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Original Article

Correlation of Methods of Glomerular Filtration Rate Estimation - 24 h Urinary Creatinine Clearance, Predicted Creatinine Clearance Method (Cockcroft-Gault), and Camera Based TC-99m-diethylenetriaminepentaacetic Acid Renography in Patients of Diabetic Nephropathy

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Abstract

Introduction: Glomerular filtration rate (GFR) is the most important measure for early detection of deterioration of renal function. Patients with diabetic nephropathy need to be intensely monitored with GFR value. Many methods are used to estimate the GFR, all having their own advantages and constraints. The correlation of these methods with each other in patients with diabetic nephropathy needs to be studied.

Purpose: To correlate three widely used methods of GFR estimation, namely 24 h urinary creatinine clearance (CrCl), predicted CrCl method (Cockcroft-Gault [CG]), and camera based Tc-99m-diethylenetriaminepentaacetic acid (DTPA) renography (by Gates' protocol), in cases of diabetic nephropathy and to study the effect of duration of disease on renal function.

Materials and Methods: A total of 52 cases of diabetic nephropathy were studied, and GFR was estimated by all the above three methods simultaneously. Correlation between variables was performed using commercially available statistical software (Minitab 15). Bland-Altman recommendations were used to compare the GFR calculated with CG formula and 24 h CrCl with the TC-99m-DTPA renogram method. Pearson's correlation was used to assess the relationship between variables.

Results: The mean GFR obtained with 99mTc-DTPA renogram, 24 h CrCl, and CG method were 63.24 ml/min ± 22.39 ml/min, 43.06 ± 13.83 ml/min, and 54.87 ± 18.25 ml/min, respectively. There was a significant correlation between GFR by 99mTc-DTPA versus endogenous CrCl ($P < 0.001$, $r = 0.830$) and 99mTc-DTPA versus CG formula ($P < 0.001$, $r = 0.919$).

Conclusion: There is a wide variation in the absolute values of GFR obtained by the three methods although all the three methods correlate well with each other. If the same method is used for follow-up, it is highly reliable in detecting the deterioration of renal function from the baseline value. However, these methods cannot be used interchangeably.

Key words: Cockcroft-Gault method, Creatinine clearance, Glomerular filtration rate, Renogram, Tc-99m-diethylenetriaminepentaacetic acid

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INTRODUCTION

Diabetic nephropathy is the leading cause of end-stage renal failure. Identification of early deterioration of renal function is most important in delaying the progression of renal damage.[1,2] Glomerular filtration rate (GFR) is the best measure of renal function and serum creatinine

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concentration alone should not be used to assess the level of kidney function.[3] There are many methods to estimate GFR. Inulin clearance is the gold standard; however, not performed in clinical practice, because of its technical complexity and limited availability. 99mTc-diethylenetriaminepentaacetic acid (DTPA) based plasma sampling methods are also very accurate but are very laborious involving multiple plasma samples.

Endogenous creatinine clearance (CrCl) estimation is widely used but is dependent on accurate collection of 24 h urine which is inconvenient and prone to collection failures and thus errors. It does not improve the estimate of GFR over that provided by prediction equations.[3] The prediction equations to calculate GFR take into account serum creatinine concentration and some or all of the following variables: Age, gender, race, and body size. The modification of diet in renal disease (MDRD) formula and Cockcroft-Gault (CG) equations are the ones commonly used. However, these calculations also have intrinsic limitations in relation to all the variables used for calculation and the fact that these equations have been derived from Caucasian population.[1,4,5]

Tc-99m-DTPA renography is being used very frequently for assessing renal function. It is a gamma camera based method simple to perform, reproducible, can assess individual kidney function and also detects additional abnormalities like obstructive uropathy. It does not require plasma sampling. The method introduced by Gate's is most commonly used.[6] Most of the clinicians nowadays have started utilizing the gamma camera based method. However, the diagnostic accuracy of this has always been debated.[7-15] There are studies comparing the formula based methods and Gate's method with the more accurate plasma sampling methods, each study having different conclusions.[4,5,16,17] Simple and accurate determination of GFR still remains a challenge as was mentioned by Swan way back in 1997.[18]

In diabetics, in whom GFR value is the most important measure for early detection of deterioration of renal function, how do all these methods correlate? It is an important question to be answered. We undertook this study to correlate these three widely used methods of GFR estimation namely formula based CG method, 24 h urinary CrCl and Tc-99m-DTPA renography in cases of diabetic nephropathy.

