

**Clinical Value of Cystatin C and Beta-trace Protein in Glomerular Filtration
Rate in Chronic Renal Disease Adult Cases With Different Degree Renal
Function Disorder: Comparison by The ^{99m}Tc-DTPA Plasma Sample Method**

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Abstract

Glomerular filtration rate (GFR) is the best indicator of the renal function. Gold standard for GFR measurement is inulin clearance (C_{in}). However, its measurement is inconvenient, time-consuming, and non-economic. Thus, in both scientific studies and routine clinical practice Nuclear Medicine methods (^{99m}Tc DTPA, ^{51}Cr EDTA) are preferred, which are highly correlated with C_{in} . In addition, recently cystatin C (Cys) and beta trace protein (BTP) are also used for this purpose. In literature, however, data are limited about the clinical value of Cys and BTP in GFR measurement in CKD cases and the results have been inconclusive. In this study, we aimed to determine the efficiency of Cys and BTP in determination of GFR in CKD patients. A total of 84 (59 males, 25 females) CKD patients aged 21 to 88 years (mean 61 years) were included criteria. GFR was calculated with three different methods: 1) using the gold standard DTPA two plasma sample method (TPSM); 2) using formula containing Cys to calculate GFR; 3) using formula containing BTP. Correlation of GFR values calculated with Cys and BTP with that obtained with DTPA TPSM was assessed. GFRs calculated with both methods were significantly correlated with that calculated with gold standard method. However, GFR values obtained with Cys had a better correlation compared to those of BTP. Using Bland Altman analysis, scatter graphics of the differences between GFR values calculated using DTPA TPSM and those calculated with Cys, BTP at a confidence interval of 95% (mean \pm 1.96SD). The GFR values obtained with Cys and BTP did not have reliable consistency. As a conclusion, we showed that GFR values calculated with Cys and BTP failed to show GFR of the CKD. Based on these results, our study demonstrated that Cys and BTP is not sufficient in reflecting GFR.

Key Words: Glomerular Filtration Rate (GFR), Cystatin C (Cys), Beta Trace Protein (BTP)

Introduction

Chronic Kidney Disease (CKD) is a nephrological syndrome secondary to chronic, progressive, and irreversible nephron injury due to various causes. In an individual with renal disease, azotemia for more than 3 months, long-standing uremic signs and symptoms, signs and symptoms of renal osteodystrophy, anemia, hyperphosphatemia, hypocalcemia, large cylinders in urine sediment, and bilateral small kidneys in radiological examinations are indicative of chronic disease. These features help make the distinction of CKD from acute renal injury (1).

Evidence of renal injury may be of structural or functional in character and can be obtained from kidney biopsy, urinalysis, blood tests, and imaging examinations. The most common and most easily detected indicator of kidney injury resulting in glomerular dysfunction is proteinuria. According to the definition of the National Kidney Foundation (NKF-KDOQI), a glomerular filtration rate (GFR) reduction below 60 ml/min/1.73 m² is sufficient to make the diagnosis of CKD, even when there are no signs of renal injury (1, 2).

GFR is the most important diagnostic tool for global assessment of renal function. With measurement of GFR, it is possible to detect renal injury and its severity, and also to assess the progression of renal disease by serial measurements. Total GFR is equal to the sum of each filtration rate of all functioning nephrons. The normal GFR value depends on a person's age, sex, and body habitus. Showing great variability even among normal persons, GFR is approximately 130 ml/min/1.73 m² in men and 120 ml/min/1.73 m² in women (1).

Various methods are used for GFR calculation and assessment. Inulin clearance (C_{in}), the accepted gold standard for GFR measurement, is the most accurate method for renal function. However, difficulties of its application, high cost, low availability, and a long measurement time hamper its routine use in clinical practice (3). In the past, evaluation of renal function has substantially been based on serum creatinine measurement. Endogenous creatinine clearance is a widely accepted method, however, its relationship with C_{in} is weak and since it is secreted from tubuli, it may lead to erroneous results in states characterized by reduced renal function.

In the measurement of GFR, blood sampling techniques following ^{99m}Tc diethylene triamine pentaacetic acid (DTPA) and chromium-51 ethylenediamine tetraacetic acid (⁵¹Cr-EDTA) injection are widely used because of the ease of application and high accuracy (4, 5). Blafox et al. (6) have demonstrated that measurement of ⁵¹Cr-EDTA clearance is the method

which gives the closest results to C_{in} . It has also been shown that ^{51}Cr -EDTA clearance and $^{99\text{m}}\text{Tc}$ DTPA clearance are in agreement with each other and $^{99\text{m}}\text{Tc}$ DTPA can be substituted for ^{51}Cr -EDTA (7). Furthermore, Rehling et al. (8) have showed that $^{99\text{m}}\text{Tc}$ DTPA clearance is very close to C_{in} , the accepted gold standard.

Cystatin C (Cys) is a non-glycosylated low molecular weight protein found in human tissues and biological fluids. It is carried unbound to any protein in plasma. It is reabsorbed from the proximal tubule and metabolized, but not secreted. It is unaffected by non-renal parameters such as age, sex, diet, and muscle mass (9). It has been shown that Cys can be used as an endogenous marker of GFR measurement as an alternative to creatinine (10, 11).

Literature data have shown that Cys does not accurately reflect GFR in patient group undergoing chemotherapy (12, 13). Aydin et al. (12) and Knight et al. (13) have shown that Cys does not accurately reflect GFR in patient group receiving immunosuppression therapy due to the nephrotoxic effects of chemotherapeutic drugs. These drugs induce tubular cell injury through nephrotoxic effects, however, when tubular cell injury is developed, reabsorption of Cys from proximal tubule and its catabolism is impaired. In contrast, we previously showed that Cys is significantly correlated to GFR values measured with $^{99\text{m}}\text{Tc}$ DTPA two plasma sample method (TPSM) in reflecting GFR in a patient group not undergoing chemotherapy (12). However, there are no studies in the literature specifically investigating whether Cys can be used as a marker of GFR in patients with renal dysfunction accompanied by varying degrees of tubular injury and not receiving any medical therapy (chemotherapy, steroid therapy etc.).

One alternative method for determination of GFR is the recently introduced serum beta trace protein (BTP). BTP is a low molecular weight enzyme that belongs to the prostaglandin D synthase group. It is found in all tissues of human body with the exception of ovaries. It is almost completely filtered from kidneys. Some studies have indicated that BTP, as Cys, may be used as a marker of GFR in adult patient populations (14). Unfortunately, there are an insufficient number of studies investigating GFR measurement with BTP in CKD. Moreover, the available studies on this subject have provided controversial results.

Moreover, there are no studies in the literature investigating the role of the GFR values calculated with Cys and BTP in reflecting renal functions of patients with CKD (14). It is also noteworthy that there are no studies examining the correlation and agreement between Cys, BTP levels and GFR values measured with $^{99\text{m}}\text{Tc}$ DTPA blood sample method for determination of

renal functions of CKD patients. In this regard, our study is the first and we believe that it will make a major contribution to the existing literature.

N-acetyl-b-D-glucosaminidase (NAG) is an enzyme that has been studied the most and has have a wide range of area of use as a highly sensitive marker of renal tubular injury among other urinary enzymes. Although NAG is present in all segments of nephron, it is particularly abundant in the lysosomes found in the proximal renal tubular cells. Its high molecular weight (130,000-140,000 Da) prevents its passage through glomerular basal membrane; thus, its daily excretion is highly stable despite minimal diurnal fluctuations. It is found in trace amounts in urine in healthy persons. NAG is a sensitive test method for determination of the severity of renal injury at a period before renal functions begin to be impaired. Although NAG is present in all segments of nephron, especially due to its abundance in the lysosomes of the proximal renal tubular cells, it is used to determine the functions of that region (15, 16). NAG activity is correlated to disease activity. Factors that promote using NAG as a marker of renal injury include the following: it is a highly sensitive marker of tubular injury in kidneys; it increases in urine earlier according to the renal function tests being used; and its amount in urine increases in parallel to the severity of the pathological changes. Thus, NAG has found a place for use in various renal conditions (17).

