guidelines K/DOQI emphasize the need to assess kidney function using predictive equations rather than just SCr; the most widely used are the Cockcroft and Gault and MDRD formulae [14]. Similarly, several formulae have also been proposed for estimating GFR based upon SCys including the Le Bricon formula [15]. In this study, a comparison has been made between the predictive equations for GFR namely the SCr based MDRD formula

and SCys based Le Bricon formula. Between the two, the Le Bricon formula showed a better correlation with DTPA clearance than the MDRD formula. White et al. [16] have also shown that the Le Bricon formula was best suited for RTR.

To conclude, the results have clearly shown that SCys is a better parameter than SCr in assessing renal function in RTR. Currently, almost all hospitals in India rely upon the results of SCr, in the immediate post transplant period, as well as for the long time follow-up of RTR. The inclusion of SCys as an additional parameter would certainly help in detection of even marginal decline in renal function and also in adjusting the dosage of immunosuppressive drugs.

The limitation of this study was that SCys was determined only once in each patient in the RTR group. Larger studies in which serial determinations of SCys during the follow-up of RTR subjects would reveal a better role of this parameter to detect rejection at an early stage.

References

1. Ojo A. Assessing post transplant renal function. *Medscape Transplant*. 2004;5(2):1–4.
2. Kassirer JP. Clinical evaluation of kidney function—glomerular function. *N Engl J Med*. 1971;285:385–9.
3. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem*. 1992;38:1933–53.
4. Levey AS, Berg RL, Gassmann JJ, Hall PM, Walker WG. Creatinine filtration, secretion and excretion during progressive renal disease. *Kidney Int*. 1989;36(Suppl 27):S73–80.
5. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of renal function. A review. *Clin Chem Lab Med*. 1999; 37:389–95.
6. Coll E, Botey A, Alvarez L, Poch E, Quintó L, Taurina A, et al. Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. *Am J Kidney Dis*. 2000;36:29–34.
7. Krishnamurthy N, Arumugasamy K, Anand U, Anand CV, Aruna V, et al. Effect of hemodialysis on circulating cystatin C levels in patients with end-stage-renal-disease. *Indian J Clin Biochem*. 2010;25(1):43–6.
8. Gates GF. Split renal function testing using Tc-DTPA. A rapid technique for determining differential filtration. *Clin Nucl Med*. 1983;8:400–7.
9. Levey AS, Greene T, Kusek JW, Beck GJ, MDRD Study Group. A simplified equation to predict glomerular filtration rate from serum creatinine. *Am Soc Nephrol Renal Week*. 2000;A0828.
10. Le Bricon T, Thervet E, Froissart M, Benlakehal M, Bousquet B, Legendre C, et al. Plasma cystatin C is superior to 24-hour creatinine clearance and plasma creatinine for estimation of glomerular filtration rate 3 months after kidney transplantation. *Clin Chem*. 2000;46:1206–7.
11. Keevil BG, Kilpatrick ES, Nicholas SP, Maylor PW. Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. *Clin Chem*. 1998;44:1535–9.
12. Risch L, Blumberg A, Huber A. Rapid and accurate assessment of glomerular filtration rate in patients with renal transplants using serum cystatin C. *Nephrol Dial Transplant*. 1999;14: 1991–6.

13. Christensson A, Ekberg J, Grubb A, Ekberg H, Lindstrom V, Lilja H. Serum cystatin C is a more sensitive and more accurate marker of glomerular filtration rate than enzymatic measurements of creatinine in renal transplantation. *Nephron Physiol.* 2003;94: 19–27.
14. National Kidney Foundation. K/DOQI clinical practice guideline to define chronic kidney disease: evaluation, classification and stratification. *Am J Kidney Dis.* 2002;39(Suppl 1):S1–266.
15. Tidman M, Sjostrom P, Jones I. A comparison between estimating formulae based upon s-cystatin C and s-creatinine and a combination of the two. *Nephrol Dial Transplant.* 2008;23: 154–60.
16. White C, Akbari A, Hussain N, Dinh L, Filler G, Lepage N, Knoll GA. Estimating glomerular filtration rate in kidney transplantation. A comparison between serum creatinine and cystatin C-based methods. *J Am Soc Nephrol.* 2006;16:3763–70.