MATERIALS AND METHODS

The study was conducted at a tertiary care hospital and cases of diabetic nephropathy as evidenced by microalbuminuria, macroalbuminuria or raised creatinine levels, reporting to medical outpatient department or admitted in the wards over 6 months and who agreed to be part of the study were included. The patients with any other preexisting renal disease or with acute complications of diabetes were excluded from the study. A total of 52 cases were studied after obtaining their consent. In all the 52 patients, 24 h urine was collected and simultaneously blood samples for serum creatinine were collected. Serum and urine creatinine were measured using a kinetic alkaline picrate assay. Age, body weight, and height were recorded on the same day.

About 24 h urine CrCl was estimated using urine/plasma creatinine ratio (UV/P) formula, where U is the concentration of creatinine in urine in mg/dl and V is the volume of urine produced per minute and *P* is the plasma creatinine in mg/dl.

CG's method-predicted CrCl was calculated according to the equation of CG formula: GFR (ml/min) = ([140 - age (years) × body weight (kg)]/[72 × SCr (mg/dl)] × 0.85 for women).

Renography - GFR assessment by 99mTc-DTPA renography was done using Gates' protocol. The GFR was calculated using Gate's formula in renal software. The patient height (in cm) weight (in kg), age (in years), and sex were fed as input according to the program. Then, the percentage of injected dose (% ID) is calculated for each kidney.

$$\% ID = (K-B) \times 100/I \times S \times D$$

Where, % ID = Percentage ID
I = Injected activity (in MBq)
S = Camera sensitivity (in Cts/min/MBq)
K = Kidney region (Cts/min)
B = Background region (Cts/min)
D = Depth correction.

$$GFR\ (ml/min/1.73\ m^2) = (\%\ ID\ left + \%\ ID\ right) \times 9.8127 - 6.8252$$

The patients were advised to be on a normal diet and well hydrated before study. They were further asked to drink 5 ml/kg of water half an hour before the study and were asked to void before start of the study. 99mTc-DTPA was prepared 30-60 min before injection using a fresh elute and a DTPA kit (supplied by Board of Radioisotope Technology, Mumbai), 5 mCi of radiopharmaceutical was injected intravenously through antecubital vein and followed by infusion of 20 ml of normal saline after obtaining the pre syringe counts for 1 min under the camera. Supine posterior imaging was done. Images were

acquired initially as 2 s frames for 1 min and then 10 s frames for 25 min. Post syringe counts were then taken in the same way as pre syringe counts. A large field of view and low energy, all purpose, and parallel hole collimator was used. Photopeak - 15% window centered over 140 keV (99mTc). The region of interest (ROI) over each kidney was drawn on the composite frames for 3 min. Elliptical background ROI was drawn below and lateral to kidney avoiding scatter, liver, spleen, and gut. Gates protocol was used for depth correction and time activity curve was generated using computer software.

The results of the UV/P, CG, and 99mTc-DTPA GFR were corrected to body surface area (BSA) of 1.73 m^2 (1.73/BSA). BSA was estimated according to Mosteller formula.

