

# Measurement of GFR by Tc-99m DTPA: Comparison of 5 Plasma Sample and 2 Plasma Sample Methods in North Indian Population

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## Abstract

*Assessment of Glomerular filtration rate (GFR) has significant impact on both prognosis and treatment of patients with renal disease. In this study we compared the two-plasma-sample method (G2S) using a MS excel spreadsheet based program, with a manual five-plasma-sample method (GS) used to measure GFR by determining Tc-99m-diethylenetriamine penta-acetic acid (Tc-99m DTPA) clearance in patients with chronic kidney disease (CKD) and healthy renal donors. The study was conducted in 148 subjects (64 men and 84 women; age range 14 to 70 yr); 59 patients of CKD and 89 prospective healthy kidney donors. Tc-99m DTPA (74-100 MBq) was injected intravenously and thereafter blood samples were obtained at 60, 90, 120, 150 and 180 min via the patent venflon. Radioactivity in the injection syringe and plasma was measured by means of a multi-well gamma counter. The correlation coefficient between the 2 methods was 0.9453, with a slope of 0.90 and an intercept of 14.72 mL/min. Bland Altman plot of disagreement showed that G2S underestimated the GFR values by 9.0 ml/min, 11.3 ml/min and 6.9 ml/min, in the entire study, CKD and healthy donor groups respectively. Our results indicate that in spite of good correlation between GS and G2S method, the G2S method constantly underestimated GFR in our study population. However, regression equation may be applied to the GFR values estimated by G2S method to match the GFR determined by GS method.*

*Keywords: GFR, Tc-99m DTPA, plasma clearance, chronic kidney disease, renal donors*

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## Introduction

Glomerular filtration rate (GFR) is an important index of functional state of kidney. Simple and accurate determination of the GFR is still a challenge in clinical practice. Twenty-four hour creatinine clearance overestimates GFR in patients with poor renal function due to a compensatory increase in tubular secretion, which limits its validity as a glomerular filtration marker (1,2). Inulin and iothalamate clearances are accepted reference standards for determining GFR, but are expensive and laborious (3). In addition, inulin clearance also requires a steady state plasma concentration and urine collection for the greatest accuracy. Moreover, it has become increasingly difficult to obtain inulin. A number of protocols for determining GFR using radionuclide methods have been developed (4-17). All these methods estimate glomerular filtration from plasma clearance of any of the several radiopharmaceuticals that are excreted by glomerular filtration following a single intravenous injection of the radiopharmaceutical. The radiopharmaceutical of choice for estimation of GFR is Cr-51 EDTA (ethylene diamine tetra acetic acid) because its clearance is considered to be closest to that of inulin. However, Tc-99m DTPA (diethylene triamine penta acetic acid) clearance also correlates well with Cr-51 EDTA clearance (4). DTPA is relatively inexpensive, provides a low radiation dose to patients and can be used for concurrent gamma camera imaging.

The GFR is calculated by dividing the net administered activity by the integral of plasma time activity curve. This process requires multiple samples from patient. Tc-99m DTPA plasma clearance dual plasma sample method has also been compared with five plasma sample method to simplify the procedure in calculations of GFR (7,8, 13-15). In the current study, GFR using two plasma sample method (G2S) and GFR using five plasma sample method (GS)

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Characteristics	Healthy Donors (n = 89)	CKD (n = 59)	Total (n = 148)
Age (mean ± S.D) (years)	43.35 ± 10.37	43.42 ± 12.69	43.39 ± 11.31
Range (years)	20-65	14-70	14-70
S.Creatinine (mean ± S.D)(mg/dl)	00.86 ± 0.12	03.02 ± 1.51	1.75 ± 1.44
Range	0.5-1.2	0.7 – 6.6	0.5 – 6.6
BUN (mean ± S.D)(mg/dl)	29.65 ± 6.97	84.29 ± 41.29	53.94 ± 38.98
Range	12-45	25 – 183	12.0 – 183.0

**Table 1:** Demographic characteristics of enrolled patients

were compared using the measurement of Tc-99m DTPA plasma clearance in patients (with chronic renal disease) and healthy individuals. The GS method is a manual slope intercept method and G2S method uses a MS excel spreadsheet based computer software and evaluates GFR reliably and with precision.

