A Comprehensive Software and Database Management System for Glomerular Filtration Rate Estimation by Radionuclide Plasma Sampling and Serum Creatinine Methods

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Abstract
Glomerular filtration rate (GFR) estimation by plasma sampling method is considered as the gold standard. However, this method is not widely used because of the complex technique and cumbersome calculations coupled with the lack of availability of user-friendly software. The routinely used Serum Creatinine method (SrCrM) of GFR estimation also requires the use of online calculators which cannot be used without internet access. We have developed user-friendly software “GFR estimation software” which gives the options to estimate GFR by plasma sampling method as well as SrCrM. We have used Microsoft Windows® as operating system and Visual Basic 6.0 as the front end and Microsoft Access® as database tool to develop this software. We have used Russell’s formula for GFR calculation by plasma sampling method. GFR calculations using serum creatinine have been done using MIRD, Cockcroft-Gault method, Schwartz method, and Counahan-Barratt methods. The developed software is performing mathematical calculations correctly and is user-friendly. This software also enables storage and easy retrieval of the raw data, patient’s information and calculated GFR for further processing and comparison. This is user-friendly software to calculate the GFR by various plasma sampling method and blood parameter. This software is also a good system for storing the raw and processed data for future analysis.

Keywords: Database, glomerular filtration rate, software

Introduction
In conventional nuclear medicine practice, technetium-99m diethylenetriaminepentaacetic acid (99mTc-DTPA) glomerular filtration rate (GFR) is a routine test performed by gamma camera method. This test forms a major bulk of the nuclear medicine studies performed across the globe. This technique is simple, easy to perform, and importantly, it provides differential renal function and renogram curve, which is a very important parameter to evaluate in various urological conditions like obstructive uropathy, renal injury because of trauma, etc. However, in conditions like chronic renal failure, acute renal failure, effect of nephrotoxic drugs, etc., the estimation of absolute GFR accurately becomes more important. There are methods to estimate the GFR by using renal blood profiles like serum creatinine, blood urea nitrogen etc., which is being used by clinicians for monitoring renal function in various nephrological conditions. Other methods such as 24 h creatinine clearance method and inulin clearance method are also used for absolute quantification of global GFR but are more difficult to utilize in routine practice. Plasma sampling is the most accurate, reliable, and reproducible technique to provide absolute global GFR. Though this technique is used worldwide, it is not utilized routinely in clinical practice since the process is complex and involves cumbersome mathematical calculations. We have tried to simplify the process of plasma sampling and developed a user-friendly software system for GFR estimation.

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estimation software” to calculate GFR by radionuclide plasma sampling method and renal Serum Creatinine method (SrCrM). It is very useful software which can store raw data, calculate GFR by various methods and compare GFR with previously stored value.

**Materials and Methods**

Gates gamma camera method and SrCrM were used to estimate GFR in our hospital, before starting plasma sampling GFR estimation. We developed and implemented the plasma sampling program in different phases and tested it in clinical condition without affecting the routine practice of GFR estimation. Various project phases are as follows:

- Workflow development
- Formula selection
- Software package selection
- Software development and features
- Software testing and validation
- Routine clinical use.

**Workflow development**

Development of most appropriate method is of utmost importance to fetch the most reliable outcome of the project. In order to develop the most reliable method, we reviewed the relevant literature published by various researchers. Various literature have emphasized the superiority of 51Cr-EDTMP over 99mTc-DTPA to estimate GFR by plasma sampling method because of less protein binding of 51Cr-EDTMP.[10,11] But at the same time, it has also been suggested that, 99mTc-DTPA is also able to produce a comparable result if radiochemical purity (RCP) is high and proper technique is used.[11-13] 99mTc-DTPA is readily available in all Nuclear Medicine Departments; it is very easy to determine RCP of 99mTc-DTPA by simple quality control (QC) technique and simultaneous gamma Camera imaging is also possible. Hence, we decided to use 99mTc-DTPA as the radiopharmaceutical for GFR estimation by plasma sampling method. The dose of 3.7 MBq 99mTc-DTPA is suggested by various literature.[12] In our study, we decided to use 18.5 MBq of 99mTc-DTPA, since we also decided to take one static image of 60 s between 2 and 4 min on gamma camera to find out the differential function of the kidneys.

