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Comparison of Glomerular Filtration Rate Measurement Methods between Radionuclide *in vivo* Scintigraphic Gates' and Plasma Sampling

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Abstract

AIM: To correlate between the radionuclide *in vitro* plasma sampling method (using single and dual blood samples) and Gates' GFR measurement using Tc-99m diethylene triamine penta-acetic acid (Tc-99m DTPA) renal scintigraphy (*in vivo* method).

METHODS: This study included 40 renal donors (group 1) and 40 patients with obstructive uropathy (group 2). Group 1 included 22 males and 18 females with an age range from 22 to 65 years, while group 2 included 24 males and 16 females with age range 27 to 64 years. Both groups subjected to renal Scintigraphy after administration of 5 mCi 99m-Tc DTPA, GFR was calculated using Gates' method (*in vivo* method), then plasma sampling was acquired at 60 mins and 180 mins post-injection of the tracer, samples were counted in well counter and GFR was calculated using *in vitro* technique either using single plasma sample (SPSM 60 mins) or dual sample (DPSM 60 & 180 min). Additionally, GFR was measured by estimated equations based on serum creatinine.

RESULTS: In group 1, the mean GFR using *in vivo* Gates' method was 115.7 ± 29 ml/min, while using the SPSM was 100.1 ± 16.1 ml/min, and the DPSM was 100.3 ± 20.1 ml/min. In group 2, mean GFR using *in vivo* method was 74.1 ± 14.5 ml/min, while using *in vitro* SPSM it was 77.5 ± 24.9 ml/min and DPSM was 76.8 ± 24.8 ml/min. There was no significant difference between mean GFR values using *in vivo* and *in vitro* methods (single or dual samples) in group 1 and 2 ($p > 0.05$). There is high significant correlation between SPSM and DPSM in groups 1 and 2 ($r = 0.90$, $r = 0.91$ respectively), moderate significant correlation was found between *in vivo* Gates' method and *in vitro* SPSM in group 1 and 2 ($r = 0.46$ and 0.57 respectively) and moderate correlation was evident between *in vivo* and *in vitro* DPSM in both groups ($r = 0.42$ and 0.68 respectively). By using the DPSM as the reference standard significant high correlation was found with SPSM and significant-high moderate correlation with *in vivo* Gates' scintigraphic method. Conclusion: *In vitro* plasma sampling considered as a reliable, accurate |method for GFR calculation yet it considered relatively complex, both single and dual sample *in vitro* techniques showed a very high correlation, and hence SPSM can replace DPSM.

CONCLUSION: Renal scintigraphy and GFR estimation using Gates' *in vivo* method is considered inaccurate, yet given its simplicity in performance it can still be used if corrected GFR is standardised for Egyptian population-based on studies with large numbers of patients from multiple centres.

Introduction

Glomerular filtration rate (GFR) is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time [1]. The Kidney Disease Outcome Quality Initiative (K/DOQI) of the National Kidney Foundation clinical practice guidelines identified GFR as the keystone for the definition and staging of chronic kidney disease including obstructive uropathies [2].

Inulin clearance has long been regarded as the 'gold standard' method for GFR calculation [3]. Yet it was restricted in clinical practice as it considered a complex technique that requires constant intravenous infusion and bladder catheterisation [4]. Evaluation of GFR using camera-based Tc-99m DTPA renal scintigraphy is a noninvasive method, less time consuming and does not require urine or blood samples collection. It also can identify the individual renal function, whereas other methods evaluate the global renal function. The major disadvantages of scintigraphy include the use of radioactive isotopes, specialised Gama camera needs, and expertise in evaluating the procedure [5].

GFR can be accurately calculated from

the rate of clearance of a tracer activity (commonly used Tc-99m DTPA) from the plasma, which considered a precise method simulating inulin clearance. Initially, multisampling technique was used yet it was exhaustive and difficult to perform in routine clinical practice. Simpler methods have been proposed in clinical practice in which the GFR is estimated from only one or two plasma samples (based on empiric relationships relating an apparent tracer volume with various GFR regression equations) rather than from a multi-sample time-activity curve [6].

