Reappraisal of single-sample and gamma camera methods for determination of the glomerular filtration rate with $^{99m}$Tc-DTPA

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The aim of this study was to assess the clinical validity of single-sample methods and gamma camera uptake methods with $^{99m}$Tc-DTPA for the estimation of the glomerular filtration rate (GFR). The study was performed in 50 patients with various degrees of renal function (29 males and 21 females; age 27–90 yrs; serum creatinine level 0.34–6.49 mg/dl). As a reference the "true" GFR (GFRt) was determined from plasma clearance by means of the two compartment model curve fitting 10 plasma samples. The GFRt of more than 30 ml/min in 46 patients was compared to the GFR which was estimated with 7 single-sample methods, two gamma camera uptake methods and 24-hour endogenous creatinine clearance (24hCcr). Close correlation was observed in all single-sample methods. The highest linear correlation was observed in the Christensen and Groth's method rewritten by Watson for a 180-min plasma sample ($r = 0.991$, $se = 5.84$ ml/min). The smallest random error was observed in the Groth and Aastad's method for a 180-min plasma ($r = 0.989$, $se = 4.31$ ml/min/1.73 m$^2$). Our method was lowest in % absolute difference analysis (mean = 4.10%). The gamma camera uptake methods correlated significantly with the GFRt ($r = 0.746-0.774$), but were less reliable than any of the single-sample methods (see $r = 15.41$ ml/min–19.14 ml/min). The lowest correlation was observed in the 24hCcr ($r = 0.698$, $se = 50.76$ ml/min/1.73 m$^2$). The single-sample method was more accurate than the gamma camera method, and the gamma camera method was more accurate than 24hCcr. The single-sample method should be recommended for the accurate determination of the GFR with $^{99m}$Tc-DTPA in a patient with mild to moderate renal dysfunction.

Key words: glomerular filtration rate, radionuclide, $^{99m}$Tc-DTPA, plasma sample method, renography

INTRODUCTION

Determination of the glomerular filtration rate (GFR) is essential in cases of renal diseases, chemotherapy, and any other disease that may lead to renal function disturbance during its clinical course. Renal clearance determined by continuous infusion and urine collection of inulin has been approved as the standard method for accurate determination of the GFR. Nonetheless, this technique is seldom applied in routine clinical practice and was only performed in research study. Instead of inulin clearance, serum creatinine and endogenous creatinine clearance are measured in clinical practice. Serum creatinine is not sensitive for the detection of mild to moderate renal dysfunction. Twenty-four hour endogenous creatinine clearance (24hCcr) is also considered to be less reliable than inulin clearance. Among single injection methods, the two-compartment model with several plasma samples is one of the approved methods for accurate determination of GFR, but this method is not practical in a routine study because it requires several blood samples and an appropriate mathematical curve fitting the plasma disappearance. A single-sample method after a single injection of $^{51}$Cr-EDTA and of $^{99m}$Tc-DTPA is now recommended as an...
alternative to multi-sample methods.\textsuperscript{7} We also proposed a new equation for a single-sample method with \(^{99m}\text{Tc-DTPA}\), which is applicable to Japanese.\textsuperscript{8,9} It requires quantitative laboratory skills such as the preparation and dilution of stock solution and counting of the separated plasma by means of a gamma well counter.\textsuperscript{10} The method has not yet been widely carried out in routine practice.

The gamma camera method is popular in routine nuclear practice. It can provide immediate calculation of individual kidney function as well as of global renal function.\textsuperscript{11} Because of its simplicity, we have used this method in routine radionuclide examinations,\textsuperscript{12} but it has been questioned how accurate the method is for the determination of glomerular function with \(^{99m}\text{Tc-DTPA}\).\textsuperscript{7,13}

This study is aimed at identifying which method is adequate in the determination of the global GFR with \(^{99m}\text{Tc-DTPA}\), taking into consideration both simplicity and accuracy.