β 2-microglobulin (B2M) is a low molecular weight, non-glycosylated peptide protein with a molecular weight of 11,800 dalton. It is found in the surface of all cell types as a continuous part of the light chain of the major histocompatibility complex of the Class I antigens. Its endogenous production is fairly stable and it is almost solely filtered by the glomeruli. B2M is easily filtered by the glomeruli. Approximately 99% of the protein is reabsorbed from the proximal tubule via pinocytosis and metabolized. Hence, B2M levels are constant in normal individuals. In patients with CKD there is a relationship between the level of GFR reduction and B2M accumulation in blood. With a reduction in GFR, B2M starts to accumulate in blood and the increase in B2M in blood has been reported to occur earlier and to a greater extent than serum creatinine increase. It has been reported that B2M is a better marker compared to the serum creatinine levels for the calculation of GFR and for showing renal dysfunction (18-21).

In the present study we aimed to: 1) show the ability of Cys and BTP in reflecting renal function in CKD patients with varying degrees of renal dysfunction by accepting GFR measured

with ^{99m}Tc DTPA TPSM as the gold standard; and 2) study the correlation between the severity of tubular function injury (as assessed by NAG and B2M levels) and Cys and BTP levels.

Materials and Method

Cases

This study enrolled a total of 100 patients with CKD (68 males, 32 females; mean age 61 ± 12 ; age range 21-88 years) who were under follow-up at Akdeniz University Faculty of Medicine, Department of Nephrology. Sixteen patients (9 males, 7 females) were excluded from the study due to taking steroid and chemotherapeutic therapy and having thyroid dysfunction (as Cys level is altered by thyroid dysfunctions).

The study was approved by the local ethics committee at Akdeniz University Faculty of Medicine in the meeting dated 28.04.2011. This study is also a project of the Akdeniz University Scientific Research Coordination Unit with the Project Number 2011.01.0103.012.

The patients who attend regular follow-ups at the Nephrology Department were informed about the study protocol and those who were willing to participate in the study gave an informed consent. Each study participant underwent spot urine and blood sampling. The obtained samples were sent to the biochemistry laboratory. The blood samples were centrifuged. The serum samples obtained from the blood samples were stored at -80°C . The patients undergoing urine and blood sampling were referred to the Nuclear Medicine Department at the same day for GFR measurement with ^{99m}Tc DTPA TPSM. The patients were injected 74-111 MBq ^{99m}Tc DTPA (binding efficiency of 95% or above) for ^{99m}Tc DTPA TPSM GFR calculation. The participants were hydrated under physiological conditions starting 30 minutes before and ending 4 hours after the injection with which blood taking process is completed.

In order to be used in ^{99m}Tc DTPA TPSM GFR calculation, 5 cc blood samples were drawn into heparinized tubes following the injection and from the non-injected arm at 120 and 240 minutes. The obtained samples were then centrifuged at 2,000 g for 10 minutes and the plasmas were separated. GFR measurement from blood samples was performed in compliance with the “Glomerular Filtration Rate Measurement Guide” prepared by “Turkish Nuclear Medicine Society Nephro-urology Working Group” (22).

Laboratory Tests

Venous blood samples of 5 cc were drawn into the tubes with gel separator. The samples were immediately centrifuged at 4,000 rpm for 5 minutes and the serum of the samples were stored at -80 ° C for 3 months for analyses. The serum samples, which are stored at -80 ° C, are thawed on the day of analysis and measurements are made.

The spot urine sample was first used for full urinalysis and then Cys, NAG, B2M, sodium, phosphate, and creatinine measurements are made.

Serum and urine Cys levels were measured with the nephelometric method in the BN II nephelometry device (Siemens Healthcare Diagnostics Ltd, USA). Serum and urine B2M levels were measured with the nephelometric method in the BN II nephelometry device (Siemens Healthcare Diagnostics Ltd, USA). Serum BUN analysis was performed with the Enzymatic, Colorimetric Method in the Cobas 8,000 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum creatinine analysis was performed with the Rate-blanked, Compensated Jaffe Method in the Cobas 8,000 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum phosphate analysis was performed with the Colorimetric Method in the Cobas 8,000 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum and urine sodium analysis were performed with the Indirect Ion Selective Electrode Method in the Cobas 8,000 autoanalyzer (Roche Diagnostics, Mannheim, Germany).

Urine protein analysis was performed with the Turbidimetric Method in the Cobas 8,000 autoanalyzer (Roche Diagnostics, Mannheim, Germany).

Serum BTP analysis was performed with the Solid-phase Sandwich ELISA Method using the “Biovendor” branded kit (Biovendor, Human Prostaglandin D Synthase (LIPOCALIN-TYPE) ELISA, Cat. No:RD 191113100R). In this method, microplates containing 96 wells pre-coated with the polyclonal anti-BTP antibody were used. The 100-time diluted serum and BTP found in the standards, which were added into the wells, were bound to these antibodies after a 1-hour incubation period and the unbound parts were removed by repeated washing. Next, a conjugate was added to the medium and a further 60-minute incubation period was waited. The unbound enzyme was removed from the medium by washing and the substrate was added. The color density that occurred after 10-minute incubation was measured at 450 nm. The amount of BTP in serum samples was calculated using the curve drawn with the help of the standards. The results were presented as ng/ml.

Urine NAG analysis was performed with the Colorimetric Method using the Diazyme branded kit. The results were presented as IU/L.

Serum free T4 and TSH were measured with the Electrochemiluminescence Immunoassay Method in the Cobas 8,000 autoanalyzer (Roche® Diagnostics, Mannheim, Germany).

GFR Measurement Methods

Three separate methods were used for GFR measurement.

^{99m}Tc DTPA TPSM was calculated with the following formula as gold standard method (23, 24):

$$\text{DTPA GFR (ml/min)} = \frac{D \ln (P_1/P_2)}{T_2-T_1} \exp \frac{(T_1 \ln P_2)-(T_2 \ln P_1)}{T_2-T_1}$$

D = total injected dose (cpm)

P₁ = Plasma activity at the time T1 (cpm/ml)

P₂ = Plasma activity at the time T2 (cpm/ml)

T₁ = 120 (min)

T₂ = 240 (min)

The value obtained by the formula was corrected with the Bröchner correction (25). All the GFR values obtained with Bröchner correction subjected to body surface area correction 1.73 m² to obtain the final GFR value (26).

GFR (ml/min/1.73 m²) was calculated with Cys (mg/l) level (27).

$$\text{Cys GFR (Hoek)} = -4.32+80.35 \times 1/\text{Cys}$$

GFR (ml/min/1.73 m²) was calculated using serum BTP (mg/l) level with the method and the formula reported by Pöge et al. (28).

$$\text{BTP GFR} = 974.31 \times \text{BTP}^{-0.2594} \times \text{creatinine } (\mu\text{mol/l})^{-0.647}$$

After all measurements, the patients were divided into 4 subgroups based on GFR values calculated with the ^{99m}Tc DTPA TPSM as proposed by K/DOQI criteria (9).

Group 1 included GFR values between 0 and 15 ml/min/1.73 m², Group 2 included GFR values between 15 and 30 ml/min/1.73 m², and Group 3 included GFR values between 30 and 60 ml/min/1.73 m², (although K/DOQI criteria separate patients into 5 groups we studied the subjects in 3 groups since the number of subjects with a GFR value of 60ml/min/1.73m² or above was low).

The correlation and the agreement within a 95% confidence interval was studied between the Cys and BTP levels and GFR values calculated with these parameters and those calculated with the ^{99m}Tc DTPA TPSM.

In addition, the study subjects were grouped into 2 groups according to NAG and B2M levels that indicate tubular injury at an early stage: Group A: NAG \leq 6.1 and Group B: NAG $>$ 6.1; 1nd group: B2M \leq 0.2 (mg/l); 2nd group: B2M $>$ 0.2(mg/l)). The correlation and the correlation differences between the GFR values calculated with Cys and BTP levels and those calculated with the ^{99m}Tc DTPA TPSM in patients with versus without renal tubular injury detected by NAG and B2M levels.

Statistical Analysis

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) 18.0 and MedCalc v. 8.2.0.1 software packages.