Another less accurate method for GFR assessment includes estimated equations based upon serum creatinine such as the Cockcroft-Gault equation (CG), the Modification of Diet in Renal Disease (MDRD) Study equations, and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. That provides a quick and simple estimate for GFR [7] however they are limited by the use of serum creatinine which depends on variations in creatinine production also they are less accurate in certain populations including diabetic patients with high GFR [8]. In light of the above factors; it was decided to compare the single and double plasma sampling method with scintigraphic Gates' *in vivo* method and estimated equations to observe the reliability of these measures in routine clinical practice.

Material and Methods

This comparative study included 80 subjects, 40 renal donors considered as control (group 1) and 40 patients diagnosed as obstructive uropathy (group 2), they were selected from the patients who were sent for routine renal study in Nuclear Medicine Unit, Cairo University during the period from July 2013 till April 2014. The study was approved by the ethical committee. The Inclusion criteria included patients above 18 years old with serum creatinine level within the normal range for both groups, while exclusion criteria included patients under 18 years old, patient with a history of marked renal impairment with GFR < 30 ml/min and high serum creatinine level (> 1.5). Both groups were subjected to full clinical history taking, and serum creatinine level is measured and recorded.

In vivo Gate's method: patients are well hydration and voiding was done just before the beginning of the study**.** Pre – injection syringe containing 185 MBq, Tc-99m DTPA (5 mCi) was counted using dual-head gamma camera (Philips-Axis) before injection. Then an intravenous bolus injection of the tracer was done followed by dynamic imaging acquisition in the posterior position. The post-injection syringe was counted at the end of study similar to pre-injection. The difference between the pre and the post-injection counts provided the total injected dose. Region of interest (ROI) for each kidney was drawn manually and semi-lunar background ROIs were placed around the lower outer renal margins. The background-corrected time-activity curve was generated, and the renal uptake of each kidney from 2 to 3 min after the injection was calculated. Afterimage acquisition, patient's weight and height were entered into the computer software system, on which all imaging data were recorded, and the GFR was automatically calculated according to the Gate's algorithm [9].

In vitro plasma sampling method: Tc-99m-DTPA plasma clearance measured by SPSM and DPSM. After scintigraphy, the site of injection on the arm was scanned under the Gamma camera. The residual radioactivity at the injection site should be less than 0.1% in all subjects, venous blood samples (10 ml) were collected in a syringe from the contralateral arm at 60 and 180 min through. The blood samples were centrifuged at 1000 g for 15 min to separate the red blood cells from the plasma, then 1 ml of plasma from the sample as well as the standards was counted in well counter of (Atom lab 960 thyroid uptake system) for 1 min after 24 hours. The decay of radioactivity was corrected. Time at which the blood sample was taken was recorded on the worksheet. The blood samples taken at 60 min and 180 min were used for the DPSM and a sample taken at 180 min was used for SPSM. Russell's method was used for *in vitro* GFR estimation [10]. Estimation equations for each patient based upon serum creatinine were calculated including the Cockcroft-Gault equation and 2009 CKD-EPI equations.

Statistical methods: All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 17 for Microsoft Windows. Data were statistically described in terms of mean ± standard deviation (± SD). Comparison of numerical variables between the study groups was made using Student t-test, Paired t-test and Chi-square test. Linear Correlation Coefficient was used for detection of correlation between two quantitative variables in one group. Also, standard linear least-squares regression analysis was used, p-values of 0.05 or less in the linear regression analysis were considered significant. Bland and Altman's analysis were referred to an agreement between the two methods for independent samples.

Results

No significant difference concerning age and gender between both groups was detected. By using

the *in vivo* scintigraphic method, the mean GFR is in group 1, and group 2 was 115.7 ± 29.0 ml/min and 74.1 ± 14.5 ml/min respectively. The difference in mean values between both groups where statistically significant (p ≤ 0.001) (Table1).