The results from the three different methods of GFR estimation were analyzed statistically. Correlation between variables was performed using commercially available statistical software (Minitab 15). Bland-Altman recommendations were used to compare the GFR calculated with Cockcroft formula and 24 h endogenous CrCl with the renal clearance of 99mTC-DTPA (gamma camera based method - Gates method). Pearson's correlation was used to assess the relationship between variables, analysis of variance, and student's *t*-test was used to find a significance of GFR by DTPA, GFR by CG method and 24 h endogenous CrCl. Results are shown as mean ± Standard deviation.

Classification of correlation of co-efficient: (a) Upto 0.1 - trivial correlation, 0.1-0.3 - small correlation, 0.3-0.5 - moderate correlation, 0.5-0.7 - large correlation, 0.7-0.9 - very large correlation, 0.9-1.0 - nearly perfect correlation and 1 - perfect correlation.

RESULTS

A total of 52 patients with diabetes mellitus (DM) with varying stages of nephropathy were included in the study. 48.1% (n = 25) were males, 51.9% (n = 27) were females, and the mean age was 56.85 ± 10.44 years (35-74 years). Their mean BSA was 1.69 ± 0.09 m^2 and mean serum creatinine and blood urea nitrogen were 1.35 ± 0.49 mg/dl and 17.08 ± 4.47 mg/dl, respectively. The distribution of cases based on duration of illness is given in Table 1.

The values of mean GFR (ml/min/1.73 m^2 BSA) by 99mTc-DTPA gamma camera based method, the endogenous GFR by 24 h urine CrCl and eGFR by Cockgroft-Gault equation, as well as other study variables, are given in Table 2.

Table 1: Distribution of cases of diabetic nephropathy based on duration of illness

Duration of illness	Number of patients (%)
1-5 years	21 (40.4)
5-10 years	21 (40.4)
>10 years	10 (19.2)
Total	52 (100.0)

Table 2: Descriptive statistics of study variables

Variables	Minimum	Maximum	Mean±SD
Age in years	35	74	56.85±10.44
Weight (kg)	6	76	62.00±9.41
BSA (m^2)	1.52	1.91	1.69±0.09
Duration of illness (years)	1	20	6.92±4.34
Serum creatinine (mg/dl)	0.6	2.8	1.35±0.49
eGFR-Cockcroft-Gault method	22	111.26	54.87±18.25
GFR-endo CrCl	17.52	76.56	43.06±13.83
GFR-99mTc DTPA renogram			
Right kidney	12.60	58.00	31.06±10.45
Left kidney	10.00	70.00	32.16±13.26
Mean GFR	24.40	128.00	63.23±22.39

BSA: Body surface area, GFR: Glomerular filtration rate, DTPA: Diethylenetriaminepentaacetic acid, CrCl: Creatinine clearance, SD: Standard deviation

Table 3: Mean GFR of 24 h urine CrCl, CG method, and 99mTc-DTPA according to age

Age in years	Number of patients	GFR by 24 h endogenous CrCl	GFR-CG method	GFR by 99mTc DTPA clearence
35-40	5	51.73±17.45	78.23±20.27	81.20±29.96
41-50	12	43.75±11.77	56.51±17.31	65.20±24.46
51-60	15	48.98±15.48	60.06±18.58	71.01±21.53
61 and above	20	36.03±9.62	44.15±9.6	51.75±13.95
Total	52	43.06±13.83	54.87±18.25	63.24±22.39
P	-	0.015*	<0.001**	0.011*

*Moderately significant (P: 0.01<P≤0.05).**Strongly significant (P≤0.01) GFR: Glomerular filtration rate, CrCl: Creatinine clearance, DTPA: Diethylenetriaminepentaacetic acid, CG: Cockcroft-Gault

To study the effect of age, the mean GFR obtained with 99mTc-DTPA, 24 h urine CrCl and Cockcroft-Gault formula were analyzed as per age (Table 3 and Figure 1). The GFR by 99mTc-DTPA was the highest in each group, followed by eGFR (CG formula) and the lowest was by 24 h urine CrCl. There was fall in GFR with an increase in age in the adult population and all the three methods correlated well with each other (P < 0.001) (Table 3 and Figure 1).