Patients are advised to drink 300 ml of water 20 min prior to the examination. 99mTc-DTPA is prepared using freshly eluted 99mTcO$_4^-$, QC is performed and RCP is noted on the GFR data sheet. After formulation of 99mTc-DTPA, patient dose and standard dose are prepared in two syringes, and they are labeled as such. Both the syringes are weighted on precision balance and counted on a gamma camera for pre and postinjection and recorded in the GFR data sheet. Injection is performed through a butterfly cannula. The standard is emptied in a graduated standard flask and diluted in 1000 ml of normal saline. Two blood samples are withdrawn at 2 h and 4 h postinjection. Blood plasma is separated by centrifuge technique and two aliquots of 1 ml each are prepared for both the sample in prelabeled test tubes. Two aliquots of 1 ml of the standard source are also prepared in prelabeled test tubes. Each sample is counted twice on a calibrated spectrometer and reading was entered in GFR data sheet.

**Formula selection**

*Body surface area calculation*

DuBois D, DuBois DF formula was used to calculate body surface area (BSA) in our software.[14]

*Calculation of injected activity to the patient*

Injected activity is calculated by the standard dilution method. We use two techniques, that is, counting and weighting technique to calculate the injected activity in the form of counts per minute.

**Counting method**

We count the patient dose and standard dose in two syringes on the gamma camera before and after injection.

**Weighing method**

We weigh the patient dose and standard dose in two syringes on precision balance before and after injection.

Following formula is used to calculate the injected dose to the patient:[15]

**Formula:**

\[ D = \frac{(D_p - R_p) \times ADS \times DS}{(D_s - R_s)} \]

Where:

- \( D \) = Administered activity to the patient (count/min).
- \( D_s \) = Count of the standard loaded syringe measured on the gamma camera or weight of the standard loaded syringe measured on precision balance.
- \( R_s \) = Count of the standard post empty syringe measured on the gamma camera or weight of the standard post empty syringe measured on precision balance.
- \( D_p \) = Count of patient syringe measured on the gamma camera or weight of patient syringe measured on precision balance.
- \( R_p \) = Count of the patient syringe after intravenous injection, measured on the gamma camera or weight of patient syringe after intravenous injection.
ADS = Count of 1 ml of the dilution of the standard measured in well counter.

DS = Dilution of the standard (e.g., 1000).

**Glomerular filtration rate by double plasma sampling method**
Russell’s method is used to calculate GFR by double plasma sampling method (DPSM) and normalized for BSA.\(^\text{[15]}\)

Because of the overestimation of the slope-intercept method related to the fact that the first exponential has been neglected, one should introduce a correction factor, using one of the following methods:

Chantler’s linear correction method is used to correct normal and high GFR values.\(^\text{[16]}\)

Brochner Mortensen’s quadratic correction method is used to correct GFR in the lower range.\(^\text{[17]}\)

**Glomerular filtration rate by single plasma sampling method**
Technetium-99m diethylenetriaminepentaacetic acid plasma clearance by single plasma sampling method (SPSM) (true GFR) is calculated by the Russell’s method.\(^\text{[15]}\)

**Serum Creatinine method for glomerular filtration rate estimation**
**Counahan-Barratt method**
Counahan-Barratt formula uses serum creatinine and height to calculate GFR.\(^\text{[2]}\)

**Cockcroft-Gault method**
Cockcroft-Gault formula uses serum creatinine, age, and weight to calculate GFR.\(^\text{[3]}\)

**Schwartz method**
Schwartz formula uses serum creatinine and height to calculate GFR. This method is not useful for adult above 18 years.\(^\text{[4]}\)

**Modified diet in renal disease method**
The modified diet in renal disease (MDRD) formula uses serum creatinine and age to calculate GFR.\(^\text{[5]}\)

**Software package selection**
Microsoft Windows\(^\circledR\) operating systems are commonly used in India and are easily available in all nuclear medicine departments. Hence, we have developed this software application on Windows\(^\circledR\) XP Professional (Microsoft Corporation-MS) 32-bit operating system installed on an Intel\(^\circledR\) Core\(^\text{TM}\) i3-2120 CPU @ 3.30 GHz, 2.0GB RAM on Lenovo Personal Computer (PC) which is being used in our department. Visual Basic 6.0, Microsoft Corporation, Redmond, WA, USA is used as a front end to develop all the forms, and Microsoft Access\(^\circledR\) database is used for database development.

