Comparison of double plasma sampling method and gates method for estimation of glomerular filtration rate

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Summary

Glomerular filtration rate (GFR) is an important indicator of renal function. Several methods have been applied to calculate GFR in clinical exams. In this study, we evaluated and compared between radionuclide plasma sampling methods (double blood samples, *in vitro* methods) and *in vivo* Gate's method using ^{99m}Tc-diethylene triamine penta-acetic acid (^{99m}Tc-DTPA) renography. *Subejct and method:* 42 patients were participated in this study, including 12 patients with obstructive uropathy (group 1) and 30 renal donors (group 2). The administered doses were in a range of 5 – 7mCi. Then, scintigraphy was performed simultaneously after injection, and GFR was calculated by Gate's method. Blood samples were collected at 60 mins and 120 mins post-injection, which were counted by a thyroid uptake system, and GFR results were determined using a double plasma sample (DPSM) method. *Result:* The mean GFRs calculated by renography in groups 1 and 2 were 85.8 ± 16.2 (ml/min) and 118.9 ± 13.9 (ml/min), respectively. Meanwhile, using the *in vitro* DPSM, the mean GFRs in group 1 and 2 were 73.8 ± 15.4 (ml/min) and 117.0 ± 13.0 (ml/min) respectively. They showed a high correlation between the two methods in the two groups (r = 0.86 and 0.71, respectively). *Conclusion:* Renography is a simple technique but considered inaccurate for determination of GFR. However, *in vitro* DPSM is rarely used in Vietnam. In this study, Gate's method corresponded well with plasma sampling method and tended to overestimate the glomerular filtration rate.

Keywords: 99m-Tc-DTPA, glomerular filtration rate, renography.

1. Background

GFR is a valuable indicator to evaluate kidney function on patients diagnosed as obstructive uropathy and renal donors. GFR is calculated by the flow rate of fluid filtered from glomerulus to Bowman's space per time unit, measured in milliliter per minute. Currently, several methods that are being used to estimate GFR include: Serum creatinine-based, renal scintigraphy using radiopharmaceutical. Inulin clearance is widely accepted as a golden standard method for the determination of GFR. Inulin is freely filtered, is not protein bound, is not reabsorbed, does not affect kidney function, and is neither secreted nor metabolized by the kidney. When injected intravenously, inulin clearance equals GFR. However, this method requires a complex technique and is time-consuming, therefore considered to be difficult for routine clinical practice [1].

Diethylenetriamine pentaacetic acid (DTPA) has the same properties as inulin: Freely filtered and less protein bound (~5%). When labelling with technetium-99m (^{99m}Tc-DTPA), not only renal scintigraphy but also plasma sampling method can

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be done to calculate GFR. Based on the two major components elimination model, the radioactivity remaining in the blood sample taken at two different times may indicate the renal glomerular filtration rate [2, 3]. Several nuclear medicine associations (British Nuclear Medicine Society - BNMS; International Scientific Committee of Radionuclides in Nephrourology - ISCORN; The European Association of Nuclear Medicine - EANM) recommend plasma sampling method as a standard method [4, 5]. Therefore, we undertook this study to compare the routine Gates method with double plasma sampling method which is not a new one but seldom used in Vietnam to investigate their correlation and practicality.

2. Subject and method

2.1. Subject

Study subjects are patients designated for renal scintigraphy at Nuclear Medicine Department, 108 Military Central Hospital. From May 2019 to July 2019, this study included 42 patients. Participants were sent for routine renal study, after that, blood samples were taken at first and second hours exactly.

Patients were divided into 02 groups: 12 patients diagnosed as obstructive uropathy (Group 1) and 30 renal donors (Group 2).

2.2. Procedure

Preparation of radiopharmaceutical

Technetium-99m is extracted from the 99-Mo/99m-Tc generator (Tekcis/Cisbio). 99mTc - DTPA was prepared in our hot-lab using a commercial cold-kit (Pentacis, Curium, France); quality control by thin-layer chromatography was applied after radio-labeling to assure radiochemistry purities not less than 95%.

Renal scintigraphy (Gate's method)

Patients were well-hydrated with 500ml of water before the test. The patients were laid down on the bed in a supine position and ^{99m}Tc-DTPA (dose: 5 - 7mCi) was given intravenously and flushed by 20ml of saline. Posterior dynamic images (1 frame per 2 seconds for 60 seconds and followed by 1 frame per 2 minutes for 30 minutes) were obtained in a 128 × 128 matrix and low energy high resolution (LEHR) collimator. Activity in the post-injection syringe was measured using the gamma camera. Region of interests (ROIs) for each kidney, cortex region, background, and aorta were manually drawn and the time-activity curve was generated by xeleris software (GE, USA). GFR was calculated automatically according to the Gate's algorithm [6] and was normalized for a body surface area (BSA) of 1.73m^2.

$$FU = \frac{(\text{renal count}) / e^{-\mu y}}{(\text{total injected dose counts})} \times 100$$