## Materials and Methods

### Patients

All patients subjected to GFR estimation from January 2004 to December 2006 were included in this study. The study population was divided into two groups, chronic kidney disease (CKD) group and healthy renal donor (D) group. CKD group included patients suffering from various degrees of renal disorders. D group included normal healthy kidney donors with no history of illness known to affect renal function. Sex, age, body weight and body height of the patients were recorded.

### Procedure

The patients were adequately hydrated prior to the study. GFR estimation in all patients was done by two different methods, (i) manual slope intercept method using five plasma samples (GS) and (ii) two plasma sample method using the MS Excel spread sheet program (G2S). For GFR measurement by slope intercept method blood samples were taken at 60, 90, 120, 150 and 180 minutes. For calculating GFR by G2S method only two blood samples were taken at 60 and 180 minutes.

A patent venflon was placed in the antecubital vein of the patient for blood sample collection. Commercially available freeze dried DTPA kits (BRIT, India) were labeled with Tc-99m pertechnetate. 74-100 MBq of Tc-99m DTPA pre-weighted dose was intravenously administrated in the contra-lateral arm of the patient. Standard was prepared by diluting duplicate syringes. Five blood samples were collected from the patent venflon at the respective times in the heparinized test tubes. The counting and calculations were performed on next day to reduce errors due to very high count rate on the test day.

After centrifugation, 1 ml plasma samples and standard were pipetted out in test tubes and weighed. Plasma radioactivity (counts per minute) was measured with multi-well automatic gamma counter (Wallac- Wizard). The average background subtracted counts of each sample and standard were weight corrected to get a final value, which

was used for calculations.

Tc-99m DTPA plasma clearance was determined by G2S method on a Microsoft excel based spread sheet program utilizing two sample formula proposed by Russell et.al.(6). The excel spread sheet program used was the same as developed and standardized already (18-19).

$$\text{GFR (ml/min)} = \{D \ln(P1/P2)/(T2 - T1) \exp[(T1 \ln P2) - (T2 \ln P1)]/(T2 - T1)\} \times 0.93$$

Where

*D*: dose of Tc99m - DTPA injected;

*T1*: time of first blood sample (60 minutes);

*P1*: plasma activity at *T1*;

*T2*: time of second blood sample (180 minutes);

*P2*: plasma activity at *T2*.

Unit for *D*, *P1* and *P2* was cpm/min/ml; unit for *T1*, *T2* was minutes.