**Software development and features**
This software is developed on a 32 bit Windows\(^\circledR\) XP Professional operating system but it is also tested on 64 bit systems and as well as the latest version of MS Windows\(^\circledR\) like Windows 7 and Windows 8 and older PCs like Pentium 4 with 500 MB RAM and Microsoft Windows\(^\circledR\) 98. This is a multi-user software system which can be used by multiple users at the same time on a local area network. This software has dedicated database to store all relevant data of patients in various tables, which is used for GFR estimation by various methods. This software can estimate GFR by SrCrM like Counahan-Barratt method, Cockcroft-Gault method, Schwartz method, MDRD method and plasma clearance method like SPSM and DPSM.

Multiple tables are developed in Microsoft Access\(^\circledR\) to store patient’s data. Multiple document interfaces feature of Visual Basic 6.0 was used to develop multiple forms. This software is self-instructional and very user friendly with separately designed Patient Registration form, requisition form, data entry form, GFR calculation, and display template. GFR calculated by any selected method can be displayed separately on the same page. There is provision to estimate absolute GFR for individual kidney by entering differential uptake obtained from gamma camera. Software has the capability to compare present GFR with previous one in terms of percentage change or graphical representation.

**Patient’s registration form**
This form is used for initial patient registration. Patient demographic data such as name, date of birth, sex, and contact details are entered through this form [Figure 1]. The patient then receives a unique patient ID number on the registration. Data entered from this form are saved in patient information table and can be retrieved on subsequent visits. The same form can be used to modify the patient details if necessary.

**Requisition form**
This form is used to create a requisition for the test and give an appointment accordingly [Figure 2]. Upon entering the patient ID, the test and date are selected, and a unique requisition number is generated for the test. Data entered in this form are saved in requisition table with unique requisition number.
**Plasma sampling data entry form**
This form is used to enter data related to plasma sampling like dilution factors, height and weight, presyringe and postsyringe weights and counts of standard and patient dose, time between injection and first and second sampling, standard count, and plasma count [Figure 3].

**Glomerular filtration rate calculation by single plasma sampling method**
This form is used to calculate GFR by SPSM. First patient ID has to be entered and then relevant requisition has to be selected [Figure 4]. Once the requisition is selected, the relevant data are retrieved from the table and entire empty field is populated by data. Next step is to select either counting method or weighting method and by clicking calculate button GFR is calculated. The GFR valued can be stored by save button and printed by clicking print button.

**Glomerular filtration rate calculation by double plasma sampling method**
This form is used to calculate GFR by two plasma sampling method [Figure 5]. First patient ID has to be entered than relevant requisition has to be selected. Once the requisition is selected, the relevant data are retrieved from the table and entire empty field is populated by data. Next step is to select counting method or weighting method and by clicking calculate button GFR is calculated. The GFR value can be stored by save button and printed by clicking print button.

**Glomerular filtration rate calculation by Serum Creatinine method**
This form is used to calculate GFR by creatinine method. First patient ID has to be entered and then relevant requisition has to be selected [Figure 6]. Once the requisition is selected, the relevant data are retrieved from the table and entire empty field is populated by data. Next step is to calculate GFR by various formulas by clicking the respective methods button available on this page. GFR calculated by these methods are displayed on the same page. The calculated GFR value can be stored in the database by clicking the save button and printed by clicking print button.

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**Figure 1:** Registration form: This form is used to register patient and patient ID is generated

**Figure 2:** Requisition form: This form is used to generate requisition for the test with a unique requisition ID

**Figure 3:** Data entry form: This form is used to enter all the data related to the test for a specific requisition ID

**Figure 4:** Single plasma sampling method (SPSM) Glomerular filtration rate (GFR) calculation form: This form is used to calculate GFR by SPSM
Glomerular filtration rate comparison various methods
This page is used to display GFR calculated by all the methods [Figure 7]. First patient ID has to be entered and then relevant requisition has to be selected. Once the requisition is selected, the GFR calculated by all the methods will be displayed on this page. This can be printed by clicking print button on the bottom of the page.

