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Comparison Between In vivo Gate's Method and in Vitro Plasma Sampling Method for GFR Measurement Using 99m-Tc DTPA

Younis, J. Moustafa, H. and Hussein, M.

Nuclear Medicine Department (NEMROCK center), Cairo University, Egypt

ABSTRACT

Objective: To compare between Gates GFR measurement using 99m Tc DTPA scintigraphy (in vivo method) as compared to in vitro blood sampling method (single and dual blood samples).**Patients and methods**: this prospective study included 40 normal individuals (group 1) and 40 patients with obstructive uropathy (group2) .The age of the group 1 ranging from: 22 to 65with mean age of 47.1±14.08 and in group 2it ranges between 27 years to 64 years with mean age of 49.12±9.1 .Group 1 included: 22 males, 18females, while group 2 included 24 males and 16 females. Both groups subjected to 99m-Tc DTPA renal scan using 8 mCi followed by blood sampling at 60 mins and 180mins post injection. Serum creatinine level was estimated for both groups and it was within normal level. **Results:** In group 1, the mean GFR using in vivo method was115.7 ± 29, and using in vitro method with single blood sample it was 100.1 ±16.1, and using dual blood sampling method it was 100.3±20.1.There was no significant difference between in vivo and in vitro methods (single sample and dual samples) for measuring GFR, in group 1 (p>0.05). In group 2, GFR using in vivo method was 74.1 ± 14.5, while using in vitro single sample method it was 77.5 ±24.9, and by using in vitro dual sample it was 76.8 ±24.8 with no significant difference using in vitro single sample and dual sample measurements. There is high significant correlation between in vitro single and dual sample in both groups,**(r=0.90)** for control group and **(r=0.91)** for patients group, while moderate significant correlation was found between in vivo and in vitro radionuclide **single** sample methods in both control and patients groups **(r=0.46 and 0.57).** Also moderate correlation was evident between in vivo and in vitro radionuclide **dual** sample methods in both groups **(r=0.42 and 0.68)**.

Conclusion: No significant difference in measurement of GFR using both in vitro and in vivo methods in control group and in obstructive uropathy group ,however there is moderate correlation between in vivo and in vitro (single and dual sample) method in both groups but there is high correlation between in vitro method using single blood sample and dual blood samples.

Keywords: Gates method, double plasma sample method, glomerular filtration rate, single plasma sampling method.

Corresponding Author: Younis, J**.** **E mail:** jehan.nuc@hotmail.com

INTRODUCTION:

Glomerular filtration rate (**GFR**) is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsuleper unit time **[1]** GFR is customarily assessed by measuring the concentrations of serum markers such as blood urea nitrogen and serum creatinine. Although widely used, these endogenous markers are not ideal and occasionally do not perform well. The other method for determining GFR is to measure the clearance of exogenous substances such as inulin, chromium-51-ethylenediaminetetraacetic acid (EDTA), technetium-99m labeled DiethyleneTriaminePenta Acetic Acid (Tc-99m DTPA) or I-125 labeled Iothalamate**[2].** Although GFR cannot be measured directly, the best method for determining GFR is measurement of the urinary clearance of an ideal filtration marker. **(3).** Camera-based renal scintigraphy is a noninvasive method to evaluate GFR, the evaluation of renal function is comparable to standard methods of GFR determination (eg, inulin clearance and creatinine clearance) **[4, 5].** 99mTc-DTPA has become a standard GFR tracer in Europe and the United States and has been recommended by the BNMS, ISCORN, and EANM **[6-8].**GFR can be calculated from the rate of clearance of tracer activity from the plasma following a single intravenous injection of a suitable radiopharmaceutical. As long as the radiopharmaceutical is excreted exclusively by glomerular filtration and is not bound to plasma protein or to any other component of blood or other tissue, the GFR can be calculated simply by dividing the administered dose by the integral of plasma time-activity curve. Initially GFR was calculated from the multisampling technique with the samples taken at different time. GFR was calculated from the dose divided by the area under the curve. Since it was exhaustive and difficult to perform in routine clinical practice, single and double plasma sampling GFR formulae were derived from multi sample technique. Fairly accurate methods have been proposed in which the GFR is estimated from only one or two plasma samples rather than from a multi sample time-activity curve. Gates computed the GFR from the scintigraphic determination of Tc-99m-DTPA uptake within the kidneys. With the above factors in mind; it was decided to compare the single and double plasma sampling method with Gates GFR and observe the reliability of these measures in routine clinical practice.

