Original Article

Can cystatin C become an easy and reliable tool for anesthesiologists to calculate glomerular filtration rate?

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Abstract

Background and Aims: The aim was to evaluate the role of cystatin C as a noninvasive and easy marker of glomerular filtration rate (GFR) estimation in voluntary kidney donors.

Materials and Methods: We retrospectively evaluated 40 voluntary kidney donors. They underwent complete biochemical and nuclear tests as a part of transplant workup. Serum cystatin C, serum creatinine, and Tc-99m diethylene-triamine-penta-acetic acid (DTPA) were used in our study. We calculated GFR using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula based on creatinine only (GFR-CKD-EPI-creat), CKD-EPI formula using creatinine and cystatin C (GFR-CKD-EPI-cyst-creat), and modification of diet in renal disease (MDRD) and CKD-EPI cystatin C equation (2012) (GFR-cyst).

Data was evaluated using the SPSS software (version 11.5). The correlation analysis and analysis of variance was used for statistical computation. Agreement was determined using analyze-it version 2.30 for MS-Excel 12+.

Results: The mean age of the donors in our study was 49.83 ± 13.06. The mean cystatin C in females was 0.72 ± 0.12, the mean cystain C in males was 0.87 ± 0.23. On correlating GFR-cyst with GFR-DTPA the Pearson correlation coefficient (ρ) was found to be 0.388 this correlation was significant with $P < 0.05$. While comparing with DTPA the correlation coefficient of GFR-CKD-EPI-creat group was 0.587 which was significant with $P < 0.01$. The correlation coefficient of GFR-CKD-EPI-cyst-creat group compared with GFR-DTPA group was 0.543 which was also significant at $P < 0.001$. GFR-CKD-EPI-creat gave the highest correlation with DTPA in our study. The correlation coefficient of GFR-MDRD group with DTPA group was 0.576 this correlation was also significant with $P < 0.01$. The results obtained were further statistically analyzed by Bland-Altman analysis the percentage error for GFR-DTPA versus GFR-cyst-creat is 29.72%; for GFR-DTPA versus GFR-EPI-creat is 30.73%; or GFR-DTPA versus MDRD is 31.63% and for GFR-DTPA versus GFR-cyst is 34.37%.

Conclusion: Cystatin C is a good endogenous marker for calculating GFR as it correlates very well with DTPA and CKD-EPI equation based GFR.

Key words: Chronic kidney disease epidemiology collaboration, cystatin C, glomerular filtration rate (GFR), Tc-99m diethylene-triamine-penta-acetic acid GFR

Introduction

The effects of anesthetics on the kidney go beyond a change in basal hemodynamics and include, for some drugs, an alteration in the ability for the kidney to autoregulate its blood flow and glomerular filtration rate (GFR). Accurate estimation of GFR is essential for anesthetist so as to forsee the effect of drugs and various anesthetic procedures on renal blood flow (RBF) and GFR. Many drugs need modification of doses in the presence of decreased GFR especially to manage critically ill patients in intensive care units, renal transplant recipients and donors after donor nephrectomy.

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Inulin clearance and radioisotope renogram are ideal methods but are time consuming and cumbersome. Serum creatinine-based GFR estimates vary depending on the individual's age, race, muscle mass, and sex. Serum cystatin C based GFR appears to be less biased because cystatin C concentration is independent of age, race, gender, and muscle mass.[1,2] We studied the role of cystatin C as a noninvasive and easy marker of GFR estimation involuntary kidney donors. In this study, we compared serum cystatin C based GFR with creatinine based GFR modification of diet in renal disease (MDRD) and Tc-99m diethylene-triamine-penta-acetic acid ([99mTc]-DTPA) GFR.

Materials and Methods

We evaluated 40 voluntary healthy kidney donors after initial clinical and biochemical evaluation between January 2011 and January 2012. The study was approved by the Institutional Review Committee and conformed to ethical guidelines of 1975 Helsinki declaration and included only those who gave a written informed consent. We excluded donors with DTPA GFR <70 ml/min/1.73 m^3, serum creatinine ≥1.5 mg/dl, impaired fasting glucose tolerance, glycosylated hemoglobin >6.