**MATERIALS AND METHODS**

**Subjects**

The study was performed in 50 patients, 29 male and 21 female, with diabetes mellitus with various degrees of renal function. Their age ranged from 25 to 90 yrs (mean \(\pm SD \) : 69.2 \(\pm 14.6 \) yrs), height from 142.7 to 175.6 cm (159.4 \(\pm 8.3 \) cm), body weight from 41 to 154.1 kg (63.5 \(\pm 17.8 \) kg) and body surface area (BS) from 1.3 to 2.59 m\(^2\) (1.65 \(\pm 0.201 \) m\(^2\)) which was estimated by Du Bois’ formula, BS (m\(^2\)) = BW\(^{0.425}\) \(\times\) H\(^{0.725}\) \(\times\) 0.007184 (BW = body weight (kg), H = height (cm)).\textsuperscript{14} The serum creatinine and BUN ranged from 0.34 to 6.49 mg/dl (0.98 \(\pm 1.09 \) mg/dl) and from 5.7 to 48.5 mg/dl (16.8 \(\pm 8.0 \) mg/dl), respectively. All patients were admitted for education and control therapy for hyperglycemia. The study was performed after informed consent was given.

**Data Acquisition**

The patient was hydrated with 300 ml of water 20 min prior to the examination. \(^{99m}\text{Tc-DTPA}\) was labeled in our hospital with a commercially available frozen-dried kit (Daichi Radioisotope Co., Tokyo, Japan) which had a labeling yield of over 95%. The radiotracer was prepared to contain 300 MBq per 2 ml in an injection syringe to which was attached a 3-way cock and butterfly needle. The injection of \(^{99m}\text{Tc-DTPA}\) was given through an indwelling butterfly needle during infusion of 20 ml of normal saline solution. Standard renal scintigraphy was carried out in supine position and all imaging data were recorded with a gamma camera (Searle ZLC 7500) and computer (Schintipac 700, Shimadzu, Japan). Ten venous blood samples were drawn at 5, 15, 30, 45, 75, 120, 150, 180, 240 and 300 min after the injection through an indwelling needle placed in the opposite arm. The patients were confined to bed throughout scintigraphy but their movement, food and oral intake of water was not restricted during the sampling of blood. Total blood was separated and plasma was counted together with a diluted standard solution of the injected radiotracer in a well scintillation counter (Autowell Gamma System ARC-380, Aloka Co., Ltd., Japan).

**Calculation of Plasma Clearance as Gold Standard**

The two-compartment model\textsuperscript{5,6} determined plasma clearance of \(^{99m}\text{Tc-DTPA}\) with multiple sample data. In this model, plasma disappearance of \(^{99m}\text{Tc-DTPA}\) fits the bi-exponential curve, \(Y = Ae^{-at} + Be^{-bt}\) (t: time after injection). The plasma clearance of \(^{99m}\text{Tc-DTPA}\) is determined by the equation, ID(ab)/(Ab + aB) (ID: total injected dose). In real curve fitting, the plasma concentration was converted to a percentage of the injected dose per liter of plasma (\%ID/L). Then the equation for calculation of plasma clearance is expressed as GFR = 100(ab)/(Ab + aB) \(\times\) 1000 (ml). Each constant of the exponential curve was automatically determined by means of the mathematical algorithm on non-linear least squares by using commercially available software (JMP v.3.1, SAS Institute Ins) (Fig. 1). As long as \(^{99m}\text{Tc-DTPA}\) is extracted from plasma solely by the kidneys, the clearance is presumed to be equal to the “true” GFR (GFRt).

Disappearance of the plasma concentration (\%ID/L) in most cases fitted the bi-exponential curve with a correlation coefficient of more than 0.997 (Fig. 1), except for only one patient (GFRt = 68.4 ml/min) with mild ascites which was disclosed on the CT scan taken after the radionuclide examination. This patient was excluded from the analysis. The GFRt in 49 patients ranged from 12.7 ml/min to 169.0 ml/min. The average \(\pm SD\) was 91.9 \(\pm 38.7\) ml/min. There were 3 patients with GFRt less than 30 ml/min (12.7, 13.1 and 22.6 ml/min, respectively). These
Table 1 Least squares linear regression and linear correlation analyses, absolute difference and % absolute difference between true GFR and estimated GFR

<table>
<thead>
<tr>
<th>Methods</th>
<th>Regression analysis</th>
<th>Absolute difference analysis</th>
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<td>24hCcr</td>
<td>1.377</td>
<td>12.43</td>
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The number in parenthesis indicates the sampling time of plasma.

r: correlation coefficient, see: standard error of estimate.