Table 1: Mean and range of GFR as measured by a radionuclide *in vivo* method in both groups

Groups	GFR in vivo						T-Test	
	Range			Mean	±	SD	t	P-value
Group 1	70.5	-	169.0	115.7	±	29.0	-8.106	< 0.001*
Group 2	42.3	-	98.1	74.1	±	14.5		

No significant difference was found between the mean GFR values using *in vitro* SPSM and DPSM in both groups; mean GFR in group 1 for the SPSM & DPSM was 100.1 ± 16.1 ml/min and 100.3 ± 20.1 ml/min respectively, while it was 77.5 ± 24.9 ml/min, and 76.8 ± 24.8 ml/min in group 2 respectively (p-value 0.6 and 0.8). However, there is a significant difference between both groups by applying each *in vitro* method (SPSM and DPSM) separately (p-value < 0.001) (Table 2).

Table 2: Mean and range of GFR as measured using radionuclide *in vitro* method (single and dual plasma samples) in group1 and 2

Groups		GFR In Vitro						
		Group 1			Group 2			P-value
Single	Range	69.3	-	122.6	33.5	-	135.8	< 0.001*
	Mean ± SD	100.1	±	16.1	77.5	±	24.9	
Dual	Range	70.9	-	138.2	39.2	-	139.6	< 0.001*
	Mean ± SD	100.3	±	20.1	76.8	±	24.8	
	T	0.446			-0.187			
	P-value	0.658			0.852			

Creatinine based equations: using CG equation in both group 1 and 2, mean value of GFR was 143.1 ± 6.4 ml/min, 104.35 ± 27.41 ml/min respectively, whereas the CKD-EPI method means GFR values were 109.41 ± 18.7 ml/min, 85.21 ± 22.39 ml/min respectively. The difference between the two equations in both groups is statistically significant (p < 0.001) as shown in (Table3).

Table 3: Mean and range of GFR as measured by creatinine-based estimated equations

Groups		GFR		T-Test
		Group 1	Group 2	P-value
CG-EQU	Range	71.0 - 198.0	53.0 - 155.0	
	Mean ± SD	143.1 ± 36.4	104.4 ± 27.4	< 0.001*
CKD-EPI EQU	Range	64.0-129.0	45.0 - 124.0	
	Mean ± SD	109.4 ± 18.767	85.2 ± 22.4	< 0.001*
Paired t-test	T	5.83	6.24	
	P-value	< 0.001*	< 0.001*	

Correlations between different methods of GFR measurement

There is a highly significant correlation between *in vitro* SPSM and DPSM in both groups, (r = 0.90) for group 1 and (r = 0.91) for patients group as demonstrated in (Figure 1).

A moderate significant correlation was found between *in vivo* and *in vitro* SPSM in both groups (r = 0.46 and 0.57). Also, a moderate correlation was evident between *in vivo* and *in vitro* DPSM in both groups (r = 0.42 and 0.68).

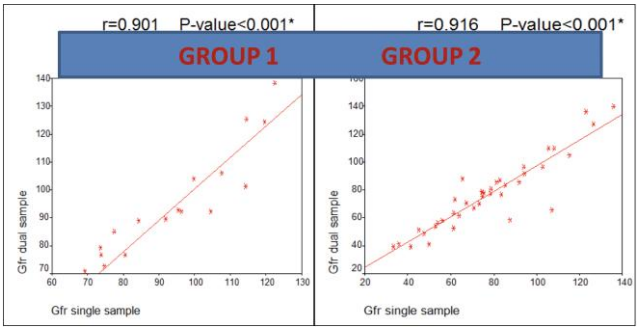


Figure 1: Scattered plot showing a linear correlation between single and dual plasma sampling using radionuclide in vitro methods for measuring GFR in both groups (r, 0.901 and 0.916) respectively and (P-value < 0.001)

Group 1 showed a low moderate significant correlation between radionuclide SPSM and DPSM *in vitro* method and CG creatinine-based equation (r = 0.43 and 0.33) respectively, while there is no significant correlation in GFR estimation between them in group 2. CKD-EPI 2009 equation demonstrates moderate significant correlation in GFR estimation compared to *in vitro* (SPSM & DPSM) in both group 1 and 2 (r = 0.46 and 0.37) and (r = 0.38 and 0.46) respectively.