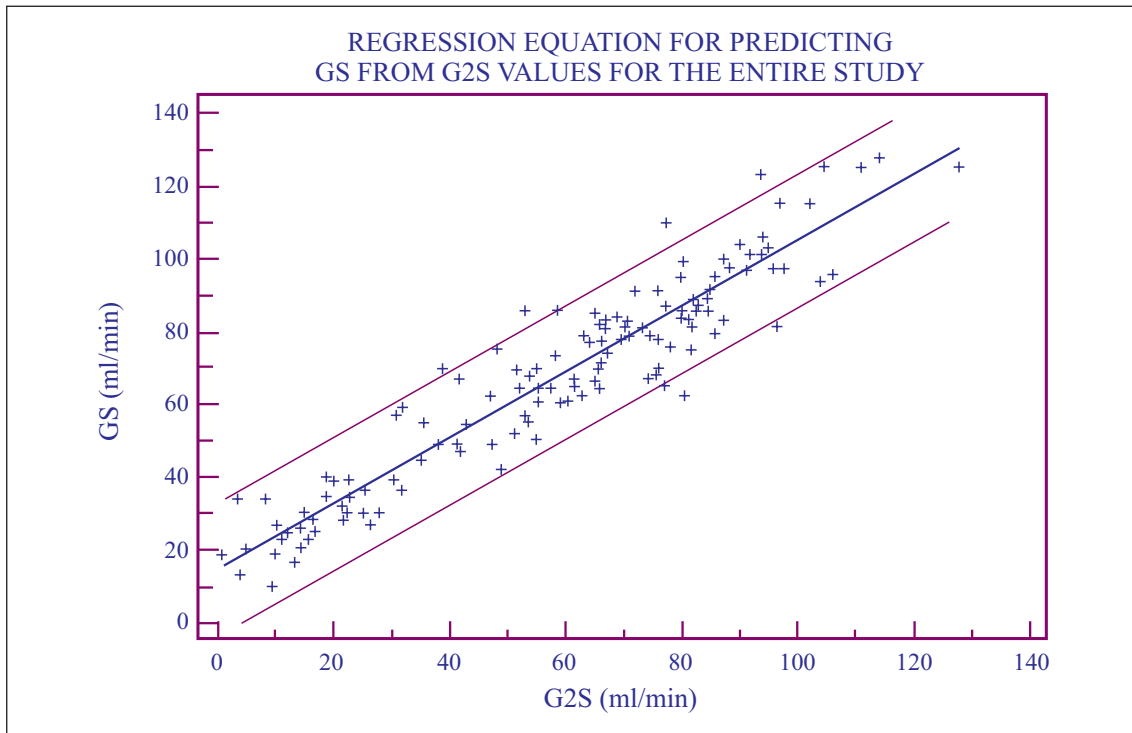
### Statistical analysis

Quantitative variables were described as mean ± standard deviation. Paired t test, Pearson's correlation coefficient and linear regression were used to describe the relationship of GS and G2S in the entire study group, CKD group and Donor group, respectively. The results were considered significant, if the p value was less than 0.05. Bland Altman plot of difference of methods against average of methods was used to express the performance of G2S and GS (20). A regression equation was also developed to estimate the GFR by GS method from G2S method.

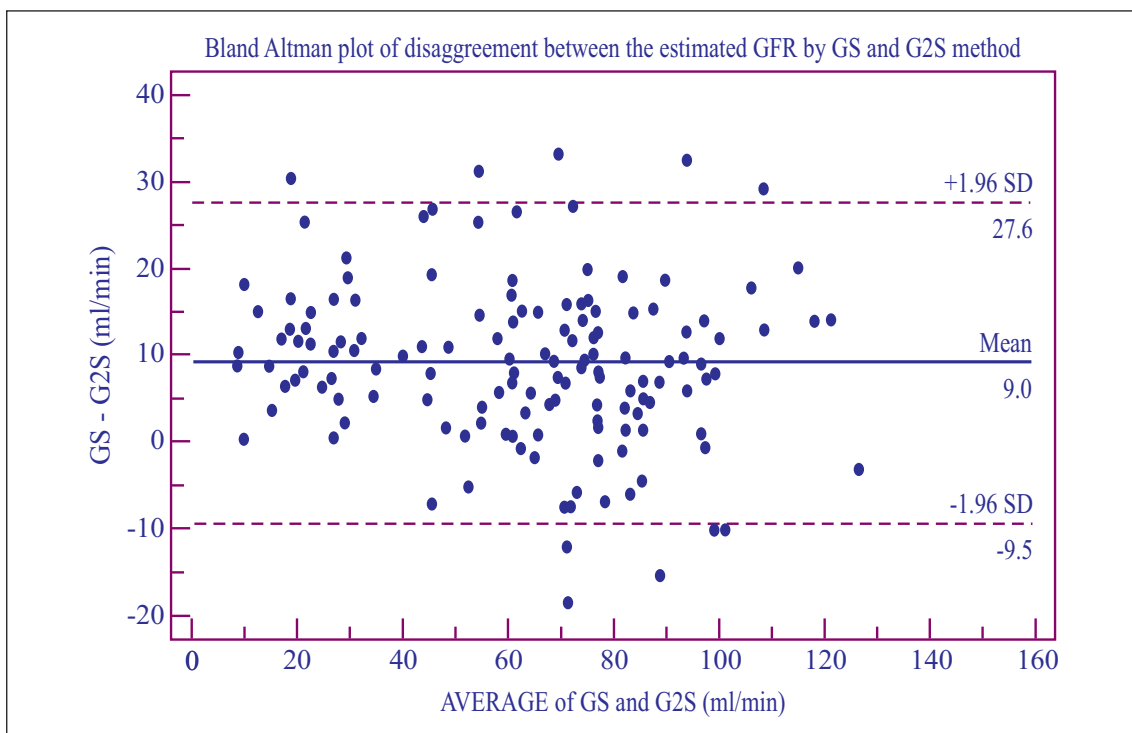
## Results

The study group comprised of 148 individuals (64 male; 84 female), aged between 14 to 70 years (mean age 43.39 ± 11.3 years). Mean serum creatinine level was 1.75 ± 1.44 mg/dL (range 0.5-6.6 mg/dL); mean level of blood urea nitrogen was 53.93 ± 38.98 mg/dL (range 12-183 mg/dL). The CKD group comprised 59 patients (38 males, 21 females, mean age 43.42 ± 12.69 years, range; 14 - 70 years) while Donor group included 89 patients (mean age; 43.35 ± 10.37 years, range; 20 - 65 years). Detailed demographic characteristics of all patients are listed in Table 1.