All results were presented as mean \pm standard deviation (SD). The correlation analysis between the GFR values calculated with our reference method, the ^{99m}Tc DTPA TPSM, and the GFR values calculated with other methods, Cys, BTP, and B2M(serum) levels were accomplished with the Spearman's correlation analysis.

A correlation analysis was done between the GFR values in the Groups 1 to 3 grouped according to the ^{99m}Tc DTPA TPSM and the GFR values calculated with the other methods.

Bland-Altman analysis method was used to determine the 95% interval of agreement. This method determined the "bias" (Mean difference) \pm 1.96 SD values of the GFR values calculated with each method according to the ^{99m}Tc DTPA TPSM. Also called the systematic error, the mean difference values were calculated as follows:

bias = $1/N(\sum dGFR)$

N: The number of cases

dGFR: The difference in GFR (other GFR - TPSM GFR)

Results

Our study enrolled a total of 84 patients with CKD (59 males, 25 females; mean age 61 ± 12 (age range 21-88 years)) who had varying degrees of renal dysfunction and did not receive steroid therapy or chemotherapy or have thyroid dysfunction (as Cys level is altered by thyroid disorders). All data that belonged to 84 subjects (NAG, B2M, Cys, BTP, creatinine, urea, BTP; GFR values calculated using Cys and GFR values calculated using ^{99m}Tc DTPA TPSM (ml/min/1.73m²)) were presented on Table 1 with mean, standard deviation, and minimum and maximum values.

There was a significant positive correlation between the GFR values calculated with ^{99m}Tc DTPA TPSM and those calculated with Cys. The r and p values obtained with the Spearman's correlation analysis were: Cys GFR ($r=0.904$, $p<0.001$), which were statistically significant.

There was a significant positive correlation between the GFR values calculated with ^{99m}Tc DTPA TPSM and those calculated with BTP ($r=0.725$, $p<0.001$).

As one can see, the GFR values obtained with Cys showed a higher correlation than the GFR values calculated with BTP. Furthermore, the comparison of the performances of Cys and BTP in reflecting GFR measured with ^{99m}Tc DTPA TPSM revealed that both methods were significantly correlated to ^{99m}Tc DTPA TPSM measured GFR ($p<0.001$). However, the correlation of Cys -measured GFR values with GFR values calculated with ^{99m}Tc DTPA TPSM was higher (73% vs. 90%) and according to these findings the data in our study has shown that Cys is superior to BTP in reflecting GFR.

Although there was a negative correlation between the GFR levels determined with the ^{99m}Tc DTPA TPSM and Cys and B2M values calculated from serum of the subjects, no significant correlation could be found between BTP and creatinine. The correlation coefficients of these parameters were as follows: Cys ($r=-0.797$, $p<0.0001$); B2M ($r=-0.762$, $p<0.0001$); BTP ($r=-$

0.090, $p=0.417$); creatinine ($r=-0.033$, $p=0.769$) (Table 2). Among all r values, the highest correlation belonged to Cys (-0.83).

After all measurements, the patients were divided into 3 subgroups based on GFR values calculated with the ^{99m}Tc DTPA TPSM as proposed by K/DOQI criteria (9).

Group 1 included GFR values between 0 and 15 ml/min/1.73 m², Group 2 included GFR values between 15 and 30 ml/min/1.73 m², and Group 3 included GFR values between 30 and 60 ml/min/1.73 m² (although K/DOQI criteria separate patients into 5 groups we studied the subjects in 3 groups since the number of subjects with a GFR value of 60ml/min/1.73 m² or above was low). The correlation and the agreement within a 95% confidence interval was studied between the Cys and BTP levels and GFR values calculated with these parameters and those calculated with the ^{99m}Tc DTPA TPSM.

The correlation values between BTP GFR and Cys GFR were separately determined for each group. Only Group 2 (DTPA GFR 15-30 ml/min/1.73 m²) showed a significant correlation between ^{99m}Tc DTPA TPSM GFR and BTP GFR ($r= 0.67$, $p<0.0001$). No significant correlations were found in other groups. Group 2 and Group 3 showed a statistically significant correlation between ^{99m}Tc DTPA TPSM GFR and Cys GFR ($r=0.82$, $p<0.0001$; $r=0.76$, $p<0.0001$, respectively) whereas Group 1 lacked a statistically significant correlation between these parameters. The correlation values calculated with Cys GFR were similar in the both groups. However, the correlation values calculated with the BTP GFR was lower than that calculated with Cys GFR (Table 3).

Bland Altman analysis was used to draw scatter plots of the difference values between ^{99m}Tc DTPA TPSM GFR values and Cys GFR, BTP GFR values within 95% confidence interval (mean $\pm 1.96\text{SD}$) (Figure 1 and Figure 2).

According to the results of the analysis, there was not a reliable agreement between the GFR values calculated with both methods and DTPA GFR values (Table 4).

The subjects were divided into 3 groups based on the ^{99m}Tc DTPA TPSM GFR values: Group 1 (TPSM GFR: 0-15 ml/min/1.73 m²); Group 2 (TPSM GFR: 15-30 ml/min/1.73 m²), and Group 3 (TPSM GFR: 30-60 ml/min/1.73 m²). Bland Altman analysis of the Cys GFR and BTP GFR values of these subjects was used to draw scatter plots of the difference values between ^{99m}Tc DTPA TPSM GFR and Cys GFR value and BTP GFR value within 95% confidence interval (mean $\pm 1.96\text{SD}$), and mean and standard deviations were calculated.

While a statistically significant correlation was found between DTPA GFR and BTP GFR in Group 2 ($r= 0.67$, $p<0.0001$), Bland Altman analysis did not reveal a reliable interval of agreement. Furthermore, while there was a statistically significant correlation between DTPA GFR and Cys GFR in Group 2 and Group 3, there was similarly no reliable interval of agreement ($r=0.82$, $p<0.0001$; $r=0.76$, $p<0.0001$).

The subjects were divided into 2 groups according to serum NAG (IU/l) level, which is a highly sensitive marker of renal tubular injury: NAG level > 6.1 (tubular injury positive) and NAG level ≤ 6.1 (tubular injury negative). Looking at the correlation value between ^{99m}Tc DTPA TPSPM GFR and Cys GFR, the r value was found 0.957 in the patients having a NAG level less than 6.1. The r value in those with a NAG level above 6.1 was 0.887. Both correlation coefficients were significantly different from each other ($p<0.0001$). This suggested that Cys reflected GFR more accurately when urinary NAG level was in normal limits (≤ 6.1), i.e. there was no tubular injury according to NAG level. The correlation between ^{99m}Tc DTPA TPSPM GFR and BTP GFR was similarly analyzed for the two separate groups. The r value was 0.801 for those with a NAG level below 6.1 and 0.694 for those with a NAG level above 6.1. There was a statistically significant difference between both groups ($p<0.0001$) (Table 6). The Bland Altman analysis was used to draw scatter plots of the difference values between ^{99m}Tc DTPA TPSPM GFR values and Cys GFR and BTP GFR values of the subjects divided according to the urine NAG level within 95% confidence intervals ($\text{mean} \pm 1.96\text{SD}$).

Although there was a statistically significant correlation between DTPA GFR and Cys GFR and BTP GFR according to urine NAG level in CKD patients (The highest value was $r=0.957$ for Cys GFR in patients with a urine $\text{NAG} \leq 6.1$), Bland Altman analysis revealed that there was not any reliable interval of agreement within 95% confidence interval.