Table 4: Linear regression between the dual sample *in vitro* technique and other methods in group1

Group 1	Standardised Coefficients		R^2
	r	Sig.	
(Dual Sample)		0.00	44.20%
GFR invivo	0.68	0.00	
(Dual Sample)		0.17	
GFR SPSM	0.90	0.00	80.74%
(Dual Sample)		0.00	8.66%
GFR CG-EQU	0.33	0.04	
(Dual Sample)		0.00	12.19%
GFR CKD-EPI EQU	0.38	0.02	

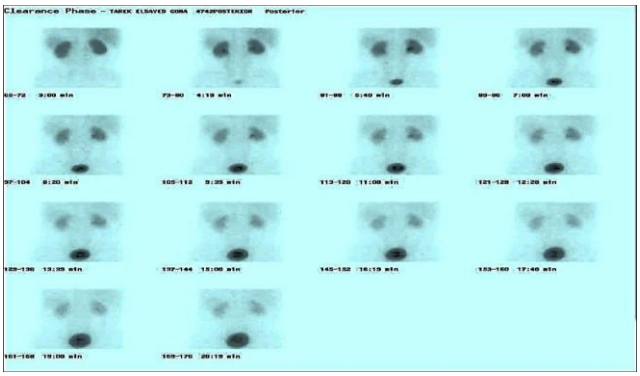
Taking the double sample radionuclide *in vitro* technique as a reference standard; linear regression analysis is considered to be significant (p < 0.05) against *in vivo* Gates' method, SPSM *in vitro* and estimated creatinine equations (CG and CKD-EPI 2009) methods respectively in control group**.** The accuracy of regression equations of dual sample radionuclide *in vitro* is highest against single sample technique (80.7%) while is moderate with *in vivo* Gates' method (44.2%) and very low against CG, and CKD-EPI creatinine-based method was (8.6% and 12.19%) respectively (Table 4 and 5).

Table 5: Correlations between DPSM and other different methods for GFR estimation in group1 (renal donors)

Methods	r -value	Correlation
DPSM & SPSM	0.91	High
DPSM & in vivo	0.68	High moderate
DPSM & CKD-EPI	0.38	Low moderate
DPSM & CG	0.33	Weak

Discussion

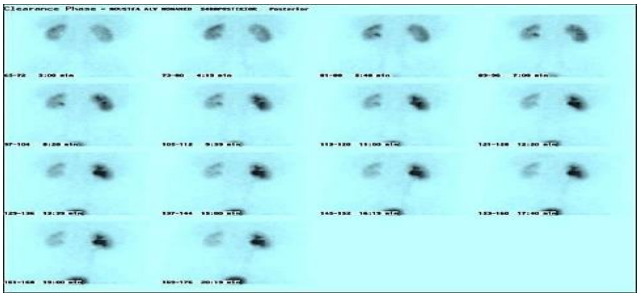
Glomerular filtration rate (GFR), the best overall index of renal function, many methods are developed to estimate GFR to obtain more accurate value and simpler procedure including the equations based on serum creatinine and serum cystatin C, and renal dynamic imaging method [11], [12]. Cr-51-EDTA and Tc-99m-DTPA are among the most commonly used radionuclide tracers for measuring GFR. Studies have shown that their renal clearance correlates well with inulin clearance which was considered the standard gold method.