The average GFR values calculated for the entire study group by GS and G2S were 66.92 ± 27.62 ml/min (95% CI; 62.34 – 71.50 ml/min) and 57.88 ± 28.95 ml/min (95% CI; 53.08 – 62.68 ml/min), respectively. Figure 1 shows the



**Figure 1.** Scatter plot of G2S against GS for the entire study group. The correlation coefficient was 0.9453 between the 2 methods, with a slope of 0.9020 and an intercept of 14.7173 mL/min.



GS-five sample method for GFR evaluation; G2S-two sample method for GFR evaluation; GFR-glomerular filtration rate.

**Figure 2.** Bland Altman plot of disagreement between the estimated GFR by G2S method and reference GFR by GS method. Solid line represents the mean difference between two methods and dashed line represents the 95% limits of agreement.

	GS method	G2S Method
All Patients (n = 148)	66.92 ± 27.62 (10-128.1)	57.88 ± 28.95 (0.69-128.0)
CKD Group (n = 59)	41.14 ± 18.39 (10-78.0)	29.80 ± 18.78 (0.69-76.0)
Donors Group (n = 89)	84.75 ± 16.63 (57.0-128.0)	77.80 ± 16.21 (52.0-128.0)

Note: Values in parentheses indicate range in ml/min

**Table 2:** Average GFR values (ml /min) in the studied patients

correlation of results between the two methods of GFR estimation. The G2S values positively correlated with the GS values with the Pearson's correlation coefficient ( $r$ ) of 0.9453 ( $P < 0.0001$ ). From regression analysis of entire study: residual standard deviation was 9.043 ml/min. The following equation may be applied to correct the GFR estimated by G2S(x) method to approximate the GFR value by GS (y).

$$y = 0.9020x + 14.7173$$

The mean GFR determined for the CKD patients by GS and G2S were  $41.14 \pm 18.39$  ml/min (95% CI; 36.30 – 45.97 ml/min) and  $29.80 \pm 18.78$  ml/min (95% CI; 24.86 – 34.74 ml/min). The correlation coefficient ( $r$ ) between GS and G2S was 0.8963 ( $P < 0.0001$  with 95 % CI = 0.8302 - 0.9375). The mean GFR determined for the donor group by GS and G2S methods were  $84.75 \pm 16.63$  ml/min (95% CI; 81.18 to 88.31 ml/min) and  $77.80 \pm 16.21$  ml/min (95% CI; 74.32 to 81.27). The correlation coefficient ( $r$ ) between GS and G2S was 0.8052;  $P < 0.0001$  with 95 % CI = 0.7153 to 0.8688. Bland–Altman plot of differences of GS and G2S against average of the two methods for the entire study group (Figure 2) revealed a mean difference of 9.0 ml/min (95% CI; 9.5 to 27.6 ml/min). Figure 3 is the Bland-Altman plot of differences between CKD-GS and CKD-G2S against average of the two methods for the chronic kidney disease (CKD) patients group, while Figure 4 represents Bland–Altman plot of difference between D-GS and D-G2S against average of the two methods for the healthy renal donor group. The GFR values for all these groups have been depicted in Table 2.