Study samples for serum cystatin C and serum creatinine were obtained on the day of GFR estimation. Both were measured immediately. Serum creatinine was measured by buffered Jaffe's kinetic reaction without the deproteinization on Cobas 6000 autoanalyzer (Roche diagnostics Ltd., GmBH, Germany). Cystatin C was determined by latex enhanced immunotubidimetry assay (a leit cystatin C Kit Agappe Diagnostic Ltd. in technical collaboration with Denka Seiken Co., Japan) on Olympus AU2700 autoanalyzer. Normal levels for individuals ≤50 years = 0.55-1.15 ng/l and for individual >50 years = 0.63-1.44 ng/l.

GFR estimation with [99mTc]-DTPA used gamma camera based Gates method.[3]

We calculated GFR using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula based on creatinine only, CKD-EPI formula using creatinine and cystatin C, and modification of diet in renal disease (MDRD) and CKD-EPI cystatin C equation (2012).[4,5]

The formulas are as follows:

GFR calculated from the MDRD formula (GFR-MDRD):

$186 \times \text{creatinine}^{-1.154} \times \text{age}^{-0.203} \times (0.742, \text{if female})$ (age in years and serum creatinine in mg/dL).

GFR calculated from CKD-EPI (CKD-EPI-creat):[6]

In males, if creatinine <0.9 GFR = $141 \times (\text{plasmatic creatinine})^{-0.411}/0.9 \times 0.993^{\text{age}}$

In males, if creatinine >0.9 GFR = $141 \times (\text{plasmatic creatinine})^{-1.209}/0.9 \times 0.993^{\text{age}}$

In females, if creatinine <0.7 GFR = $144 \times (\text{plasmatic creatinine})^{-0.329}/0.7 \times 0.993^{\text{age}}$

In females, if creatinine >0.7 GFR = $144 \times (\text{plasmatic creatinine})^{-1.209}/0.7 \times 0.993^{\text{age}}$

Cystatin C based GFR (GFR-cyst) was calculated using CKD-EPI cystatin C Equation (2012):[7]

$\text{GFR} = 133 \times \min(\text{Scys}/0.8, 1)^{-0.499} \times \max(\text{Scys}/0.8, 1)^{-1.328} \times 0.996^{\text{age}}$ [×0.932 if female]

Scys is serum cystatin C.

The CKD-EPI creatinine-cystatin C (GFR-cyst-creat) based GFR was derived from the Inker equation:[7]

$\text{GFR} = 135 \times \min(\text{Scr}/k, 1)^{-a} \times \max(\text{Scr}/k, 1)^{-0.601} - \min(\text{Scys}/0.8, 1)^{-0.375} \times \max(\text{Scys}/0.8, 1)^{-0.711} \times 0.995^{\text{age}}$ [×0.969 if female] [×1.08 if black]

Scr is serum creatinine and Scys is serum cystatin C. k is 0.7 for females and 0.9 for males a is –0.248 for females and –0.207 for males.

Statistical analysis

We used SPSS software (version 11.5) to analyze data. Correlation analysis and analysis of variance technique was used for statistical computation. The results obtained were also analyzed by Bland-Altman analysis which is used for assessing between two measurements of the same clinical variable. Agreement was determined using Analyze-it version 2.30 for MS-Excel 12+.

Results

The mean age of the donors in our study was 49.83 ± 13.06. The gender wise mean values of various variables are shown in Table 1. The mean creatinine in females was 0.78 ± 0.12 and in males was 0.95 ± 0.17. The mean cystatin C in females was 0.72 ± 0.12, the mean cystain C in males was 0.87 ± 0.23. Both cystatin C and creatinine values were higher in males. All the GFR had a higher value in the female population [Table 1]. The mean GFR calculated out of the GFR-cyst, GFR-CKD-EPI-creat, GFR-CKD-EPI-cyst-creat,