MATERIAL AND METHODS :

This prospective study included 80 subjects, 40 control (group 1) who were volunteers and 40 patients diagnosed as obstructive uropathy (group 2) selected from the patients who were sent for routine renal study in Nuclear Medicine Unit (NEOMROCK Center), Cairo university during the period from July 2013 till April 2014. The study was approved by the ethical committee. The Inclusion criteria included volunteers andpatients above 18 years old and with different sex with serum creatinine level within normal range for both groups, whileexclusion Criteria included patients under 18 years old, patient with history of marked renal impairment with GFR <30ml/min, pregnant patients, high serum creatinine level (>1.5).Both groups were informed about the nature of the study, subjected to full clinical history taking and serum creatinine level is measured. Both groups were subjected to in vivo radionuclide renal scintigraphy and GFR measurements according to Gate's method using Tc-99m DTPA .In vitro plasma sampling (single and dual) method for GFR estimation, estimated GFR according to Cockcroft-Gault equation and 2009 CKD-EPI creatinine based equations.**Methodology and data collection:** We compared the single plasma sampling method(SPSM), double plasma sample method (DPSM), Gates camera method and bothequations (CG equation and 2009 CKD-EPI creatinine equation).

In vivo Gate's method: Patient preparation included good hydration (patient drinks 300-500 ml water) and voiding prior to beginning of study. Dual head gamma camera was used in this work for imaging process (Philips-Axis)**.**Technology &processing:Pre–syringe containing 185 MBq, Tc-99m DTPA (5 mCi) was performed before injection. After a bolus of intravenous injection of Tc99m -DTPA, the dynamic imaging acquisition was carried out in the posterior position using dual head gamma camera Philips axis. The post-injection syringe was counted at the end of in vivo study similar to pre-injection. The pre-count minus the post-count provided the total injected dose. Region of interest (ROI) for each kidney was drawn manually. The semi-lunar background regions of interest were placed around the lower outer renal margins as initially outline with a light pen. The background-corrected time–activity curve was generated, and the renal uptake of unilateral kidney for 1 min from 2 to 3 min after the injection was calculated. After image acquisition, patient's weight and height were entered into an online computer, on which all imaging data were recorded.

The in vivo GFR was automatically calculated by commercially available computer software according to the Gate's algorithm **[9].2].**

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 contra lateral arm at 60 and 180min through an in-dwelling venous cannula. The blood samples were centrifuged and 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 blood samples were centrifuged at 1000 g for 15 min to separate the red blood cells from the plasma. A test dose of 1 ml of plasma was pipette meticulously by taking care to avoid disturbing the interface between the plasma and the red cells. Decay of radioactivity was corrected. Time at which the blood sample was taken was recorded on the worksheet **[10].**

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 done 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 was referred to agreement between the two methods for independent samples.

RESULTS:

This prospective study included 80 participants. The studied groups included, 40 controls (group 1) and 40 obstructive uropathy patients (group 2). No significant difference concerning age and gender between control and patients groups.Radionuclide GFR in vivo (camera based) in group 1 and group 2 showing mean (± SD) 115.7 ± 29.0 and 74.1 ± 14.5 respectively. The difference in mean values between both groups where statistically significant (p=<0.001).Radionuclide GFR in vitro (single and dual samples) the mean GFR in group 1 was 100.1 ± 16.1, 100.3 ± 20.1, while it was 77.5 ± 24.9, and 76.8 ± 24.8 in group 2 respectively. The difference in mean value between radionuclide in vitro single plasma sample and dual methods in control

group and patients group is found to be insignificant (p value 0.6 and o.8). However there is a significant difference using single sample radionuclide in vitro method in both groups and same for the dual plasma sample method, (p value <0.001) (**table 1).**

Table 1: Mean and range of GFR as measured using radionuclide in vitro method (single and dual plasma samples) in control and patients groups.