## Discussion

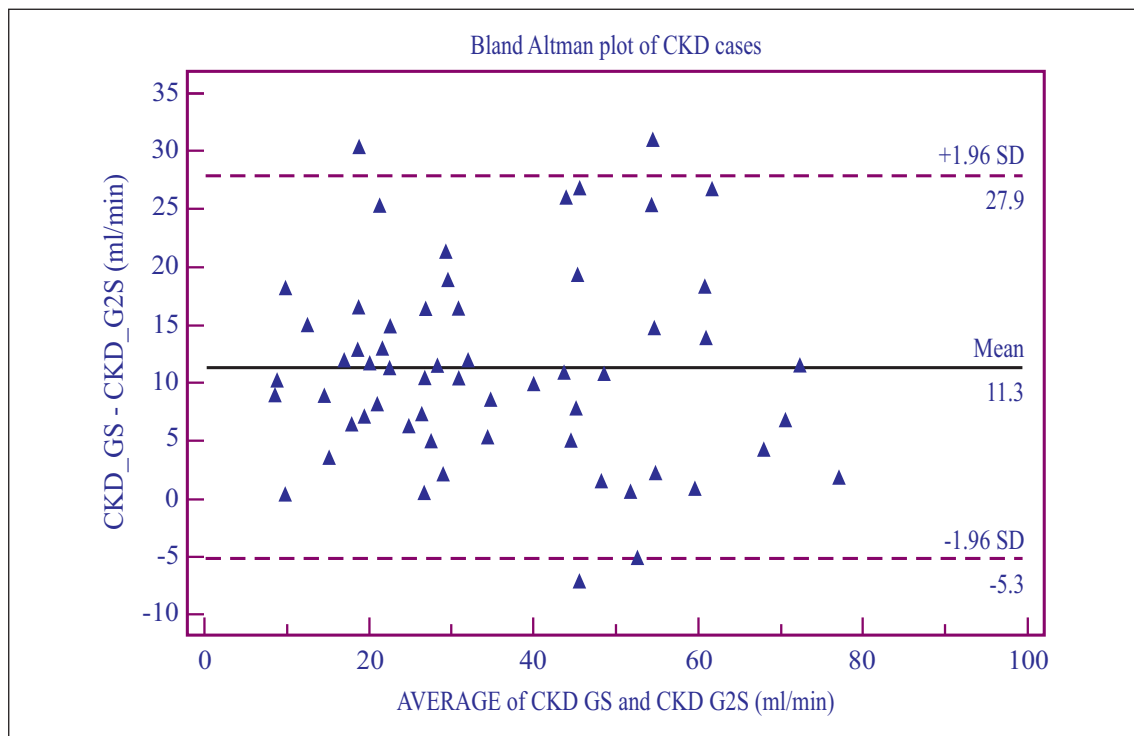
With the easy availability of techniques for intervention, the need for simple and accurate methods for measurement of renal function has increased. Serum creatinine concentration depends on muscle mass and is not usually elevated beyond the normal range until the GFR has fallen by at least 50%. Compensatory increase in tubular secretion during CKD limits the validity of endogenous serum creatinine clearance as a measure of GFR (1-3). Inulin clearance remains the gold standard as a GFR tracer (3), but it is expensive, time-consuming and requires a steady state plasma concentration and urine collection for the greatest accuracy (7). In addition, it has become increasingly difficult to obtain inulin. Multiple sample plasma clearance

method after a single injection of radioactive marker has proven to be more accurate than the other methods for quantitative renal function assessment (7-9). The radiopharmaceutical of choice for GFR estimation is Cr-51 EDTA because its clearance is considered to be the closest to that of inulin (4). However, Tc-99m DTPA clearance correlates well with Cr-51 EDTA (4). It may be noted that multiple plasma sample method is cumbersome in practice. Hence alternative or modified methods are routinely used. One of them is the two plasma sample method, which is derived from the empirical analysis of the relationship between the reference GFR and the volume of distribution and the plasma concentration at the sampling time. The routine GFR measurement at our centre is a slope intercept multiple sample manual method. To simplify and enhance the final process of GFR calculations, in addition to GS method we calculated GFR by G2S spreadsheet program also. The idea was to find out the difference in GFR values and if two sample spread sheet method can substitute 5 sample method of GFR calculation without resulting in any significant difference in the final results, as well as clinical management.

A computer software programme for the determination of GFR using two samples has already been validated in a previous study (18-19). Several equations can be used to calculate GFR values; however Russell formula had been used in the present computer software to calculate G2S values (6). However there has been no agreement study published in literature using computer program. Also the present software has not yet been tested in chronic kidney disease patients and donor patient group. So we proposed to study the agreement as well as validate the computer software in our set up.