It has been reported that B2M is also a good marker for renal tubular dysfunction (29). The reference range of urinary B2M is 0.01-0.2 mg/l. We thus divided the study subjects into 2 groups according to B2M values: Group 1 had a urinary B2M level ≤ 0.2 mg/l and Group 2 had a urinary B2M level > 0.2 mg/ml. We studied the correlation between the gold standard method, ^{99m}Tc DTPA TPSPM GFR, and Cys GFR and BTP GFR values for these 2 groups. In the first group r was equal to 0.849 ($p<0.01$) in the correlation assessment with Cys GFR. In Group 2, it was found 0.840 ($p<0.01$) in the correlation analysis made with Cys GFR. These two correlation values were significantly different ($p<0.0001$). The correlation analyses made with BTP GFR

revealed r values of 0.698 ($p < 0.01$) and 0.433 ($p < 0.01$), for Group 1 and Group 2, respectively. There was also a statistically significant difference between the correlation values of the two groups ($p = 0.02$) (Table 7). The Bland Altman analysis was used to draw scatter plots of the difference values between ^{99m}Tc DTPA TPSM GFR values and Cys GFR and BTP GFR values of the subjects divided according to the urine B2M level within 95% confidence intervals ($\text{mean} \pm 1.96\text{SD}$). Although there was a statistically significant correlation between DTPA GFR and Cys GFR and BTP GFR according to urinary B2M level in CKD patients (the highest value was $r = 0.892$ for Cys GFR in patients with a urinary $\text{B2M} \leq 0.2$), Bland Altman analysis revealed that there was not any reliable interval of agreement within 95% confidence interval.

Discussion

A number of previous studies have shown that GFR measurement methods using ^{99m}Tc DTPA or ^{51}Cr -EDTA blood samples for showing renal functions gave similar results with the continuous inulin infusion method (23, 25, 30, 31). Rehling et al. (8), in a study where inulin was accepted as the gold standard, reported a ^{99m}Tc DTPA - C_{in} ratio of 0.97 and showed that the ^{99m}Tc DTPA clearance was fairly correlated to C_{in} . They also reported in the same study that the GFR value measured with ^{99m}Tc DTPA was on average only 3.5 ml/min higher compared to C_{in} . In light of these findings, we also accepted ^{99m}Tc DTPA TPSM as the reference method and compared the GFR values obtained by using Cys and BTP with the GFR values calculated with TPSM in an attempt to investigate whether these methods could also be reliably used in the clinical practice.

Various studies with different patient populations investigated the role of Cys in measurement of GFR (32). However, its use in CKD patients is both limited and debated. Franka et al. (33) reported that in CKD patients Cys gave higher but acceptable GFR values while Maillard et al. (34) and Pöge et al. (35) suggested that Cys should be preferred to serum creatinine measurement.

Coll et al. (32) reported that serum Cys level had a higher sensitivity compared to serum creatinine (93.4% vs. 86.8%) for measurement of GFR. They stressed the importance of Cys for detecting early stage renal failure in mild renal injury. Various studies (36) found that serum creatinine, Cys, and B2M levels showed a very close correlation with ^{51}Cr EDTA clearance (36, 37).

In addition, Oner et al. (38) compared serum creatinine, Cys, and BTP values for the determination of renal function in 89 transplant recipients and showed that the serum Cys value was a better marker for GFR determination compared to BTP.

Rule et al. (39) observed a strong correlation between Cys and GFR values particularly in 204 patients with a renal pathology ($r^2=0.853$). In that study, Cys showed a better correlation than creatinine, but the authors pointed that it is affected by factors such as inflammation and immunosuppression and thus cannot be used as an ideal marker of GFR.

In our study, ^{99m}Tc DTPA TPSM GFR was found 31.9 ± 14.3 and Cys GFR was found 46.8 ± 17.3 . When the correlation between the GFR value calculated with the gold standard ^{99m}Tc DTPA TPSM and with other methods was analyzed, it was found that the highest correlation values in patients with CKD was with Cys GFR value ($r=0.904$, $p<0.001$).

BTP is an alternative method for GFR determination, which has recently been introduced. Various studies have shown that GFR reduction leads to a progressive elevation in BTP level. Some studies have reported that BTP has similar accuracy with serum creatinine, Cys, and B2M in detecting renal dysfunction. Kobata et al. (40) demonstrated that serum BTP is an accurate marker for detection of early-stage renal failure in patients with Type 2 diabetes mellitus. It was also reported that it can be used as an alternative endogenous GFR marker in renal transplant recipients receiving steroid therapy (14). However, Oner et al. (38) reported that BTP did not accurately reflect GFR in cases with renal transplantation.

Pöge et al. (14) studied the role of BTP in reflecting GFR in 187 renal transplant recipients. They accepted the GFR calculated with ^{99m}Tc DTPA GFR method as the gold standard and compared the GFR values calculated with three separate formula using BTP with the GFR values calculated with the re-expressed MDRD formula. They observed that the GFR values calculated with BTP were not significantly superior to the GFR values calculated with the re-expressed MDRD formula.

In our study, on the other hand, there was a statistically significant correlation between the gold standard method ^{99m}Tc DTPA TPSM and the Cys GFR ($r=0.904$, $p<0.001$) and that correlation was better than that observed with BTP GFR ($r=0.725$, $p<0.001$). However, the Bland-Altman analysis revealed no acceptable agreement between ^{99m}Tc DTPA TPSM GFR and Cys GFR or BTP GFR within a 95% confidence interval.

After grouping the subjects according to their GFR values (Group 1, 2, 3) we observed that there was no statistically significant correlation between DTPA GFR and Cys GFR in Group 1, while Group 2 ($r=0.821$, $p<0.0001$) and Group 3 ($r=0.764$, $p<0.0001$) showed statistically significant correlations for the parameters in question. Only Group 2 ($r=0.671$, $p<0.0001$) showed a statistically significant correlation between ^{99m}Tc DTPA TPSM GFR and BTP GFR, albeit to a lower degree compared to Cys.

Since Bland-Altman analysis in each of the three groups revealed that the lower and upper limits of the mean difference $\pm 1.96\text{SD}$ between ^{99m}Tc DTPA TPSM GFR and Cys GFR or BTP GFR were not within the acceptable limits within the 95% confidence interval, we suggest that these parameters are not suitable for clinical use.

In the present study we found that Cys showed a better correlation with ^{99m}Tc DTPA TPSM GFR compared to BTP in both the entire study population and in the subgroups (Group 1,2,3) created by the severity of renal dysfunction. However, the GFR values calculated with both methods had no acceptable agreement with ^{99m}Tc DTPA TPSM GFR. Under the light of these results, we believe that Cys and BTP are not suited for determination of renal functions in patients with CKD.

We also grouped the study subjects based on urinary NAG and B2M values, which are the markers of tubular injury. The correlation value between ^{99m}Tc DTPA TPSM GFR and Cys GFR was analyzed and r value was found 0.957 in patients with a NAG normal level (≤ 6.1). In the subjects with elevated urinary NAG level (>6.1), on the other hand, the r value was 0.887. These two coefficients were significantly different ($p<0.0001$), suggesting that Cys reflects GFR more accurately when urinary NAG level is within normal limits, i.e. there is no tubular injury. Nevertheless, Bland-Altman analysis revealed no acceptable agreement between ^{99m}Tc DTPA TPSM GFR and Cys GFR within 95% confidence interval. Bland-Altman analysis similarly showed no significant agreement between ^{99m}Tc DTPA TPSM GFR and BTP GFR within 95% confidence interval, although the two GFR values were significantly, albeit less than Cys, correlated to each other. Comparison of the subgroups created by the urinary B2M level demonstrated that there was a statistically significant correlation between ^{99m}Tc DTPA TPSM GFR and Cys GFR and BTP GFR in all cases with a normal or high B2M level, although Bland-Altman analysis showed no agreement within 95% confidence interval.

Conclusion

In the literature, there are no studies with respect to the comparison between ^{99m}Tc DTPA TPSM GFR with the Cys GFR or BTP GFR values in patients with CKD. In this regard, our study is the first to do this comparison. We found that Cys GFR and BTP GFR showed a high correlation with the gold standard method ^{99m}Tc DTPA TPSM GFR in patients with CKD who had varying degrees of renal dysfunction.

No studies so far has studied the extent to which the gold standard ^{99m}Tc DTPA TPSM GFR and Cys or BTP are affected by the degree of renal tubular injury in reflecting GFR. We grouped the study subjects according to urinary levels of NAG and B2M, which are the markers of renal tubular injury. We observed that when NAG levels were within normal limits, ^{99m}Tc DTPA TPSM GFR and the GFR values calculated with Cys and BTP showed a high correlation. However, the GFR values calculated with either method showed no acceptable agreement with the GFR values calculated with the ^{99m}Tc DTPA TPSM. Under the light of the above data, we suggest that Cys and BTP do not accurately reflect accurate GFR in CKD patients with or without tubular injury, and therefore the gold standard ^{99m}Tc DTPA TPSM should be used for determination of renal function in patients with all stages of CKD.