Groups		GFR In Vitro						T-Test	
		Controls			Patients			t	P-value
Single	Range	69.3	-	122.6	33.5	-	135.8	-4.799	<0.001*
	Mean±SD	100.1	±	16.1	77.5	±	24.9		
Dual	Range	70.9	-	138.2	39.2	-	139.6	-4.648	<0.001*
	Mean±SD	100.3	±	20.1	76.8	±	24.8		

Creatinine based equations: using CG equation in both group 1 and 2 mean value of GFR was 143.10 ± 36.42, 104.35 ± 27.41, whereas CKD-EPI method mean GFR values was 109.41 ±1 8.77, 85.21 ± 22.39 respectively. The difference between the two equations in both control and patients groups is statistically significant (p<0.001) as shown in (**table 2).**

Table 2: Mean and range of GFR as measured by creatinine based estimated equations.

Groups		GFR						T-Test	
		Controls			Patients			t	P-value
CG-EQU	Range	71.0	-	198.0	53.0	-	155.0	-5.377	<0.001*
	Mean±SD	143.1	±	36.4	104.4	±	27.4		
CKD-EPI EQU	Range	64.0	-	129.0	45.0	-	124.0	-5.173	<0.001*
	Mean±SD	109.4	±	18.767	85.2	±	22.4		
Paired t-test	T	5.83			6.24				
	P-value	<0.001*			<0.001*				

There is high significant correlation between in vitro single and dual sample in both groups, **(r=0.90)**for control group and **(r=0.91)** for patients group as demonstrated in **(fig.1)**.

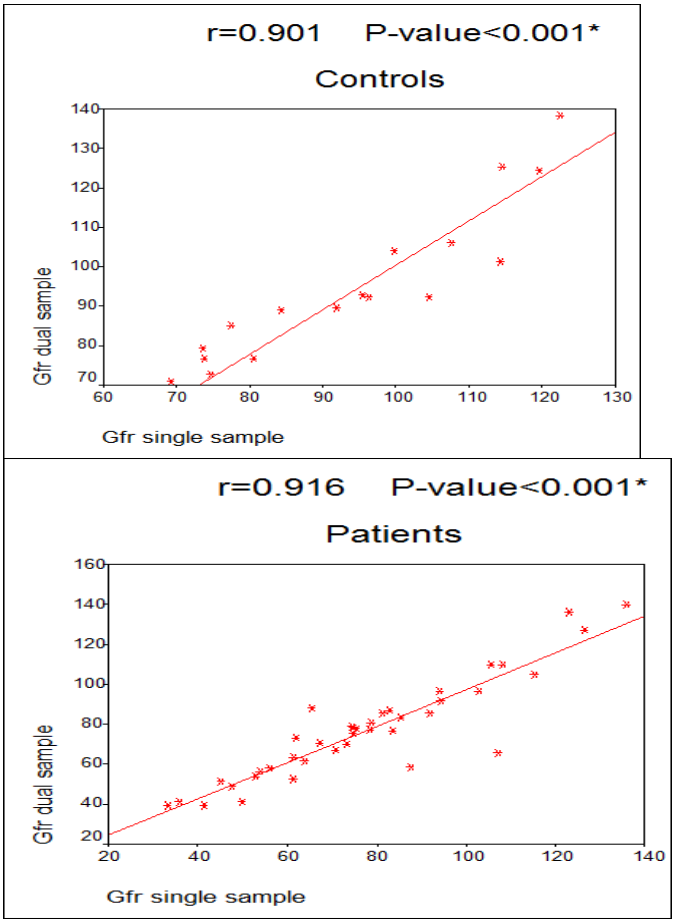


Fig.1: correlation between single and dual plasma sampling using radionuclide in vitro methods for measuring GFR.