MDRD, and GFR-DTPA methods were 105.82 ± 15.92, 91.05 ± 16.03, 99.88 ± 15.10, 82.15 ± 15.15, and 85.56 ± 14.17 respectively. The mean GFR was highest in the cystatin C group, followed by the CKD-EPI-cyst-creat group, GFR-CKD-EPI-creat group, DTPA group, and CKD-EPI group. On inter-group correlation GFR-cyst with GFR-DTPA the Pearson correlation coefficient (ρ) was found to be 0.388 this correlation was significant with $P < 0.05$. To determine the agreement for these two tests we applied the Bland-Altman method. The 95% limits of agreement (–12.48, 53.01) contains 92.5% different scores. The mean difference (bias) of the measurement between GFR-cyst and GFR-DTPA is 20.26. The standard deviation (SD) of the difference is 16.7, Correlation — absolute versus average was –0.08, $P < 0.0001$ [Figure 1]. While comparing with DTPA the correlation coefficient of GFR-CKD-EPI-creat group was 0.587 which was significant with $P < 0.01$. On applying the Bland-Altman analysis for agreement, the 95% limits of agreement (–21.6, 32.6) contains 97.5% of difference scores. The bias of the measurement between GFR-CKD-EPI-creat and GFR-DTPA is 5.49. The SD of the difference is 13.8. Correlation of absolute versus average was –0.18. The P value was 0.016 [Figure 2]. The correlation coefficient of GFR-CKD-EPI-cyst-creat group compared to GFR-DTPA group was 0.543 which was also significant at $P < 0.001$. The 95% limits of agreement (–13.2, 41.8) contains 93% difference score. The bias of the measurement between GFR-CKD-EPI-cyst-creat and GFR-DTPA was 14.3. The SD of the difference was 14. Correlation of absolute difference versus average difference was 0.00, $P < 0.0001$ [Figure 3]. GFR-CKD-EPI-creat gave the highest correlation with DTPA in our study. The correlation coefficient of GFR-MDRD group with DTPA group was 0.576 this correlation was also significant with $P < 0.01$. The 95% limits of agreements (–29.9, 23.1) contains 97.55 of the scores. The bias of the measurement between GFR-MDRD and GFR-DTPA was 3.4. The SD of the difference was 13.5. Correlation of absolute difference versus average difference was –0.01. The P value was 0.12. On the correlation, the GFR-CKD-EPI-cyst-creat with MDRD group the correlation coefficient was 0.794 which was significant with $P < 0.01$. On correlating GFR-cyst to GFR-CKD-EPI-creat, the coefficient was 0.507 which was also significant. On comparing the two CKD-EPI group, the correlation coefficient was 15.926 with $P < 0.01$. Comparing the MDRD with GFR-cyst the correlation coefficient was 0.45

Table 1: Gender wise mean values of various variables

Variables (mean)	Gender	
	Females	Male
Age (years)	49.17	54.50
Serum creatinine (mg/dl)	0.78	0.95
Cystain C	0.72	0.87
GFR-Cyst	107.11	96.8
GFR-CKD-EPI-creat	91.6	87.2
GFR-CDK-EPI-cyst-creat	100.8	93.4
GFR-MDRD	81.68	85.4
GFR-DTPA	84.92	90