Note: FU: fractionated uptake. The renal count was calculated from the renal uptake between 2 and 3 min in the renography. μ: Attenuation coefficient of Tc-99m (0.153). y: Kidney depth (cm), which was calculated as described in Tonnesen's formula [7]

The GFR, in ml/min, was calculated as:

$$GFR = 9.75621 \times FU - 6.19843$$

In vitro plasma sampling methods

When renal scintigraphy was finished, the first blood sample (about 10ml) was collected intravenously from the opposite arm to prevent radiation contamination at 60-min post-injection and the second one was taken at 120-min post-injection. The blood samples were centrifuged at 10,000rpm for 10 minutes to separate plasma and red blood cells. A standard solution was prepared by diluting the same amount of ^{99m}Tc - DTPA (5 - 7mCi) radioactivity in 1000ml water. Then, 1.0ml of plasma samples and standard solution were counted in a thyroid uptake system (Atomlab 960, Biodex, USA) for 1 minute.

Double plasma sampling method (DPSM)

GFR was calculated by using Russell's method [8]:

Note: D: Dose (cpm - counts per minute).

P1: Radioactivity of the sample at T1 (cpm/ml).

P2: Radioactivity of the sample at T2 (cpm/ml).

The final result was also normalized for BSA by using the Haycock formula.

$$BSA = 0.024265 \times height\ (cm)^{0.3964} \times weight\ (kg)^{0.5378}$$

Measuring the sample counts

The standard and test samples are taken with correct volume of 1.0ml and stored in the test vial. Counts of samples and background were measured using Atomlab 960 Thyroid Uptake System (Biodex) in 1 minute. The samples were prepared and measured on the same day, the counts were corrected with the half-life ($t_{1/2}$) of 99m-Tc isotope.

Statistical analysis

The one-way analysis of variance (ANOVA) and Pearson correlation were performed using SPSS program (Statistical Package for the Social Science) version 26 and Microsoft Excel 365.

3. Result

Forty-two patients including 16 females and 26 males participated in the study with mean age 41.4 ± 13.3 (24 - 69), average height 161.4 ± 7.7cm, average weight 58.2 ± 7.8kg. Patients were divided into 2 groups: Group 1 (12/42) included patients with abnormal kidney function (kidney stones, hydronephrosis, renal pelvis dilatation) and Group 2 (30/42) included patients with normal kidney function (renal donors). Mean GFR using Gate's method and DPSM on 42 patients were 110.8 ± 21.3 (ml/min) and 106.2 ± 24.0 (ml/min). In Group 1 and 2, mean GFR using Gate's method and DPSM were 85.8 ± 16.2 (ml/min), 73.8 ± 15.4 (ml/min), 118.9 ± 13.9 (ml/min) and 117.0 ± 13.0 (ml/min), respectively (Table 1).

Table 1. Mean GFR using gate's method and DPSM

	Gate's method	DPSM	p
Group 1	85.8 ± 16.2	73.8 ± 15.4	<0.05
Group 2	118.9 ± 13.9	117.0 ± 13.0	0.33
p-value	p<0.05	p<0.05	

The difference in mean values between Gate's method and DPSM in 2 groups was statistically insignificant (p<0.05). For patients with normal kidney function (Group 2), the difference in mean values between 2 methods was statistically significant (p=0.33). The Bland and Altman's analysis for the global difference in the DPSM and Gate's method on 42 patients showed a different mean value of -4.7 (confident interval 95% [CI] = -8.1 ÷ -1.3). Acceptance limit is from -26.6 to 17.1 (Figure 1).

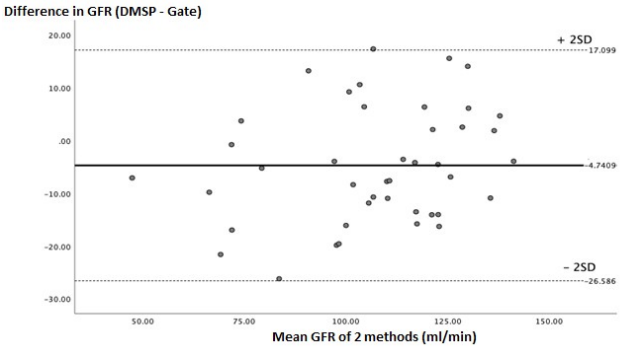

Figure 1. Bland and Altman plots of difference in GFRs by DPSM and Gate's method. The solid lines indicate the mean difference and 95% of agreement (2sd)

Compare the correlation of the two methods on two groups of patients, in Group 1, patients with abnormal kidney function, there is a high correlation between two methods với with r = 0.857 (p<0.001). However, in Group 2, patients with normal kidney, Gate's method and DPSM only showed a moderate correlation with r = 0.711 (p<0.001) (Figure 2 and 3).