Glomerular filtration rate comparison at various intervals
This page is used to display GFR calculated on different intervals [Figure 8]. First patient ID has to be entered then GFR method has to be selected. Once the GFR method is selected the GFR calculated by that method will be displayed in the form of the graph where Y-axis represents the GFR in ml/min and X-axis displays dates. This can be printed by clicking print button on the bottom.

Print Page
This page is used to display GFR calculated by all the methods [Figure 9]. First patient ID is to be entered then relevant requisition has to be selected. Once the relevant requisition is selected the GFR calculated by all the methods will be displayed on this page. This can be printed by clicking print button on the bottom.

Software testing and validation
Multi-layer validation processes were adopted to validate the software; (a) validation for data entry, (b) validation for calculation with dummy data, (c) validation for calculation for real data; (d) validation of the technique by comparing with gamma camera GFR.

Figure 5: Double plasma sampling method (DPSM) Glomerular filtration rate (GFR) calculation form: This form is used to calculate GFR by DPSM
Figure 6: Serum creatinine method (SrCrM) Glomerular filtration rate (GFR) calculation form: This form is used to calculate GFR by SrCrM
Figure 7: Glomerular filtration rate (GFR) review form: This form is used to display GFR calculated by all the method. This form is also used for printing
Figure 8: Glomerular filtration rate (GFR) comparison form: This form is used to display GFR calculated on different intervals in the form of graph
Validation for data entry
Data are entered through data submission page and verified from updated data in the database table for correct entry followed by correct retrieval of data from the database table to the relevant page.

Validation for calculation with dummy data
Software was tested for data flow, data entry, and data validation. Software is also tested for its calculation accuracy using dummy data and verified by manual calculations and calculation performed by using Microsoft Excel spreadsheet developed in-house for this purpose.

For SrCrM, GFR 10 random values of Age from 5 years to 15 years, creatinine values from 0.7 to 3.2, height from 140 cm to 180 cm and weight from 40 kg to 80 kg were randomly generated and the GFR was calculated by this software and for the same value GFR was also calculated by online software available.

Validation of the plasma sampling method by comparing with gamma camera glomerular filtration rate
We have compared GFR value estimated by plasma sampling method using our software with that of GFR value estimated by gamma camera method which was considered to be standard of care in our institution at that point of time. Thirty patients’ blood sampling GFR is compared with that of GFR calculated by gamma camera method. Those 30 patients GFR were also calculated by weighing and counting methods.

Routine clinical use
This software was tested for 30 GFR procedures, and the result was compared with that of gamma camera method. After preliminary comparison, this software was put on the use for routine GFR estimation by plasma sampling method. This software was also used for GFR estimation by SrCrM whenever it was required. Software is also being used in various clinical trials being conducted in our hospital. We have satisfactorily performed more than 300 procedures on this software.

Results
Our software can be installed on all the available versions of 16 bit to 64 bit Microsoft Windows® like Windows® 98, XP, 7 and 8 installed on PC with lowest 256 MB RAM and processing power as low as 1.2 GHz and more. The software can also be installed on any Windows® tablet, netbook or laptop for flexibility. Data entry validation process is checked, and it is working properly. Total 10 dummy patient’s data were entered through the various forms available and checked for correct entry in the database table and found to be absolutely correct. Same data were retrieved and matched with the data in the database table and found to be absolutely correct.

With above data, GFR was calculated by the software using all the methods and matched with the GFR calculated manually for plasma sampling and by online software for SrCrM. Software available on The Nephron Information Center was used for calculation of GFR by Schwartz method, Counahan-Barratt method, Cockcroft-Gault method and MDRD.[18] The GFR calculated by same method by our software and online software found to be the same and correlation coefficient for all the methods were $r = 1$. For single plasma sampling GFR the correlation coefficient was $r = 1$, for double plasma sampling also the correlation coefficient was $r = 1$. GFR for a total of 30 patients, 18 male and 12 female, who came to us for prechemotherapy GFR evaluation, was calculated using this software for clinical validation. The average Serum Creatinine value was 0.8, average weight 48 kg, average height 156 cm, and the average age of the patient was 45 years.