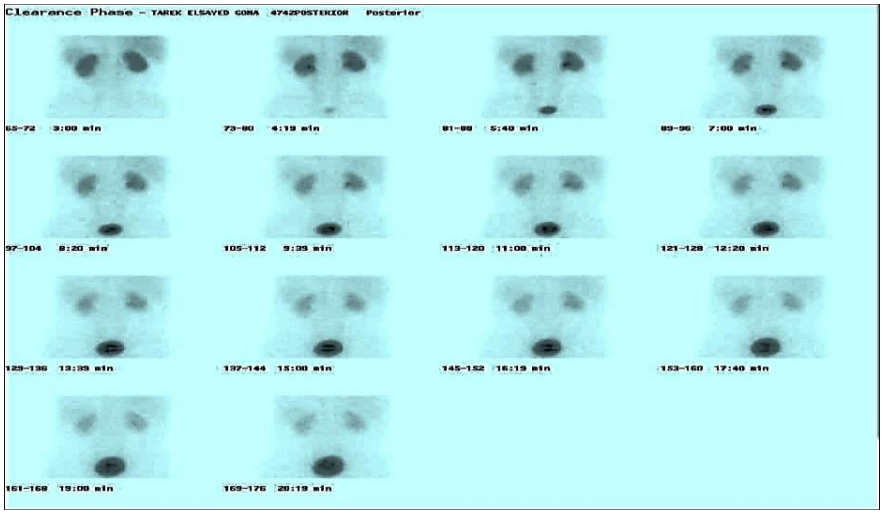
While moderate significant correlation was found between in vivo and in vitro radionuclide **single** sample methods in both control and patients groups **(r=0.46 and 0.57).** Also moderate correlation was evident between in vivo and in vitro radionuclide **dual** sample methods in both groups **(r=0.42 and 0.68)**.There is no significant correlation between radionuclide in vitro GFR estimation and CG creatinine based equation in patients group, while low, moderate significant correlation between radionuclide SPSM and DPSM in vitro method **(r=0.43 and 0.33)** in control group respectively. Moderate significant correlation found between radionuclide in vitro(SPSM &DPSM) and CKD-EPI 2009 creatinine based equation in both control and patients group

(r=0.46 and 0.37)and **(r =0.38 and 0.46)** respectively.Taking the double sample radionuclide in vitro technique as a reference; linear regression analysis is considered to be significant ($p<0.05$) against in vivo Gates' radionuclide, SPSM in vitro

and estimated creatinine equations(CG and CKD-EPI 2009) methods respectively in control group as shown in (**table 4).** The accuracy of regression equations of dual sample radionuclide in vitro is highest against single sample technique (R2=80.7%) followed by moderate correlation with in vivo Gates' method (R2=44.2%) and whereas no correlation was found against CG and CKD-EPI creatinine based methods was (R2=8.6% and 12.19%) respectively.

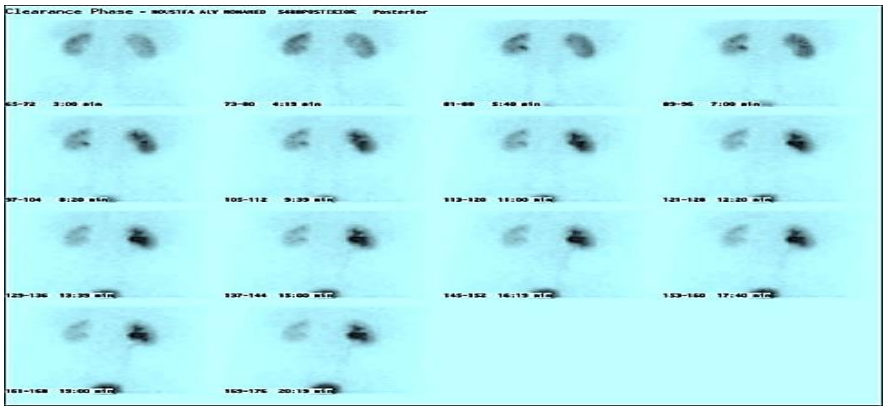
Table 3: linear regression between dual sample in vitro technique and other methods in control group.

Controls	Unstandardized Coefficients		Standardized Coefficients	T-test		R^2
	B	Std. Error	Beta	t	Sig.	
(Dual Sample)	46.13	9.89		4.67	0.00	44.20%
GFR invivo	0.47	0.08	0.68	5.65	0.00	
(Dual Sample)	-12.31	8.89		-1.38	0.17	80.74%
GFR single sample	1.13	0.09	0.90	12.83	0.00	
(Dual Sample)	74.08	12.49		5.93	0.00	8.66%
GFR CG-EQU	0.18	0.08	0.33	2.17	0.04	
(Dual Sample)	55.48	18.27		3.04	0.00	12.19%
GFR CKD-EPI EQU	0.41	0.16	0.38	2.50	0.02	



(Fig.2) Male donor, 41 years old with normal GFR value by different methods.