As disease duration of diabetes in a patient can affect renal function, the findings were analyzed for various disease durations of diabetes. The mean GFR obtained with 99mTc-DTPA, 24 h urine CrCl and CG formula according to disease duration between 1 and 5 years was 75.27 ± 23.71 ml/min, 50.87 ± 14.29 ml/min, and 66.78 ± 18.43 ml/min, respectively; for disease duration

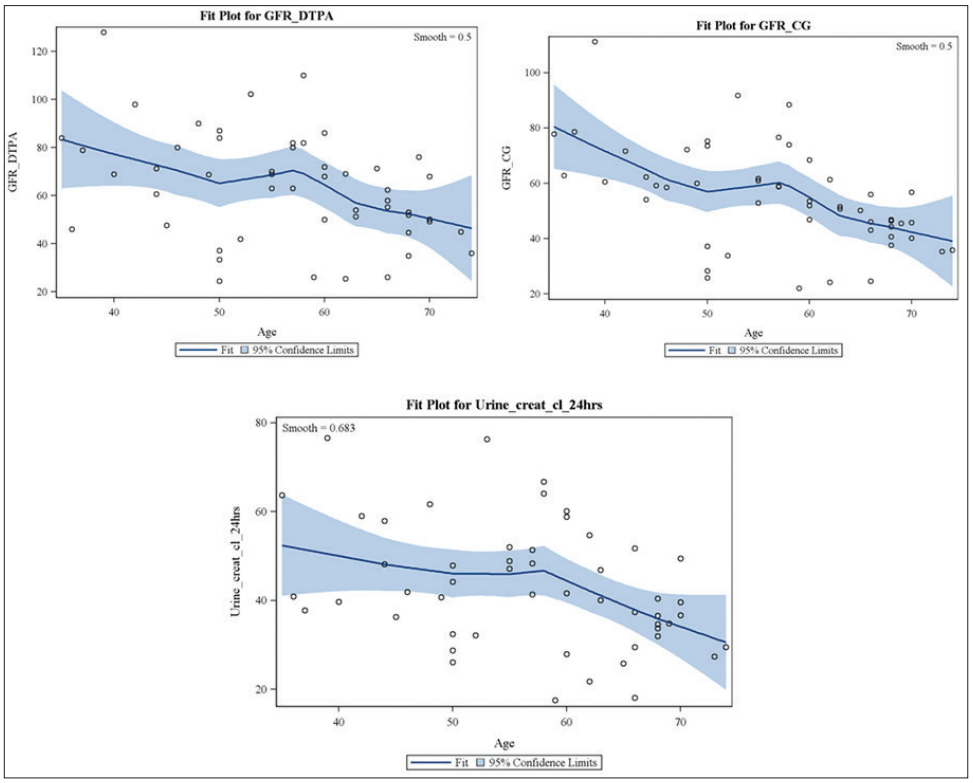

Figure 1: Fit plot graphs showing relation of glomerular filtration rate by 99mTc diethylenetriaminepentaacetic acid, Cockcroft-Gault formula, and 24 h urine creatinine clearance with age

between 5 and 10 years it was 57.61 ± 19.31 ml/min, 38.18 ± 11.07 ml/min, and 48.22 ± 14.16 ml/min, respectively and for disease duration >10 years was 49.79 ± 12.25 ml/min, 36.89 ± 10.89 ml/min, and 43.81 ± 10.84 ml/min, respectively (Table 4). The decrease in GFR with the duration of illness was significant in all the three methods.

The mean GFR obtained with 99mTc-DTPA camera based method was 63.24 ml/min ±22.39 ml/min, whereas by 24 h urine CrCl was 43.06 ± 13.83 ml/min ($P = 0.015$) and by CG formula was 54.87 ± 18.25 ml/min (Tables 2-4). The mean values of eGFR by CG formula were nearer to the mean GFR obtained with 99mTc-DTPA camera based method. GFR from 24 h urine CrCl was the lowest. GFR values by 99mTc-DTPA (Gates method) were >15.2% compared to CG formula and GFR values by CG method were >21.5% compared to 24 h urine CrCl.