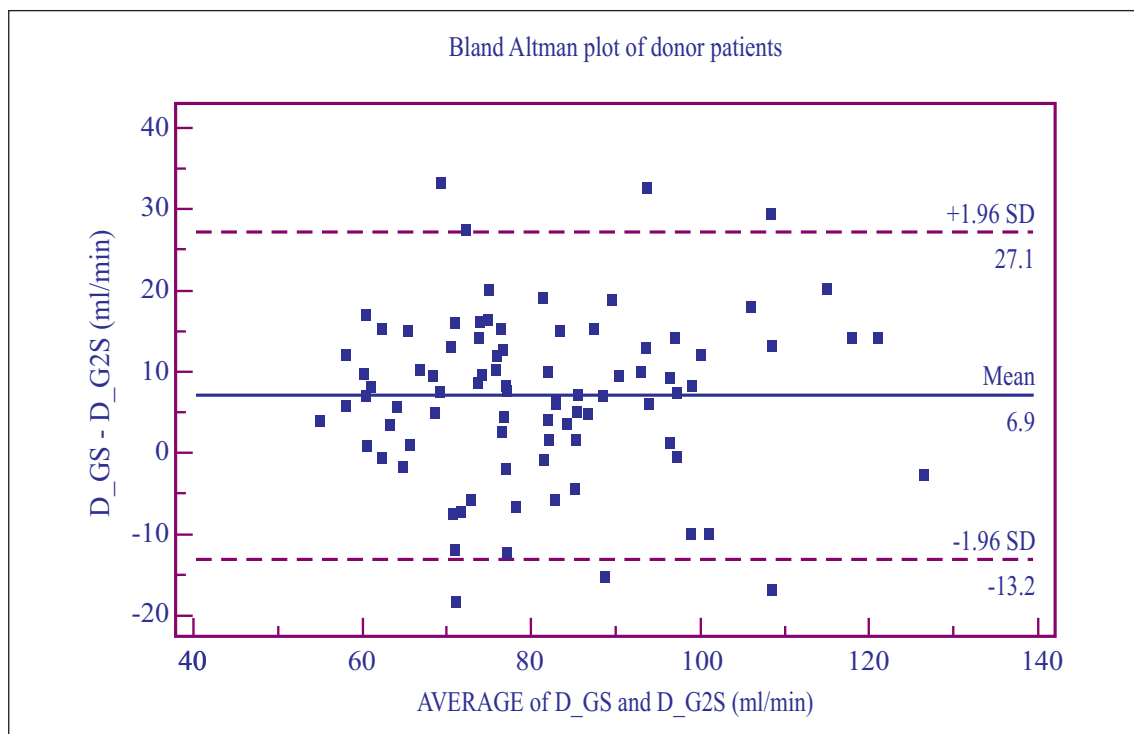
Our results showed that the GFR measured by G2S was lower than that measured by reference GS method in all the study groups. The GFR values determined by GS method almost perfectly correlated with the G2S for the mixed group and CKD patients, while the correlation was only good in the donor group. Similar results have been reported in literature before (20).

Bland Altman plot of disagreement showed that the two plasma sample method based on the MS excel spreadsheet program (G2S), underestimated the GFR values by 9.0 ml/min, for the entire study group (Figure 2). The G2S method also underestimated GFR in patients belonging to the CKD group as well as in healthy donors by 11.3 and 6.9



CKD-GS is five sample method for GFR evaluation in CKD patients; CKD-G2S is two sample method for GFR evaluation in CKD patients.

**Figure 3.** Bland–Altman plot of differences between CKD-GS and CKD-G2S against average of the two methods for the chronic kidney disease (CKD) patients group. The mean difference was 11.3 ml/min, 95 % confidence interval (-5.3ml/min, 27.9 ml/min).



D-GS is five sample method for GFR evaluation in donor patients; D-G2S is two sample method for GFR evaluation in donor patients.

**Figure 4.** Bland–Altman plot of difference between D-GS and D-G2S against average of the two methods for the healthy renal donor group. The mean difference was 6.9 ml/min, 95 % confidence interval (-13.2ml/min, 27.1 ml/min).

ml/min, respectively (Figures 3 and 4). GFR values were noted to be widely scattered in all the CKD patients, which is consistent with the reported literature (7). Literature suggests the use of five plasma sample method for the GFR estimation whenever special accuracy is needed (7). The GFR values calculated for the entire study group and healthy donors were also scattered, suggesting that the G2S values do not agree with the reference GS values either. We also have noted that it is possible to reach the GFR values obtained by GS method by applying the regression equation to the GFR values obtained by G2S method.

### Conclusion

Our results indicate that in spite of good correlation between GS and G2S method, the G2S method constantly under estimated GFR in our patient as well as healthy control population. To obtain reliable reference GFR values, it is desirable that five sample method (GS) be used in clinical trials. However, regression equation can be applied to GFR values calculated by G2S method to achieve more accuracy in routine day to day clinical practice.

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