Glomerular filtration rate calculated by this software for SPSM and DPSM are compared with that of GFR calculated by gamma camera method and shown in Table 1. The correlation coefficient between GFR calculated by SPSM and gamma camera method is 0.91, for DPSM and gamma camera method is 0.93, and SPSM and DPSM is 0.90. GFR calculated by weighing and counting method for single and DPSM is correlated and shown in Table 2. The correlation coefficient between GFR calculated by SPSM using counting and weighting method is $r = 0.977$ and by DPSM by using counting method and weighting method is $r = 0.984$. This software is used for research as well as routine work in our department satisfactorily. GFR of more than 300 patients has been estimated by plasma sampling method using this software with satisfactory results.
Discussion

Glomerular filtration rate estimation is performed by various methods like SrCrM, 24 h creatinine clearance method, inulin clearance method, radionuclide gamma camera method, and radionuclide plasma sampling method.\[^{1-10}\] There are various methods to follow-up patients with renal disease or patients likely to develop renal problems because of administration of nephrotoxic drugs. Serum creatinine value by itself is not sufficient to predict renal damage, so GFR calculated by SrCrMs are the most common and easiest method used by most of the clinicians for follow-up of patients in these conditions.\[^{24}\] But GFR estimated by SrCrM is also not able to predict the early renal damage. Therefore, in most institutions, the estimation of GFR is performed by Gates gamma camera method, when available to follow-up those patients who are suspected for deterioration of renal function. In addition, though GFR estimation by plasma sampling method and inulin clearance method are considered the gold standard, they are not frequently used because of technical difficulties.\[^{7,8}\] The number of online software are freely available on the internet to perform the GFR calculations by SrCrM. But these software cannot save raw data or processed data for future use. GFR estimation by Gates gamma camera method is a simple, standard, and very popular technique used in most of the Nuclear Medicine Departments because of availability of Gates software on all the modern gamma cameras provided by all the vendors. Coupled with the ease in performing the technique, this protocol has established itself as a routine practice in nuclear medicine centers around the world.\[^{19}\] The Gates method can efficiently calculate the differential function but is not so reliable to calculate the absolute GFR correctly, because of its inherent issues of injection technique, regions of interest definition, operator dependence, etc. Plasma sampling method is a very reliable technique to estimate absolute GFR correctly and works accurately even in markedly impaired renal function. But the estimation of plasma sampling is a cumbersome process, and unavailability of proper software and database system makes it even more difficult to routinely perform in Nuclear Medicine Department. We have developed “GFR estimation software” to address all the related issues of plasma sampling method and SrCrM.

Russell’s method has been used for the calculation of GFR by one and two plasma sampling method.\[^{15}\] GFR calculated by Russell’s method is further corrected for BSA. Plasma clearance curve follows bi-exponential curve; first exponential is rapid clearance, and second clearance is slow. Second clearance curve is used for GFR estimation by slope-intercept method used for GFR estimation by two plasma sampling method, and first exponential plasma clearance curve is not considered. This introduces minor error in GFR calculation and can be corrected by two methods; linear correction method for higher GFR value and quadratic correction method for lower GFR value. We have made a provision for these corrections in our software and all the values are displayed so that physician can use the value judiciously.\[^{16,17}\]

The most popular four formulae used for GFR estimation by SrCrM are listed, and there is provision to display all the values on the same page and print-outs of the same can also be taken.

We correlated the GFR calculated by this software using various formulae and GFR calculated by online software and manual method for the same datasets. The excellent correlation coefficient $r = 1$ for all the methods show that this software stores and uses the data properly to produce the correct result.