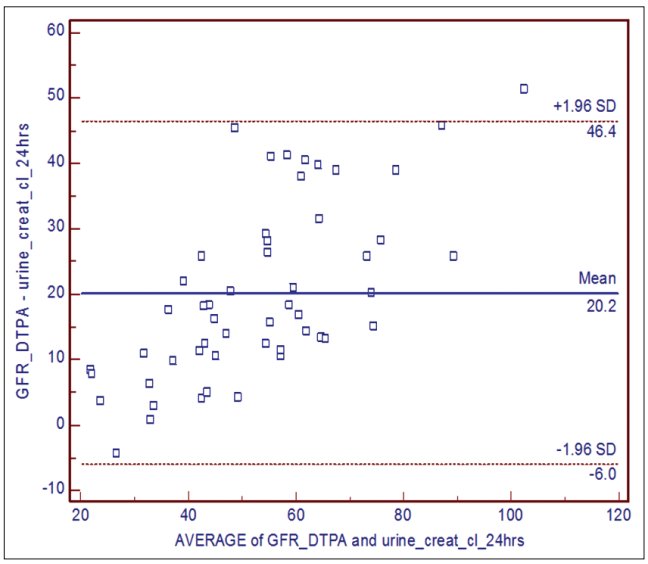

Figure 2: Bland-Altman plot for correlation of glomerular filtration rate by diethylenetriaminepentaacetic acid and 24 h urine creatinine clearance

GFR by all the three methods was normally distributed by D'Agostino and Pearson omnibus normality test. There was a significant correlation between GFR by 99mTc-DTPA versus end CrCl ($P < 0.001$), 99mTc-DTPA versus CG formula ($P < 0.001$) (Figures 2 and 3). The coefficient of correlation (r) of 99mTc-DTPA (Gates' method) and endogenous CrCl was $r = 0.830$ (very large correlation) and coefficient of correlation (r) of 99mTc-DTPA and Cockcroft formula, $r = 0.919$ (nearly perfect correlation). An assessment of the plots (Bland-Altman plot) of GFR estimation by Cockcroft-Gault formula and 24 h urine CrCl compared with Tc-99m-DTPA clearance by camera based method (Gates method) showing that they correlate well (Figures 2-4).

Table 4: Mean GFR by 24 h urinary CrCl, CG method, and 99mTc-DTPA according to duration of illness

Duration of illness	Number of patients	GFR by 24 h urine CrCl	GFR-CG method	GFR by Tc-99m-DTPA scan
1-5 years	21	50.87±14.29	66.78±18.43	75.27±23.71
5-10 years	21	38.18±11.07	48.22±14.16	57.61±19.31
>10 years	10	36.89±10.89	43.81±10.84	49.79±12.25
Total	52	43.06±13.83	54.87±18.25	63.24±22.39
P	-	0.002**	<0.001**	0.003**

**Strongly significant (*P*≤0.01). GFR: Glomerular filtration rate, CrCl: Creatinine clearance, DTPA: Diethylenetriaminepentaacetic acid, CG: Cockcroft-Gault