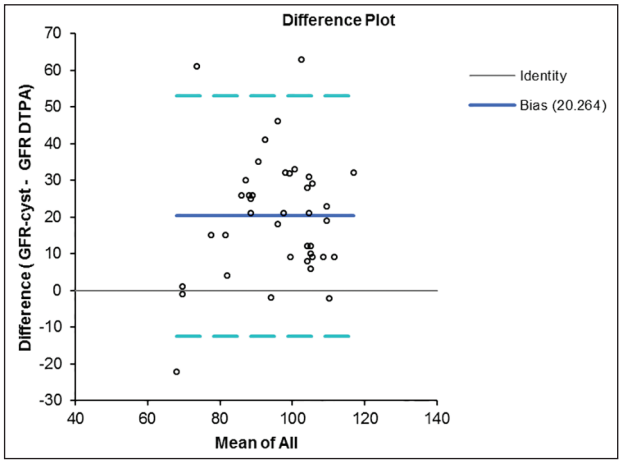

Figure 1: Plot showing agreement between GFR-cyst and GFR-DTPA

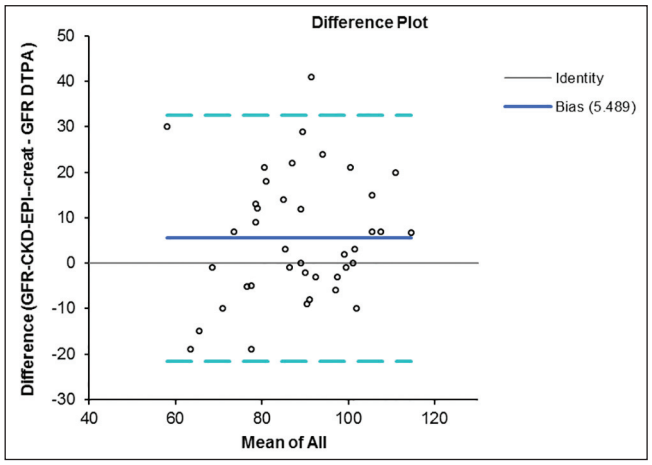

Figure 2: Plot showing agreement between GFR-CKD-EPI-creat and GFR-DTPA

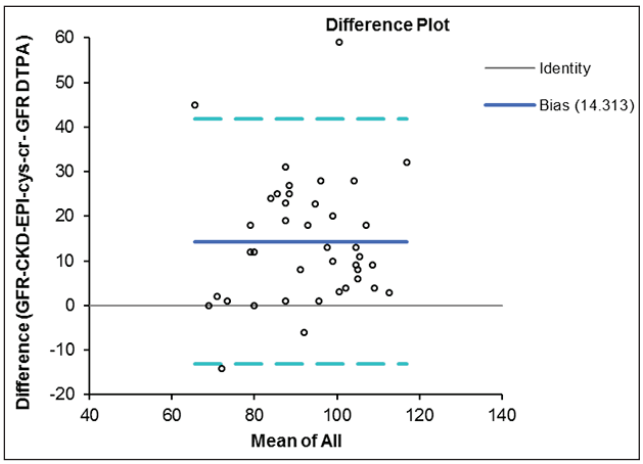

Figure 3: Plot showing agreement between GFR-CKD-EPI-cyst and GFR-DTPA

which was also significant with $P < 0.01$. On comparing, the MDRD with GFR-CKD-EPI-creat the correlation coefficient was 0.94 which was also significant with $P < 0.01$.

Discussion

The purpose of our study was to identify the utility of GFR based on cystatin C and compare it with other creatinine-based GFR and DTPA based GFR. GFR is commonly assessed in the intensive care unit (ICU). Many pharmacological agents are eliminated by the kidney, and their dosage is adjusted for kidney function. There are various ways of estimating GFR. The differences in GFR estimates by various methods used indicate that the GFR method used in settings like the patients undergoing a donor nephrectomy, critically ill patients in intensive care unit, may influence the treatment.[8]

An ideal marker of GFR is defined as an endogenous substance that, produced at a constant rate is freely disposed of by the kidney only by glomerular filtrations, without being either secreted or reabsorbed by tubular cells.[9]

The gold standard for the estimation of GFR is based on the clearance of exogenous substances such as inulin, iohexol, ^{51}Cr-EDTA, 99mTc-DTPA or [^{125}I]-iothalamate, which involve laborious and invasive, time-consuming techniques. The 24 h urine creatinine clearance with urine collection has various drawbacks like; it is cumbersome, and associated with long turn-around times, which may delay initiation and adjustment of treatment. Thus, these modalities are less suitable for patients in the transplant and intensive care unit requiring rapid decisions and actions. Hence, endogenous markers are usually preferred. Determination of plasma concentrations of digoxin, gentamycin, tobramycin, and vancomycin are all among the top 40 test requests in the intensive care unit, and they are all influenced by the GFR. Even though concentration of these drugs can be measured, initial therapy is started based on GFR estimates. Furthermore, levels of several widely used pharmacological agents with renal elimination are not routinely assessed. Examples of such drugs used in critical care are H_2-antagonists, beta-blockers, and antibiotics such as penicillins and cephalosporins.[8] During last few decades, serum creatinine has been the most frequently employed marker to estimate GFR. Creatinine is completely filtered by the glomerular membrane and is not reabsorbed or metabolized by the kidney, although it is partly secreted by the proximal tubule. Tubular secretion raised creatinine clearance by 10-20%, reaching 50% in cases of advanced CKD.[10]