![Graphs](https://example.com/graphs)

Fig. 2 Scatter plots of GFR by single-sample method (GFRs) against true GFR for 10 samples in 7 single-sample methods. The number in parenthesis indicates sampling time of the plasma.

patients were also excluded from the linear regression and linear correlation analysis and absolute difference analysis between GFRt and GFR estimated by other methods.

The analytical study was then carried out in 46 patients with a GFR more than 30 ml/min (34.7 ml/min–169.0 ml/min, 97.3 ± 34.7 ml/min).
Fig. 3 Scatter plots of GFR by gamma camera uptake method (GFRgc) against true GFR for 10 samples.

Fig. 4 Scatter plots of 24 hour endogenous creatinine clearance against true GFR for 10 samples.

**Single-Sample Methods**

The GFR was estimated by the 7 previously proposed single-sample methods of Christensen and Groth's (CGr)\(^1\) rewritten by Watson,\(^1\) Constable et al. (Con),\(^2\) Dakubu et al. (Dak),\(^3\) Groth and Aasted (GrA),\(^4\) Jacobsson (Jac),\(^5\) Russell, et al. (Rus)\(^6\) and the authors (Itoh).\(^7\) Each formula is shown in Appendix 1. GFR (GFRsp) by single-sample methods was calculated and was expressed as two different dimensions of ml/min and ml/min/1.73 m\(^2\) according to those formulae with commercially available software (Microsoft Excel 5.0, Microsoft).

**Gamma Camera Uptake Methods**

The global GFR (GFRgc) was estimated by two gamma camera uptake methods using formulae proposed by Gates\(^8\) and one of the authors (Itoh).\(^9\) These were programmed in our computer that was provided by a commercial company. Each formula is shown in Appendix 2. All data were derived from a routine processing of the renogram by three technicians who were not giving special attention to the study.

**Twenty-four Hour Endogenous Creatinine Clearance**

Twenty-four hour endogenous creatinine clearance (24hCcr) which was carried out within a week either before or after the radionuclide study was referred to. Urine excreted during one day, from 6.00 to 6.00 the next morning was collected. The 24hCcr value was determined from the urine concentration (nmol) of creatinine by urine volume (ml/1440 min), which was divided by plasma concentration of creatinine (mg/ml), and was conventionally normalized for the average Japanese body surface area of 1.48 m\(^2\) (ml/min/1.73 m\(^2\)). The enzyme method was used for the measurement of creatinine in our hospital.

**Statistical Analysis**

Least squares linear regression and linear correlation analyses were performed with commercially available software (JMP v.3.1, SAS Institute Inc.). In this analysis, the regression coefficient (r), standard error of estimate (see), intercepts and slope of the regression equation were determined. Statistical values such as mean, standard deviation (sd) and standard error of estimate (see) of absolute difference and percent absolute difference were calculated with Statview J-5.0 (SAS Institute, Inc.) or Excel v.5.0 (Microsoft, Inc.).

**RESULTS**

Analyses of correlation and absolute difference between true GFR (GFRt) and estimated GFR (GFRsp) by single-sample methods

At sampling times between 120 min and 300 min, all 7 single-sample methods showed a close linear relation between GFRt and GFRsp and resulted in a correlation coefficient greater than 0.970 (Table 1) and a standard error of the estimate smaller than 9.95 ml/min or 7.90 ml/min/1.73 m\(^2\). As examples, Figure 2 shows a scatter plot of GFRsp at 180 min against GFRt in all 7 single-sample methods. The CGr for the 180 min sample showed the highest linear correlation coefficient (r = 0.991) and the
GrA for the 180 min sample showed the smallest standard error of the estimate (4.31 ml/min/1.73 m²). In addition, absolute difference and percent absolute difference between GFRt and GFRsp were calculated according to ABS(GFRt - GFRsp) and ABS(GFRt - GFRsp)/GFRt × 100 (%), respectively. The author's method (Itoh) with a 180 min-sample had the lowest absolute difference (mean ± se = 3.37 ± 0.53 ml/min/1.73m²) and % absolute difference (mean ± se = 4.10 ± 5.10%) (Table 1), but these were not significantly different from CGr and GrA in Student's t-test.