GFR In vivo	GFR SPSM	GFR DPSM	GFR-CG equ	GFR-CKD EPI equ
121.2	114.6	125.27	183	125

Figure 2: Male donor, 41 years old with normal GFR value by different methods

Plasma clearance of Tc-99m-DTPA using *in vitro* plasma sampling method correlates well with inulin clearance (standardised estimation error is 3.5 ml/min) [13], [14]**.** Based on study results, the DPSM in a mono-compartment model is more accurate in GFR determination than the SPSM [15], this method is taken as a reference in our study as inulin clearance was not available for our setup.



GFR In vivo	GFR SPSM	GFR DPSM	GFR-CG equ	GFR-CKD EPI equ
65.5	87.59	78.59	88	52

Figure 3: Male patient 54 years old, complaining of right loin pain 2 months ago, diagnosed radiologically as right renal stone with grade II to III backpressure. There is a normal value of GFR using in vitro method as compared to in vivo method

Similarly, because of the satisfactory accuracy and relative simplicity of 99mTc-DTPA dual plasma sample clearance, this method was taken as the reference approach in determining GFR by the Nephrology Committee of Society of Nuclear Medicine [16].

The results of the present study demonstrate that the DPSM correlate well with the SPSM in both groups (r = 0.91). Similar results were reported in a study by Mulligan et al., [17]. The DPSM using Russell's formula considered as a reliable method for the valid estimate of true GFR. Also, in a study by Itoh et al., [18] Russell's SPSM was compared with 10 sample method, and the coefficient was 0.971. Furthermore, Zuo et al., [19] reported that the DPSM should be used when GFR is less than 45 ml/min.

In our study, GFR ranged 33.5-135.8 ml/min with a mean value of 77.5 ± 24.9 ml/min using SPSM, while using DPSM ranges 39.2-139.6 ml/min with mean GFR value of 36.8 ± 24.8 in obstructive uropathy group. The Gates *in vivo* [20] method was considered feasible and very simple when compared to the plasma sampling method, which was a bit complex yet more accurate.

Jackson et al., [21] reported that the Gates method tended to overestimate GFR in comparison to the dual sample *in vitro* method. Itoh [22] also reported overestimated GFR values with the Gates method and indicated that the overestimation might be attributable to insufficient correction for background activity in the kidney**.** In the present study *in vivo* GFR measurement using the Gates method also tends to overestimate GFR, the value ranges 42.3-98.1 ml/min with a mean value of 74.1 ± 14.5 ml/min in group2. GFR estimation was performed in 133 patients using: A) gamma camera uptake method (modified Gates, Gates); B) predicted creatinine clearance method (Cockcroft-Gault, CG); and C) single- or two-plasma clearance method (PSC). The PSC was chosen as a reference (Same as in the current study). In comparison with the GFR by PSC, the Gates tended to overestimate the GFR, as found in our study. This study concluded that The Gates correlates well with the PSC, while in our study, it showed a moderate correlation.

Itoh et al., [22] showed that GFR estimation using by *in vitro* method is better than the CG method, which tended to underestimate the GFR. In our study GFR values using CG method ranges from 71-198 with a mean value of 143.1 ± 36.4 in group1 with low, moderate correlation (r = 0.33) in both SPSM &DPSM. The estimated creatinine equations show a weaker correlation than Gates as compared to the *in vitro* techniques. However, in group1 the DPSM and *in vivo* camera-based method showed a mean difference of -15.43 ± -8.92(95% confidence interval CI). Whereas for CKD-EPI method, the mean difference was -9.09 ± 1.37, 95% CI. Accordingly, we concluded that both the Gates *in*

vivo and the CKD-EPI equation tended to overestimate GFR, especially in the range of high GFR (group 1).

In conclusion, dual sample *in vitro* method (DPSM) was considered as the reference with good correlation with the SPSM. Whereas neither Gates method nor CKP-EPI predicted creatinine equation could calculate GFR accurately as they tend to overestimate GFR measurement, especially in the range of high GFR. Our study was limited by the small number of patients. Gold standard "inulin" *in vitro* GFR measurement was not available for comparison. Also, normal GFR in the Egyptian population has not been standardised specially in children were *in vitro* SPSM and DPSM will be a proper method for GFR.

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