Unlike SrCrM, plasma sampling method involves complex procedures from patient preparation to the GFR

| Table 1: Comparison of GFR calculated by single plasma method, double plasma sampling method using this software and gamma camera method |
|---------------------------------|--------|--------|--------|--------|--------|
| GCM\(^a\) | SPSM\(^b\) | DPSM\(^c\) | DPSM-SPSM-GCM\(^d\) | DPSM-SPSM-GCM\(^d\) | DPSM-SPSM-GCM\(^d\) |
| Average (ml/min) | 92.70 | 94.15 | 95.12 | 1.53 | 2.47 | 1.09 |
| Minimum (ml/min) | 76.54 | 77.38 | 78.98 | -4.40 | -4.58 | -7.89 |
| Maximum (ml/min) | 109.20 | 108.11 | 116.20 | 9.53 | 7.79 | 9.94 |
| Standard deviation | 8.89 | 8.73 | 10.14 | 3.73 | 3.77 | 4.47 |

\(^{a}\)GCM: Gamma camera method; \(^{b}\)SPSM: Single plasma sampling method; \(^{c}\)DPSM: Double plasma sampling method; \(^{d}\)DPSM-SPSM-GCM: Difference between DPSM and GCM; \(^{e}\)DPSM-SPSM-GCM: Difference between DPSM and SPSM; GFR: Glomerular filtration rate

| Table 2: Comparison of GFR calculated by single plasma using weighing and counting method and double plasma sampling using weighing and counting method by this software |
|------------------|--------|--------|--------|--------|--------|
| SPSM\(^a\) | Counting | Weighting | Counting | Weighting | Counting | Weighting |
| Average (ml/min) | 94.082 | 94.148 | 95.040 | 95.117 | 0.066 | 0.077 |
| Minimum (ml/min) | 79.150 | 77.380 | 77.480 | 78.980 | -3.370 | -2.640 |
| Maximum (ml/min) | 108.520 | 108.110 | 114.580 | 116.200 | 2.840 | 3.200 |
| Standard deviation | 8.866 | 8.727 | 9.931 | 10.139 | 1.899 | 1.811 |

\(^{a}\)SPSM: Single plasma sampling method; \(^{b}\)DPSM: Double plasma sampling method; \(^{c}\)DDPSM: Difference between GFR calculated by single plasma sampling using weighing and counting method; \(^{d}\)DDPSM: Difference between GFR calculated by double plasma sampling using weighing and counting method; GFR: Glomerular filtration rate
calculation. We follow the standard recommendation for patient preparation and injection process provided in the literature. Two plasma samples between 1 h and 5 h with a 2 h interval between the two samples has been recommended in the literature. Pandey and Pant, have suggested the early sampling time of 1 h and 3 h after injection whereas Hephzibah et al., have suggested 2 h and 4 h sampling. Russell et al., have suggested 1 h and 3 h sample for two plasma sampling and 3 h sample for single plasma sampling for patient convenience. However, they have also suggested a delayed second sampling between 3 h and 5 h for improved accuracy in GFR calculation by DPSM and delayed sampling between 3 h and 5 h for single plasma sampling. Christensen and Groth, have also suggested the sampling between 3 h and 5 h for single plasma sampling. In our protocol, we collect two samples at 2 h and 4 h for DPSM and a 4 h sample for GFR calculation by single sampling method.

Performing plasma sampling GFR calculation by weighing method requires the procurement of additional equipment, that is, precision balance. We performed the plasma sampling GFR estimation by both the counting as well as the weighing methods and found good correlation between GFR calculated by both methods. Therefore, the software works equally well with both methods offering flexibility in the protocol that can be followed.

Software developed by Pandey and Pant, in MS Excel is user-friendly software. This software requires MS Excel installation on MS Windows® computer system. This software is able to calculate absolute global GFR but is unable to calculate differential function and absolute GFR for individual kidney. This software also cannot store patient’s data for future use. Our software can be installed on any PC with MS Windows® and no other additional software installation is required to run it. This is multiuser software and can be used on the local network by multiple users simultaneously. This software is password protected and can be used by authorized user only. Our software is able to provide absolute global GFR and individual kidney GFR. There is provision to save raw data and calculated GFR for future use and comparison during next follow-up. The software makes GFR calculation by plasma sampling and blood profile method very easy and simple. This software is able to do multipoint GFR comparison and is able to save data in a dedicated database. Patient’s data is saved with respective unique Patient ID and unique requisition ID. There is provision to transfer data in MS Excel format which allows easy data portability.

After the multilayered validation of this software and plasma sampling process, GFR calculation by two plasma sampling method has been incorporated for routine clinical use in our department.

**Conclusion**

This is a robust software program which makes the entire process of GFR estimation by various methods very simple. User friendliness of the software interface enables ease of use by getting rid of complex calculations and data maintenance.

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