Correlation between true GFR (GFRt) and GFRgc by gamma camera uptake methods

Figure 3 shows the relationship between GFRgc by the gamma camera methods and GFRt (Fig. 3). The GFRgc in both methods correlated well with GFRt (r = 0.774 in Gates and 0.745 in Itoh) and was not significantly different, but the coefficients of correlation between them were lower than those in all single-sample methods (Table 1). In addition, the means of absolute differences and % absolute difference between GFRt and GFRgc were also greater than that obtained by the single-sample method. It was significantly different from those in all single-sample methods (p < 0.01).

Correlation between true GFR (GFRt) and 24 hour endogenous creatinine clearance (24hCcr)

The 24hCcr, after the standardization of dimension for ml/min/1.73 m², also correlated well with the GFRt (r = 0.698) (Fig. 4) but was lowest in linear correlation coefficient and largest in the standard error of the estimate among comparative studies. The means of absolute difference and % absolute difference were significantly lower than in all the compared methods (p < 0.01) (Table 1).

DISCUSSION

Many methods for determining the GFR with radionuclides have been reported and some of them have been commonly used in clinical practice. Renal function tests performed in routine clinical practice should be simple and accurate. All single-sample methods for samples between 120 min and 300 min had a higher linear correlation coefficient and smaller standard error of the estimate than the gamma camera methods and 24hCcr (Table 1). And the mean of absolute difference and % absolute difference in single-sample methods was also smaller than that in the gamma camera uptake method and 24hCcr. These results indicate that the single-sample method is more accurate for the determination of a GFR of more than 30 ml/min.

Although the parameters and values for the dimensions in the converting equations in each of the 7 single-sample methods (Appendix I) are different, an apparent value of distribution of the radiotracer (CGr, Con, Dak, Jac and Rus) or plasma concentration per injected dose (GrA and Ito) is used. Our primary question was whether these equations can be adapted to Japanese, who are seemingly different from western people in physique and distribution volume. We then proposed the original single-sample converting equation that was derived from the same study population and bi-exponential model in this paper. It cannot therefore be emphasized that our method showed the smallest means of absolute and % absolute difference among all comparative single-sample methods. Nonetheless, it is remarkable that the other 6 comparative single-sample methods had a very high linear regression and
correlation coefficient. Among them, Christensen and Groth's and Groth and Aasted's methods for samples from 180 min to 300 min should be recommended as a single-sample method with $^{99m}$Tc-DTPA. These results are closely comparable with those in a recent consensus report. Our results also indicate that Christensen and Groth's equation rewritten by Watson and Groth and Aasted's method are universal for the estimation of GFR in adult Japanese as well.

The gamma camera uptake method is simple and very convenient to use in immediate analysis of any individual kidney function as well as global renal function. Two gamma camera methods correlated well with the GFR but were less accurate than any of the single sample methods. We wonder why this method was more accurate than 24hCcr, because the conversion equation in both gamma camera methods was obtained from the linear regression analysis on 24hCcr (Gates) or 2hCcr (Itoh). Our equation was based on evaluation with different equipment and different measurement of 2hCcr in a different hospital from the present. In addition, the 24hCcr alone was performed on another day, while all parameters concerning radionuclide study were obtained from a single examination on the same day. The GFR in humans is thought to be a dynamic parameter that is diet-dependent and can be altered by hemodynamic maneuver. In a patient with diabetes mellitus, these factors may cause the GFR to vary from day to day. It is reported that direct measurement of renal depth can improve the accuracy of GFR estimated by means of the gamma camera method.

We estimated renal depth from body (kg)/height (cm). It cannot therefore be generalized that the gamma camera method is not accurate in the determination of the GFR. Our results suggest that 24hCcr is not suitable as a quantitative standard parameter in a comparative study with a gamma camera method with $^{99m}$Tc-DTPA.