In conclusion, we demonstrated that Cys and BTP cannot accurately measure GFR, an important marker for monitoring patients with CKD, although they are able to suggest impaired renal function, and that they are therefore not suitable markers of renal injury in subjects with or without renal tubular injury.

Acknowledgement

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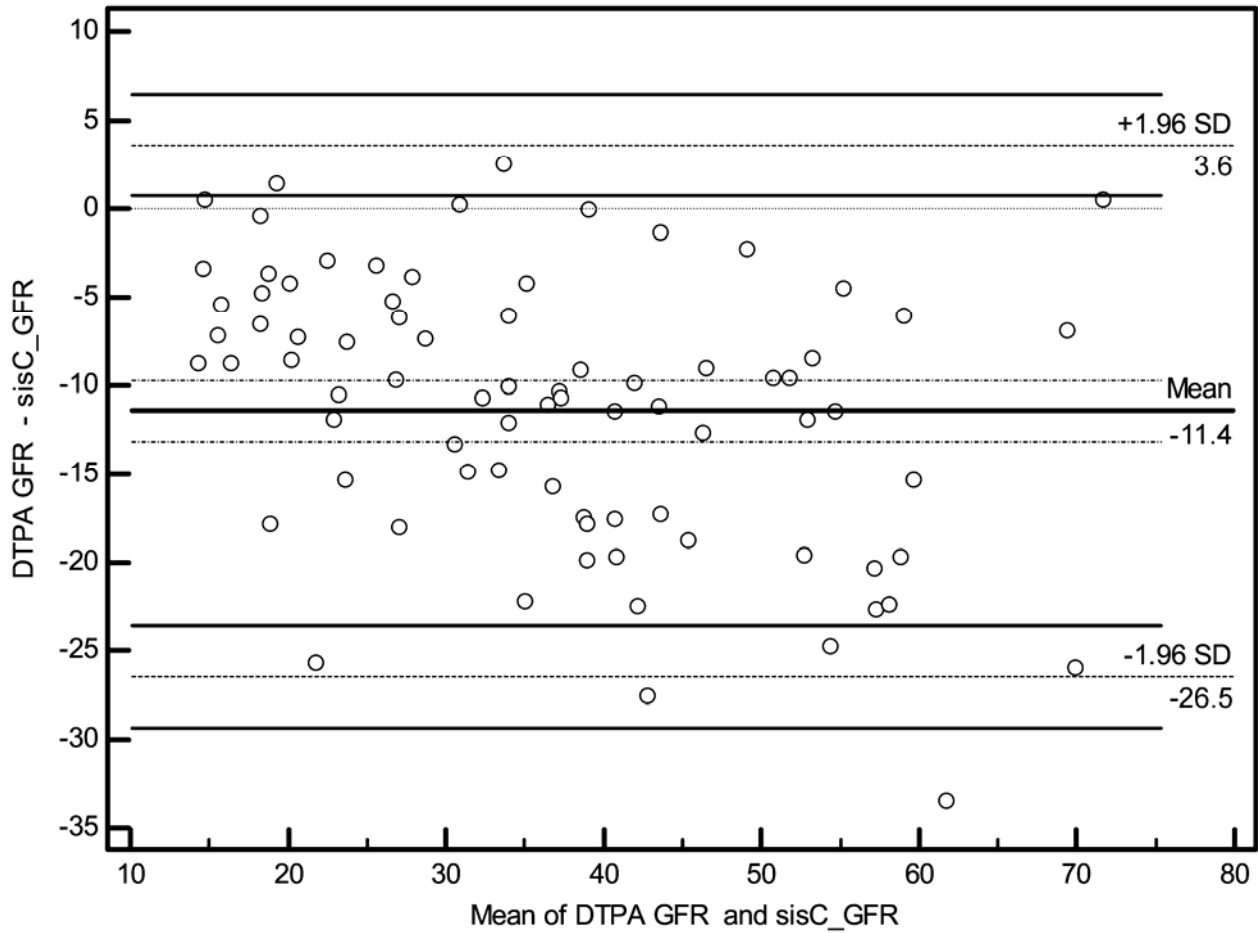


Figure 1. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and Cys C GFR values at a confidence level of 95%. Cys C, cystatin C; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentaacetic acid.

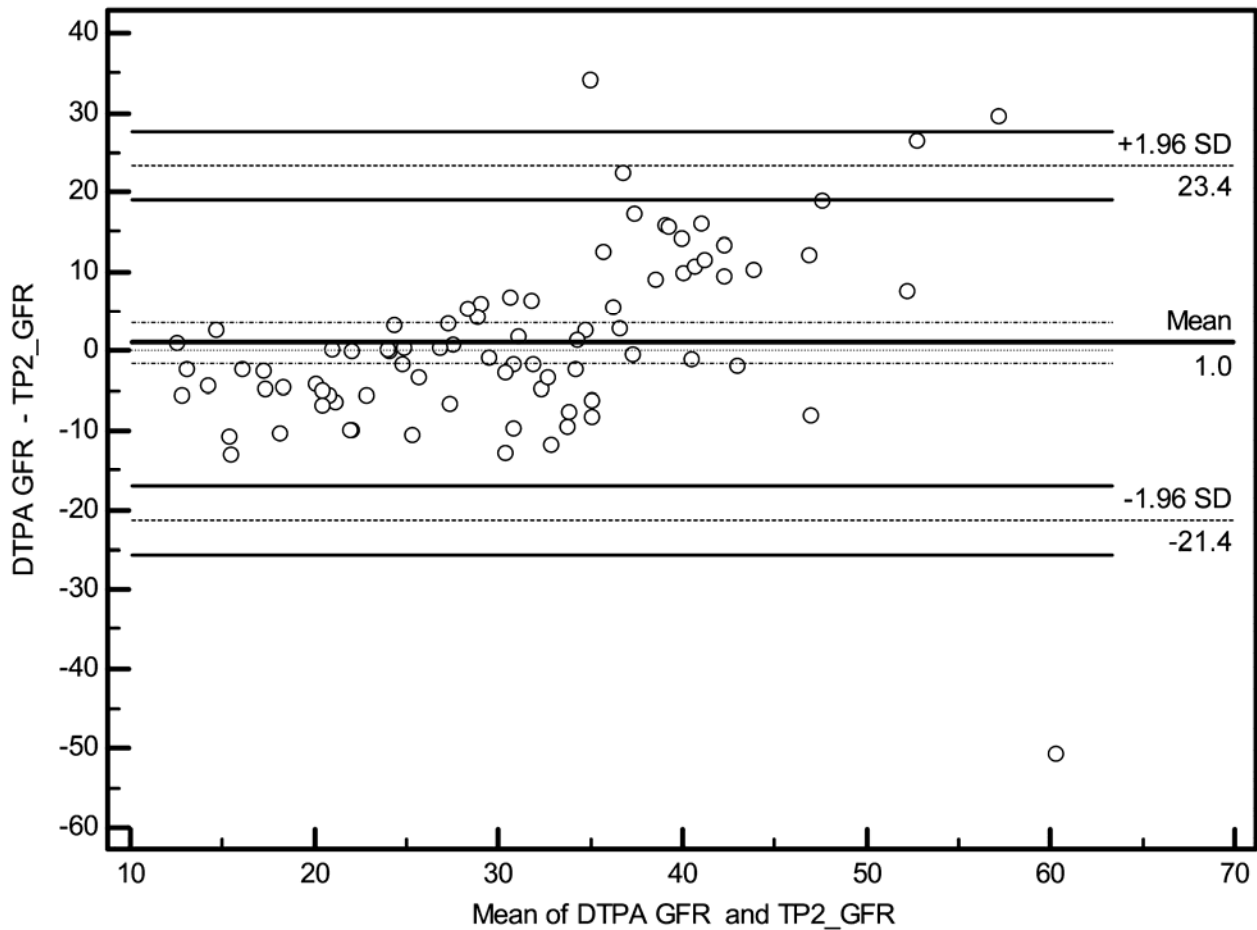


Figure 2. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and BTP GFR values at a confidence level of 95%. BTP, beta-trace protein; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentaacetic acid.