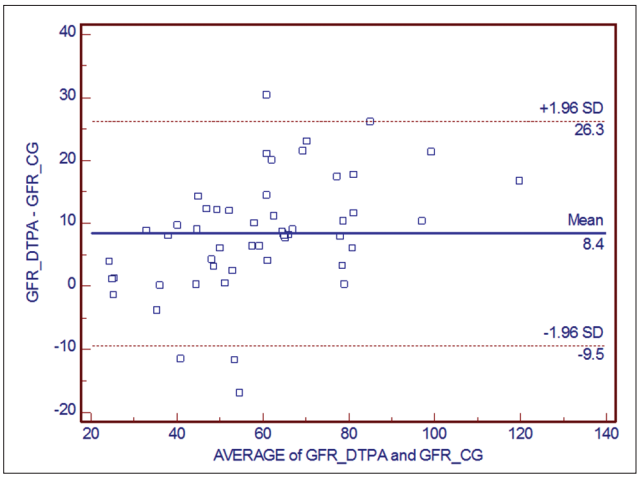

Figure 3: Bland-Altman plot for correlation of glomerular filtration rate by 99mTc diethylenetriaminepentaacetic acid and glomerular filtration rate by Cockcroft-Gault method

DISCUSSION

Diabetic nephropathy is the leading cause of end stage renal failure. Approximately, 20-30% of all diabetics will develop evidence of nephropathy. GFR is generally considered the best measure of renal function in health and in disease.[1,2] However, simple and accurate measurement of GFR still remains a challenge.[18] The correlation of the commonly used methods of GFR estimation exclusively in the patients of diabetic nephropathy needs to be evaluated. We undertook this study, to correlate the three commonly used methods - 24 h CrCl, CG formula based method, and Tc-99m-DTPA renography in the patients of diabetic nephropathy.

Our results show that GFR value obtained by Tc-99m renography (Gates method) is highest, followed by the CG method with intermediate values and the 24 h clearance method showing the least values. This trend was seen in all the subsets of diabetics. The question remains as to which of these is the accurate value. We have not compared the values with any gold standard method to answer this question. However, all the three methods showed good correlation with each other. There was a significant correlation between GFR by 99mTc-DTPA versus 24 h CrCl ($P < 0.001$), 99mTc-DTPA versus CG formula ($P < 0.001$). The coefficient of correlation (r) of 99mTc-DTPA (Gates' method) and endogenous CrCl was $r = 0.830$ (very large correlation) and coefficient of correlation (r) of 99mTc-DTPA and Cockcroft formula, $r = 0.919$ (nearly perfect correlation).