The ideal method for the assessment of the GFR is feasible with no limitation in any clinical situation and is adaptable for adults as well as children. Although the single-sample method after the single-injection of radionuclide was proved to be accurate in the determination of the GFR, its adaptation has been restricted to some clinical situations such as severely decreased renal function with a GFR of less than 20–30 ml/min and peripheral edema. In a recent study on 8 single-sample methods, Groth and Aasted's method with a 4-hour sample was better than other methods in GFR ≥ 30 ml/min as well as < 30 ml/min. Sample time in a steady state GFR of less than 20 ml/min should be recommended to be 24 hour after the injection. As general agreement on indication for a single-sample method, the lower GFR level in the individual, the later the appropriate time of plasma sampling and the less the reliability of the estimate. In addition, the 120 min and 240 min two-sample method is reported to be more accurate than the single-sample method. These clinical limitations should be appreciated when the single-sample method is adopted for routine clinical practice.

From the clinical aspect, the shorter the sample time, the more convenient the test is for outpatients. Our formula alone can be used during an unspecified time of blood sampling between 120 and 240 min, but accuracy of the estimate depends on the sample time and the best time for plasma sampling is 180 min post-injection. Bubeck et al. propose a universal formula which is applicable to adults as well as children in the determination of tubular function by means of a single-sample method with $^{99m}$Tc-MAG3. In his original formula, the apparent volume of distribution is corrected with the standard body surface area of 1.73 m². The normalized plasma concentration in our formula is a reciprocal of that of Bubeck's formula. We presume that our new formula may have universality for the determination of the GFR with a single sample in children as well, but the sample time in children may be different from that in adults. The most appropriate plasma sampling time for adults appears to be 180 min to 240 min. On the other hand, it was reported to be from 90 min to 120 min in children. Further investigation is required to assess the application of our formula to children.

Serum creatinine clearance seems to be unreliable in the quantitative measurement of GFR in a severely decreased renal functional state. The single-sample method with $^{99m}$Tc-DTPA is also inaccurate in the estimation of a GFR less than 20 ml/min–30 ml/min, but it is obvious that the method is more reliable and accurate in the estimation of the GFR in patients with normal to moderately decreased renal function. Our final goal is, of course, to establish a simple and accurate radionuclide method that can easily be performed in routine clinical practice to estimate renal function of any severity. As such, a single-sample method with $^{99m}$Tc-DTPA should be primarily indicated to evaluate the GFR in a patient in a subclinical state without clinical symptoms of renal failure or with normal conventional serological tests (Fig. 5).

CONCLUSIONS

The reliability of previously proposed quantitative methods with $^{99m}$Tc-DTPA was compared in 46 patients with a GFR higher than 30 ml/min. All single-sample methods can provide great accuracy in the estimation of the GFR. On the one hand, the gamma camera methods are less reliable than any of the single-sample methods. Christensen and Groth's method rewritten by Watson and Groth and Aasted's method should be recommended in routine practice because of the closest correlation among those compared in the present study. Our formula may possibly be used as a universal equation for the determination of the GFR with $^{99m}$Tc-DTPA in adults as well as children. Single-sample methods can provide simple and accurate determination of GFR with $^{99m}$Tc-DTPA in the subclinical state of mild to moderate renal dysfunction in
routine clinical practice.

ACKNOWLEDGMENTS

We thank Mr. Katsuura and Mrs. Takahashi of Hokkaido University Hospital, Section of Nuclear Medicine, for their assistance in the measurement of blood samples. Mr. Ibara, Mr. Oka and Mr. Sasaki of JR Sapporo General Hospital for their assistance in processing the renal scintigraphy and the renogram, and Dr. H. Shuke of Asahikawa Medical University, Department of Radiology, for his advice on calculation of plasma clearance with the two-compartment model.

REFERENCES


Appendix 1

1. Christensen & Groth’s formula rewritten by Watson

\[ \text{GFR (ml/min)} = \left( -b + \sqrt{b^2 - 4ac} \right) / 2a \]
\[ a = \tau(0.00000171 - 0.0012) \]
\[ b = \tau(-0.000775t + 1.31) \]
\[ c = \text{ECVln(ECV/V_t)} \]

\[ \text{ECV (extracellular volume) = 8116.6BSA - 28.2} \]
\[ V_t = \text{tracer apparent volume (ml) of distribution at time } t \]
\[ t = \text{sample time (min) (180 < t < 300)} \]
\[ \text{BSA = body surface area (m²)} \]