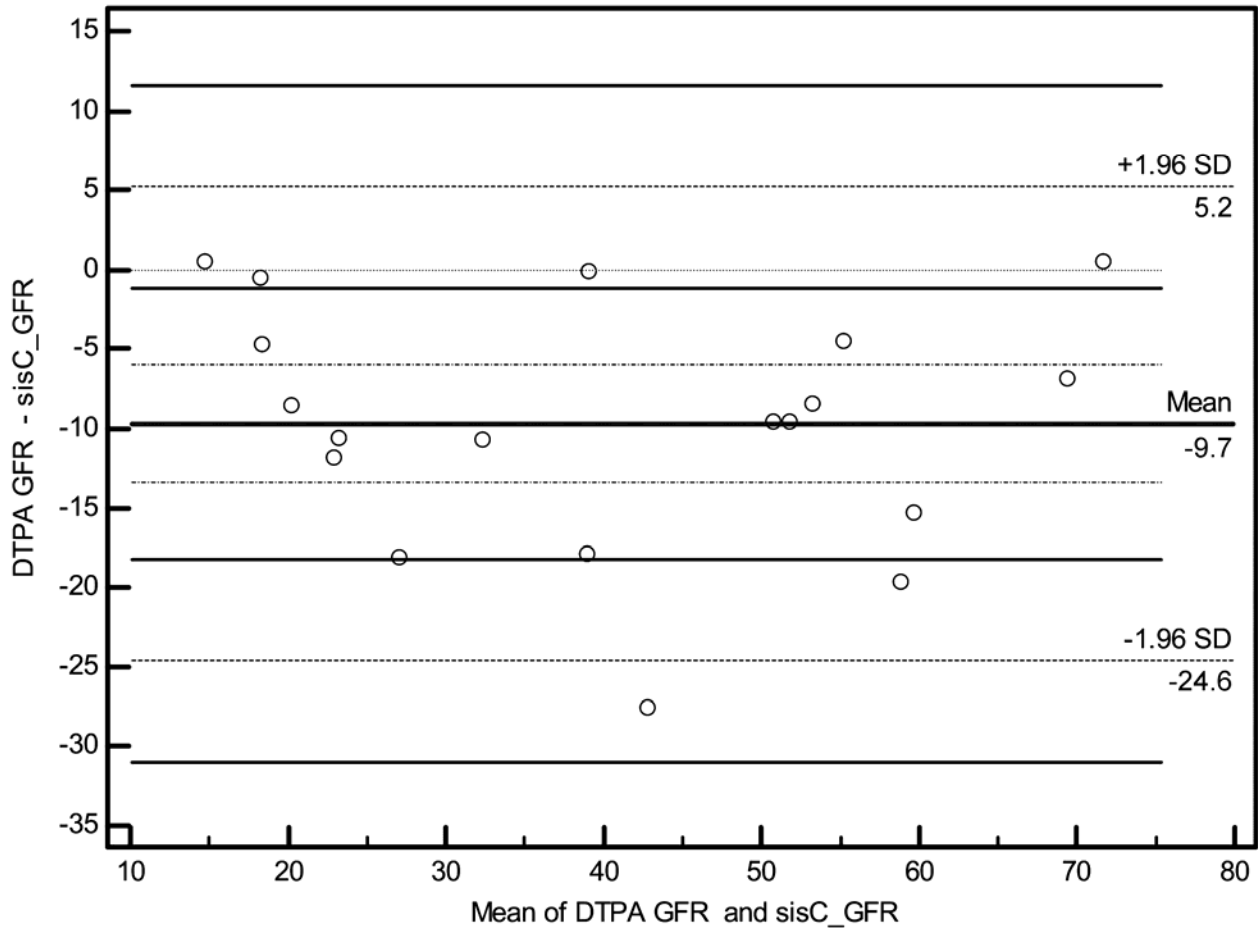


Figure 3a. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and Cys C GFR values, urine NAG(IU/L)≤6.1, at a confidence level of 95%. Cys C, cystatin C; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentaacetic acid; NAG, N-acetyl-b-D-glucosaminidase.

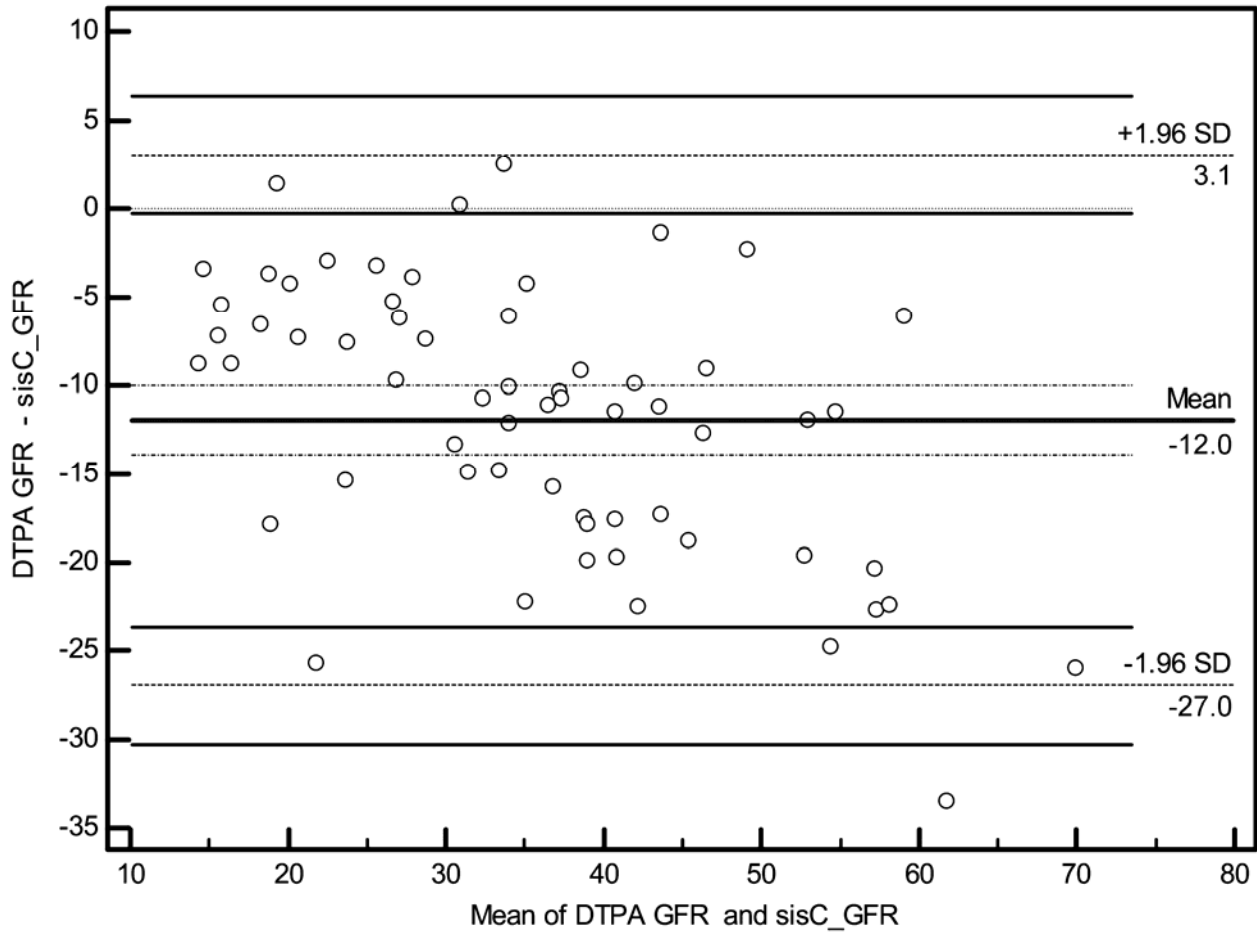


Figure 3b. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and Cys C GFR values, urine NAG(IU/L)>6.1, at a confidence level of 95%. Cys C, cystatin C; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentaacetic acid; NAG, N-acetyl-b-D-glucosaminidase.