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



(Fig 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 back pressure. There is normal value of GFR using in vitro method as compared to in vivo method.

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

DISCUSSION:

Glomerular filtration rate (GFR), the best overall index of renal function in health and disease, can be evaluated by several approaches. Many methods are developed to estimate GFR in order to obtain more accurate value and simpler procedure, including the equations based on serum creatinine and on serum cystatin C, and renal dynamic imaging method[11–15]. 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; the99m-Tc DTPA to inulin ratio was 0.97. Further, plasma clearance of Tc-99m-DTPA correlates well with inulin clearance (standardized estimation error is 3.5 ml/min)[16, 17]. The alternative methods used, such as DPSM and SPSM were derived from the relationship between the reference GFR and the volume of distribution and plasma concentration at sample time [18, 19]. Based on Study results that proved DPSM in a mono-compartment model to be more accurate in GFR determination than the SPSM [20], this method is taken as a reference in our study as it was not possible in our setup to acquire inulin for calculating the GFR.In view of the satisfactory accuracy and relative simplicity of 99mTc-DTPA dual plasma sample clearance method was taken as the reference approach in determining GFR by the Nephrology Committee of Society of Nuclear Medicine [21]. The results of the present study demonstrate that the DPSM correlate well with the SPSM in both control and patients groups **(r=0.91).**Similar results

were reported in a study by **Mulligan etal [22].** The DPSM using Russell's formula has been vouched as a reliable method for the valid estimate of true GFR. Also, in a study by **Itohetal [23]** Russell's SPSM was compared with 10 sample method and the correlation coefficient was 0.971. Furthermore, **Zuoetal[24]** reported that the DPSM should be used in order to obtain reliable reference GFR values, when GFR is less than 45 ml/min/1.73 m^2.In our study. GFR ranged 33.5-135.8 with mean value of 77.5±24.9 using SPSM, while GFR using DPSM ranges 39.2-139.6 with mean GFR value of 36.8±24.8 in obstructive uropathy group.The Gatesin vivo[25] method was considered feasible and very simple when compared to the plasma sampling method, which was very cumbersome yet more accurate. **Jackson etal**[15] reported that the Gates method tended to overestimate GFR in comparison to the dual sample in vitro method. **Itoh**[26] 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. **Russell etal.** Suggested that the Gates method with a simple background activity correction is less accurate than the methods with more sophisticated background activity correction for the calculation of GFR[27]**.** In the present study in vivo GFR measurement using Gates method also tends to overestimate GFR, the value ranges 42.3-98.1 with mean value 74.1±14.5 in obstructive uropathy group. Similar data was reported by **Hephzibahet al[28].**In a study done by **Itoh et al[26]** Tc-99m-DTPA renography was performed in 133 patients. The GFR was determined simultaneously by 3 methods; (1) gamma camera uptake method (modified Gates, Gates); (2) predicted creatinine clearance method (Cockcroft-Gault, CG); (3) single- or two-plasma clearance method (plasma sample clearance method, PSC). The PSC was chosen as a reference. 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 ourstudy it showed moderate correlation. **Itoh et al[26]** showed that GFR estimation using by in vitro method is better than CG method which tended to underestimate the GFR. In our study GFR values using CG method ranges from 71-198 with mean value of 143.1±36.4 in control group with low moderate correlation (r=0.33) in both SPSM &DPSM. The estimated creatinine equations show weaker correlation than Gates as compared to the in vitro techniques. However, in control group the dual sample in vitro method and in vivo camera based method showed 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 (control group).

To conclude, clinician's final decision regarding obstructive uropathy or any other renal diseases requires an accurate GFR measurement. 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.

Limitations of the study: The number of patients in our study was small. Gold standard "inulin" or Cr-51-EDTA in vitro GFR measurement were not available for comparison. Also normal GFR in the Egyptian population has not been standardized specially in children where in vitro SPSM & DPSM will be proper method for GFR.

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