2. Constable et al.

\[ \text{GFR (ml/min)} = 24.5 \sqrt{V_t - 6.3} - 67 \]
\[ V_t = \text{tracer apparent volume (L) of distribution at sample time } t \]
\[ t = \text{sample time (min) (240 min)} \]

3. Dakubu et al.

\[ \text{GFR (ml/min/1.73 m²)} = 95.33\ln(V_t) - 270.99 \]
\[ V_t = \text{tracer apparent volume of distribution/1.73 m² at sample time } t \]
\[ t = \text{sample time (hour) (3h)} \]

4. Groth and Aasted’s formula

\[ \text{GFR (ml/min/1.73 m²)} = (0.213t - 104) \cdot \ln \left( \frac{Q_t}{Q_0} \right) + 1.88t - 928 \]
\[ Y_t = \text{radioactivity of plasma at sample time } t \]
\[ A = \text{body surface area (m²) estimated by Du Bois’ nomogram} \]
\[ Q_0 = \text{total injected dose (cpm)} \]
\[ t = \text{sample time (min); t = 180 min for 3 h} \]

5. Jacobsson’s Formula

\[ \text{GFR (ml/min)} = \frac{\ln(Q_t/V^C)}{V^C + 0.0016} \]
\[ Q_0 = \text{total injected dose (cpm)} \]
\[ V^C = \text{calculated volume of distribution (ml)} \]
\[ = 0.246BW (g) \]
\[ C_t = \text{plasma activity at time } t (\text{cpm/ml}) \]
\[ Q_t/C_t = \text{tracer apparent volume of distribution at time } t \]
\[ t = \text{sampling time (240 min)} \]

6. Russell et al.

\[ \text{GFR (ml/min)} = \text{Aln}(D/P) + B \]
\[ A = -0.278t + 119.1 + 2450/t \]
\[ B = 2.886t - 1222.9 - 16820/t \]
\[ D = \text{total injected dose counts (cpm)} \]
\[ P = \text{plasma radioactivity counts (cpm/ml)} \]
\[ D/P = \text{tracer apparent volume of distribution at time } t \]
\[ t = \text{sampling time (180 min)} \]

7. Itoh et al.

\[ \text{GFR (ml/min/1.73 m²)} = A + B\ln(P) \]
\[ A = 463.1217 - 3.458t + 0.01205t^2 - 0.0000154t^3 \]
\[ B = -212.601 + 1.42518t - 0.004834t^2 + 0.0000062t^3 \]
\[ P = \text{percent total injected dose/L of plasma/1.73 m²} \]
\[ t = \text{sampling time (min) (120 < t < 300 min)} \]

Appendix 2

1. Gates’s Method

\[ \text{GFR (ml/min)} = 9.81272\%RU - 6.82519 \]
\[ \%RU = \frac{\left( Cr/\text{e}^{0.153L} \right) + \left( Cl/\text{e}^{0.153L} \right)}{C_{pre} - C_{post}} \]

2. Itoh and Arakawa Method

\[ \text{Cr (ml/min)} = 13.15\%RU^{0.787} \]
\[ \%RU = \frac{\left( Cr/\text{e}^{0.153L} \right) + \left( Cl/\text{e}^{0.153L} \right)}{C_{pre} - C_{post}} \]

In both

\[ %RU = \% \text{ uptake/total injected dose at 2–3 min postinjection} \]
\[ Cr = \text{counts in the right kidney for 1 minute at 2–3 min postinjection} \]
\[ Cl = \text{counts in the left kidney for 1 minute at 2–3 min postinjection} \]
\[ C_{pre} = \text{pre-injection counts/min} \]
\[ C_{post} = \text{post-injection counts/min} \]

In Gates’ method

\[ R = \text{right renal depth (cm)} = 13.3X + 0.7 \]
\[ L = \text{left renal depth (cm)} = 13.2X + 0.7 \]
\[ X = \text{body weight (kg)/height (cm)} \]

In Itoh’s method

\[ R = \text{right renal depth (cm)} = 13.63X^{0.996} \]
\[ L = \text{left renal depth (cm)} = 14.0285X^{0.7554} \]
\[ X = \text{body weight (kg)/height (cm)} \]

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Annals of Nuclear Medicine