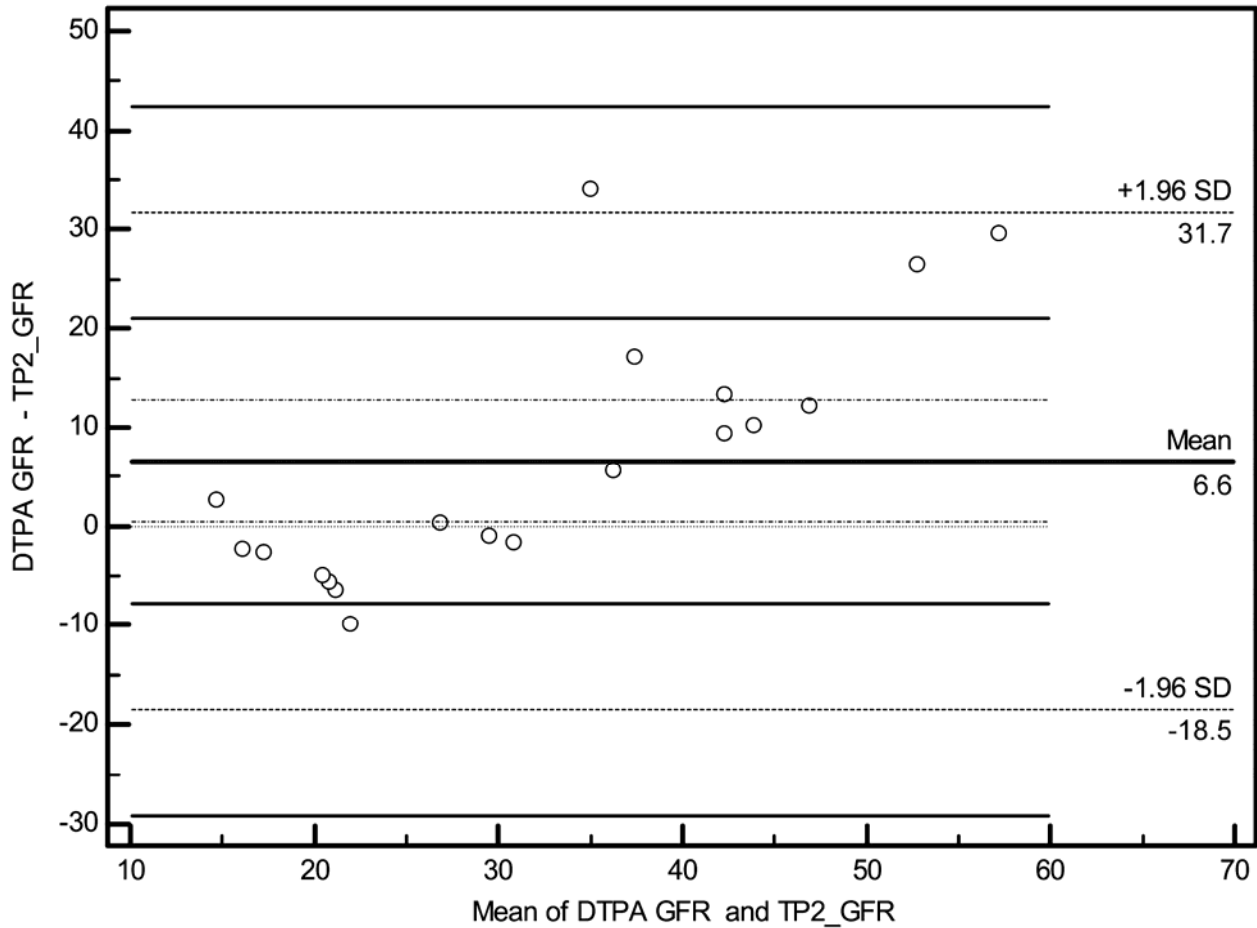


Figure 4a. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc-99m GFR and BTP GFR values, urine NAG(IU/L)≤6.1, at a confidence level of 95% BTP, beta-trace protein; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentacetic acid; NAG, N-acetyl-b-D-glucosaminidase.

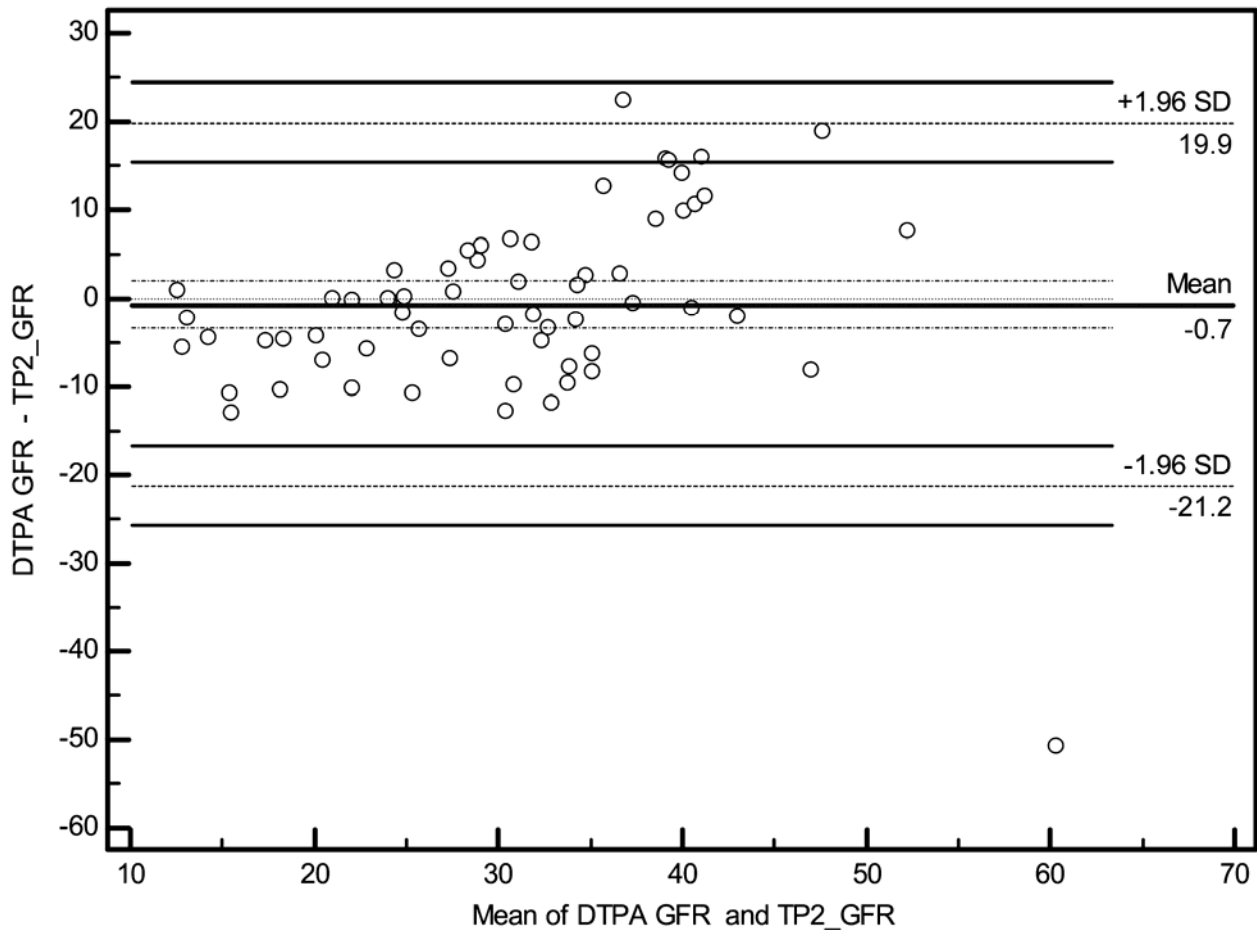


Figure 4b. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and BTP GFR values, urine NAG(IU/L)>6.1, at a confidence level of 95%. BTP, beta-trace protein; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentacetic acid; NAG, N-acetyl-b-D-glucosaminidase.