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. Cystatin C is freely filtered through the glomerular membrane and is reabsorbed and metabolized but not secreted by the proximal tubule.[11]

Serum cystatin C concentration appears to be independent of muscular mass, sex, age or nutritional status.[1,2] It is a better marker of GFR in special clinical conditions such as hepatic cirrhosis, diabetes mellitus and the elderly.[12,13]

The use of cystatin C instead of creatinine will increase the number of patients identified with decreased GFR. The discrepancy between the two methods may influence the pharmacological treatment of the patients and shows that there is a need to improve GFR measurements in intensive care. Various studies have shown that formulae to estimate renal function in ICU subjects with normal serum creatinine concentrations are inaccurate.[14]

In our study, we evaluated 40 voluntary kidney donors because they underwent complete biochemical workup and nuclear tests without incurring extra cost. The number of females was higher in ($n = 35$) our study. The mean value of serum creatinine and serum cystain C was higher in the male population. The mean GFR calculated by all the methods was higher in females of that group. GFR calculated by cystatin C has a higher value comparing to other groups. We used GFR from DTPA as the gold standard and correlated it with GFRs of another group. The value of cystatin C mean GFR was found to be higher than our reference. Review of literature further revealed that Gate's method of estimation of GFR tends to underestimate GFR at higher values and overestimates at lower values in comparison with plasma sample method.[1] Cystatin C based GFR appears to be higher. Han *et al.* reported cystatin based GFR to be higher and more specific for renal function recovery after live kidney donation.[15]

Addition of cystatin C in CKD EPI formula also gave GFR in the higher range. The mean GFR calculated by CKD-EPI formula using creatinine alone was 91.05 ± 16.03, on using the CKD-EPI formula with both creatinine and cystatin C as variable the GFR was 99.88 ± 15.10. Cystatin C appears to be a better determinant of GFR in our study. However, when we compared the various GFR groups with DTPA all methods had a significant correlation. The results obtained were further statistically analyzed by Bland-Altman analysis also which is used for assessing between two measurements of the same clinical variable. The results of Bland-Altman are expressed in terms of bias and limits of agreement (2SD), that means, if bias is low than accuracy is high. Limits of agreement refer to how precise the measurements are. So if they are narrow; the precision is high; if large than, the precision is low. An ideal result, therefore, would have a very small bias with tight limits of agreement. Critchley and Critchley, criteria, were also applied, which proposed that the percentage error (PE) of the limits of

agreement, as compared with the population mean, be used to describe the agreement and that this could be used as a cutoff for whether to accept a new technique. The two techniques are considered comparable if the difference in values measured by them is within ±15%. A paired *t*-test was also applied to test the difference of mean between the two groups. An ideal result should be a small bias with tight limits of agreement but according to this study, it contradict the ideal result because all the four parameters have high mean bias as well as large limits of agreements. The PE for GFR-DTPA versus GFR-cyst-creat is 29.72%; for GFR-DTPA versus GFR-EPI-creat is 30.73%; or GFR-DTPA versus MDRD is 31.63% and for GFR-DTPA versus GFR-cyst is 34.37%, which all does not meets the criteria of Critchley and Critchley of 30%. The results obtained were similar by correlation analysis also. Our finding were similar to findings of other authors.[16] In a meta-analysis in 2002, Dharnidharka *et al.*[12] published that cystatin C is better than serum creatinine as a marker of the renal function. On the other hand, in 2007, Zahran *et al.*[17] performed a literature review with 43 studies of renal transplantation and patients with primary renal disease; and found a large number of researches in favor of cystatin C to estimate GFR, but still there are many studies that show that there are no advantages of cystatin C over creatinine. Thus, our study adds evidence to the role of cystatin C as valid, easy and reliable marker of renal function unbiased of muscle mass, age, and sex.

Conclusion

Cystatin C is a good endogenous marker for calculating GFR as it correlates very well with DTPA and CKD-EPI equation based GFR. However, large-scale studies needs to be done to assess its superiority over other creatinine-based equations. Moreover, this study was performed in patients with normal GFR and without major renal risk factors, and the results cannot be directly extrapolated to patients with acute kidney injury or chronic renal disease.

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