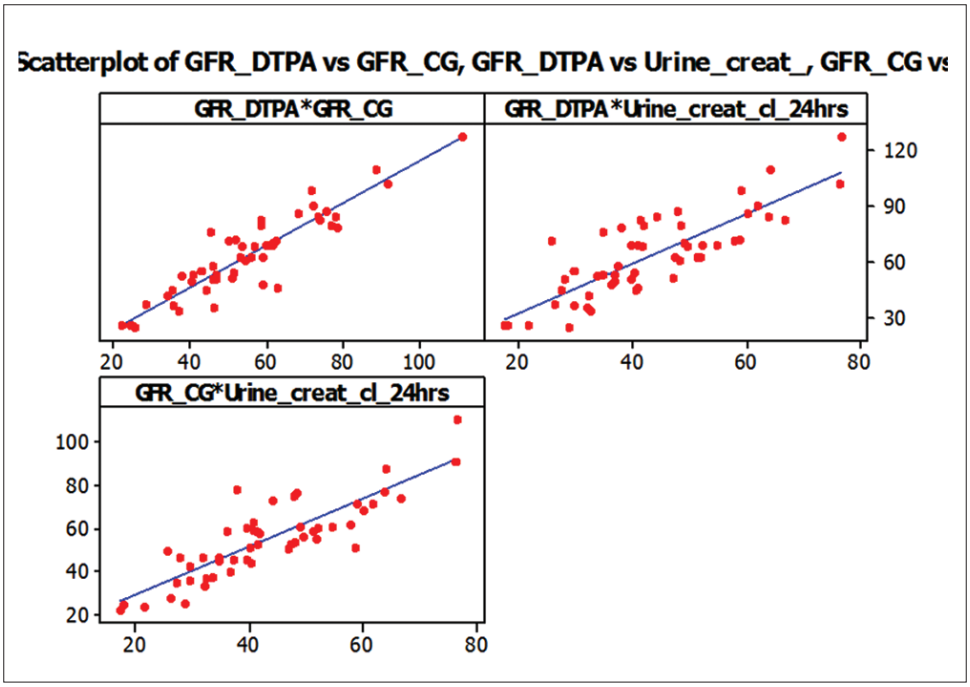

Figure 4: Scatterplots of glomerular filtration rate values of 99mTc diethylenetriaminepentaacetic acid (Gates' method) versus Cockcroft-Gault, 99mTc diethylenetriaminepentaacetic acid (Gates' method) versus 24 h urine creatinine clearance, and Cockcroft-Gault versus 24 h urine creatinine clearance

There are many studies available in the literature comparing these three methods, each study having different opinion about the correlation among them and the relative superiority of each method.[4,5,16,17] Many studies have revealed that Gates method overestimates the GFR.[13,14] Itoh in his study of 133 patients with a wide range of renal function, compared Tc-99m-DTPA renography (Gates method) and CG method using plasma sample clearance method as reference. He concluded that Gates method tended to overestimate the GFR and contrarily CG tended to underestimate the GFR. He concluded that Tc-99m-DTPA renography is not suitable for GFR estimation.[16] Our study has shown similar trend.

Prasad *et al.* in their study involving 897 patients with a wide range of renal function concluded that Gates method correlates better with plasma sampling method than formula based MDRD method, although overall both the methods showed poor correlation with the reference method making them suboptimal for clinical use. Both these methods overestimated GFR at lower levels of renal function and underestimated it at higher levels of renal function.[17]

There are many formula based methods described to calculate and predict the GFR by incorporating serum creatinine level and various biometrical variables.[19] MDRD and C-G formulae are most commonly used. We studied the CG method in our study. However, it has been debated whether the equations correctly depict the GFR.[16,19,20,21] Virga *et al.* evaluated the performance of 12 different creatinine-based equations in predicting the CrCl in patients on peritoneal dialysis and found that Gates, Virga, and 4-MDRD showed best results while the CG formula revealed a rather poor reliability.[22] Actually, there are so many variables involved in calculating the GFR by the formula based methods, starting from the accurate measurement of serum creatinine itself and the various biometric parameters that the accuracy will always be doubtful.

In Tc-99m-DTPA renography, the method introduced by Gates is most commonly used, because of greater ease of administration, simplicity, accuracy, and precision of measurement.[6] The GFR is calculated without blood or urine sampling. It has the added advantage of greater reproducibility and assessment of individual kidney function. It also detects additional renal abnormalities like obstructive uropathy. However, even in this, there are so many variables involved such as background correction, ROI drawn, state of hydration of patient, and calibration of camera. A simple change in the ROI drawn leads to a huge difference in GFR value.

Hence, the search for an ideal and simple way to measure GFR which can be close to inulin clearance and radioisotope plasma sampling methods still continues. Our study had a few limitations as we did not use the reference gold standard method and the sample size was limited to the duration of the study.

However, this study clearly shows that there is a good correlation in the three methods of measuring GFR (99mTc-DTPA, 24 h urine CrCl and Cockcroft-Gault formula) and a significant fall in mean GFR obtained with age and with an increase in disease duration. This indicates that irrespective of the method used, the same method should be used for subsequent follow-up to accurately detect fall in GFR.

CONCLUSION

There is a wide variation in the absolute values of GFR obtained by the three methods (24 h CrCl, CG method of eGFR calculation, and Tc-99m-DTPA renography). Hence, it still remains questionable as to which is the most accurate method and it is also clear that these methods cannot be used interchangeably in the same patient for follow-up. However, one finding is evident that if the same method is used for follow-up, it is highly reliable in detecting the deterioration of renal function from the baseline value. The study also shows the steady decline in GFR with age and duration of diabetes and reiterates the importance to follow-up the patients of DM with GFR value for early detection of deterioration in kidney function.

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