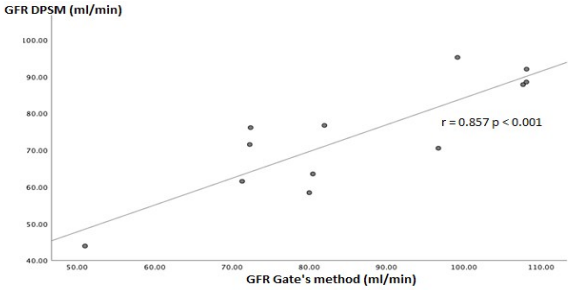

Figure 2. Scatter plots of GFR estimated by DPSM against that by Gate's method in Group 1. The line indicates the regression

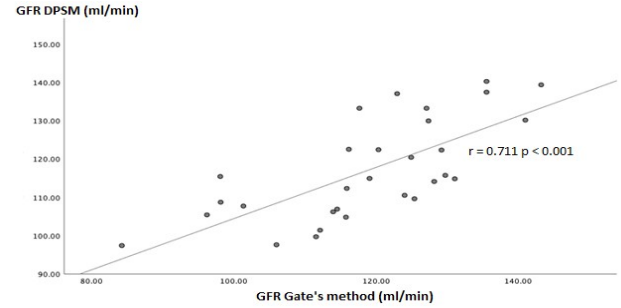

Figure 3. Scatter plots of GFR estimated by DPSM against that by Gate's method in Group 2. The line indicates the regression

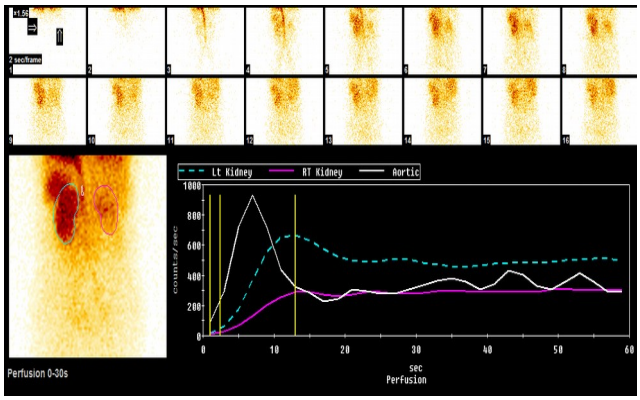

Figure 4. Renal scintigraphy with perfusion and function graphs4. Discussion

Glomerular filtration rate is one of the most important indexes for renal function assessment, in clinical practice, many methods are currently used and developed for estimating GFR, for instance: renal scintigraphy, serum creatinine-based, double plasma sampling method. They have shown a high correlation with inulin renal clearance which is the gold standard measurement [9].

In Vietnam, Gate's method or renal scintigraphy on γ camera system and serum creatinine method using Cockcroft-Gault formula are more common methods for GFR estimation than in vitro plasma methods. Each method has its own advantages and disadvantages. In the Cockcroft-Gault method, the quantification of GFR is based on creatinine in the blood, while creatinine is influenced by many factors such as age, sex, weight, as well as inaccuracies in patients with liver disease, edema, or obesity. Moreover, the ratio between creatinine and glomerular filtration rate is not predictable in pathological cases [10, 11]. On the other hand, Gate's method evaluating GFR based on the count of the radioactive 99m-Tc-DTPA filtered in the kidney is visual and could assess of individual kidney function [12]. However, the disadvantages of the Gate's method are related to physical properties such as radiation background, half-life, system dead time, correction level and quality of radiopharmaceuticals.

According to the European Association of Nuclear Medicine (EANM), plasma sampling method uses Cr-51-EDTA pharmaceutical. In USA with Society of Nuclear Medicine and Molecular Imaging (SNMMI), I-125-Iothalamate and 99m-Tc-DTPA are more popular. In this study, Gate's method and DPSM are combined, after finishing the process on SPECT system, two blood samples were collected at correct times. Correlation between Gate's method and DPSM is assessed, the results showed that two methods has a high correlation with r = 0.89. Group 1 and 2 also showed a high correlation when compared between two methods (r = 0.86 and 0.71, respectively), however, the difference in mean values in Group 1 was statistically insignificant ($p<0.001$) while the difference in mean values between two method2 in Group 2 was statistically significant ($p=0.33$) with an average difference of 12.0 ± 9.4.

The mean GFR value measured with the Gate's method was 110.8 ± 21.3 (ml/min) and with the DPSM was 106.2 ± 24.0 (ml/min). GFR value obtained by Gate's method is 4.74 (ml/min) higher than the DPSM, which is similar to some studies of foreign authors [6].

In the clinical practice, the Gates method is favorable due to its conventional technique and time saving, especially for assessment of renal function before kidney transplant. However, the renography is not the best choice for all cases. In addition, the DPSM is also recommended in cases where the GFR is too low (< 30ml/min), however the disadvantage of this method is the inconvenience of prolonged waiting times (up to 24 hours in the case of patients with very low GFR levels).

5. Conclusion

Double plasma sampling method has been shown the high possibility for clinical application to evaluate the GFR in parallel with traditional methods. This method can be combined with renal scintigraphy after the patient has been completed with the SPECT scan, or can be used in cases when the scan using SPECT system is unavailable.

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