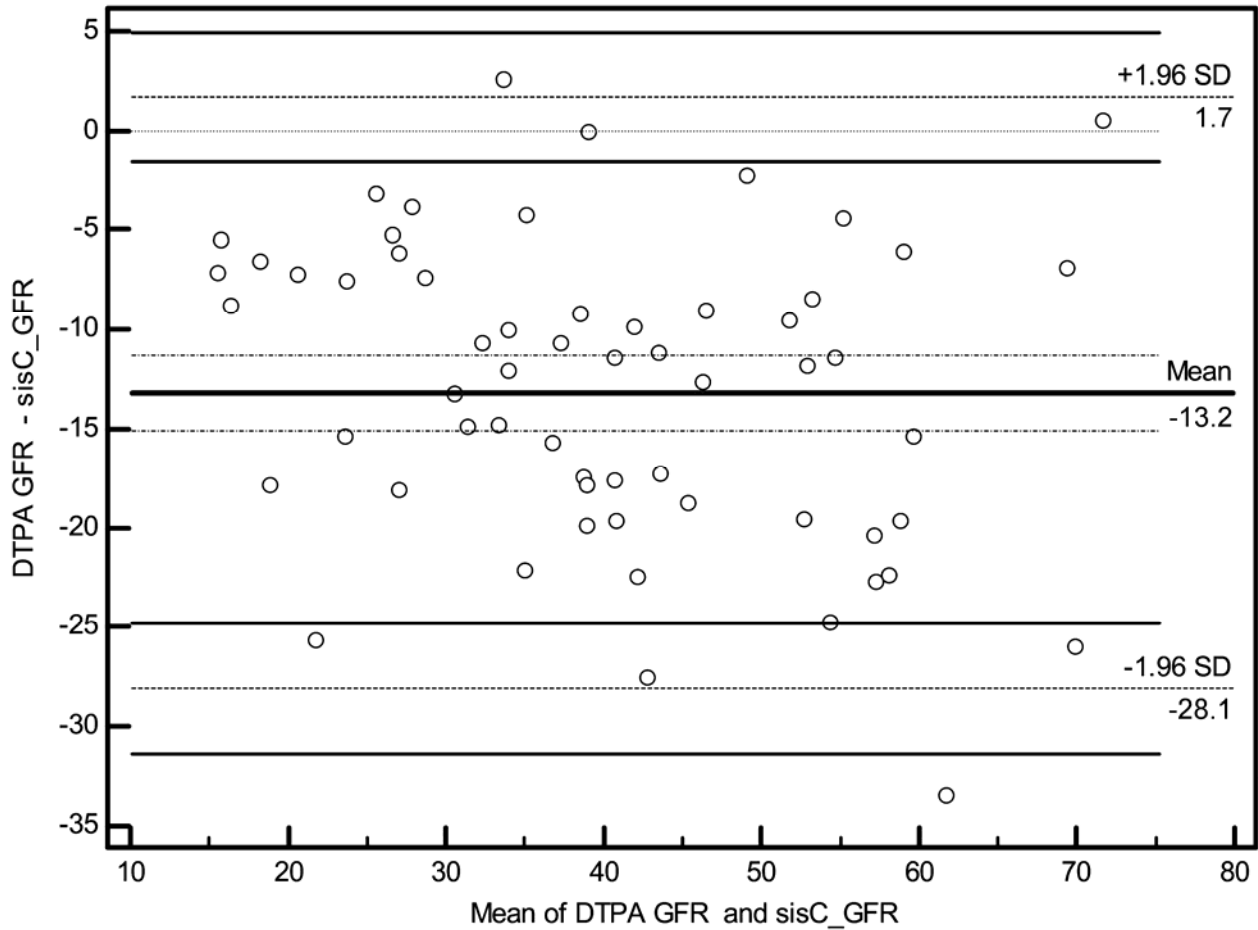


Figure 5a. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and Cys C GFR values, B2M(Urine) ≤ 0.2 (mg/l), at a confidence level of 95%. Cys C, cystatin C; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentacetic acid; B2M, $\beta 2$ microglobulin.

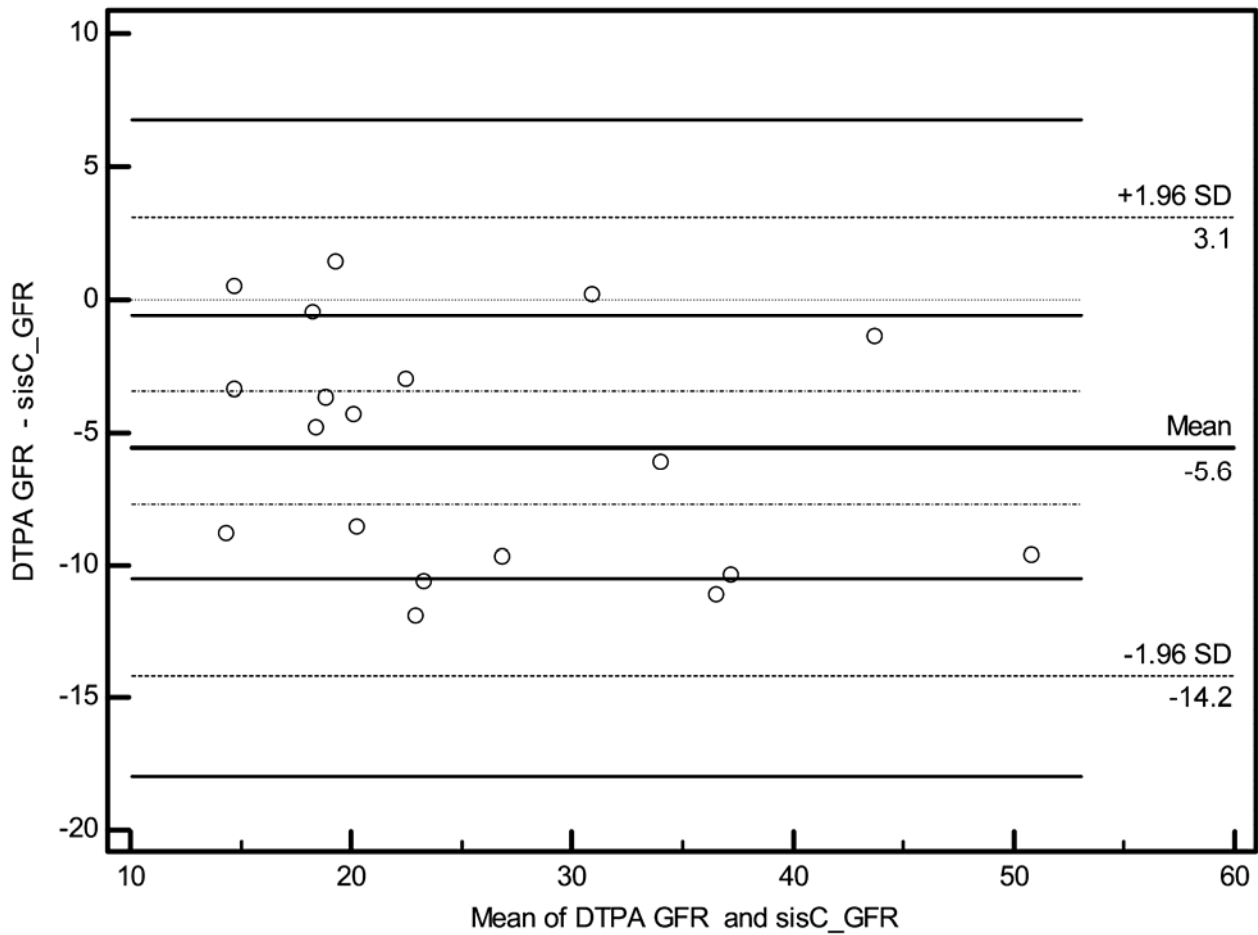


Figure 5b. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and Cys C GFR values, B2M(Urine)>0.2 (mg/l), at a confidence level of 95%. Cys C, cystatin C; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentaacetic acid; B2M, β 2 microglobulin.

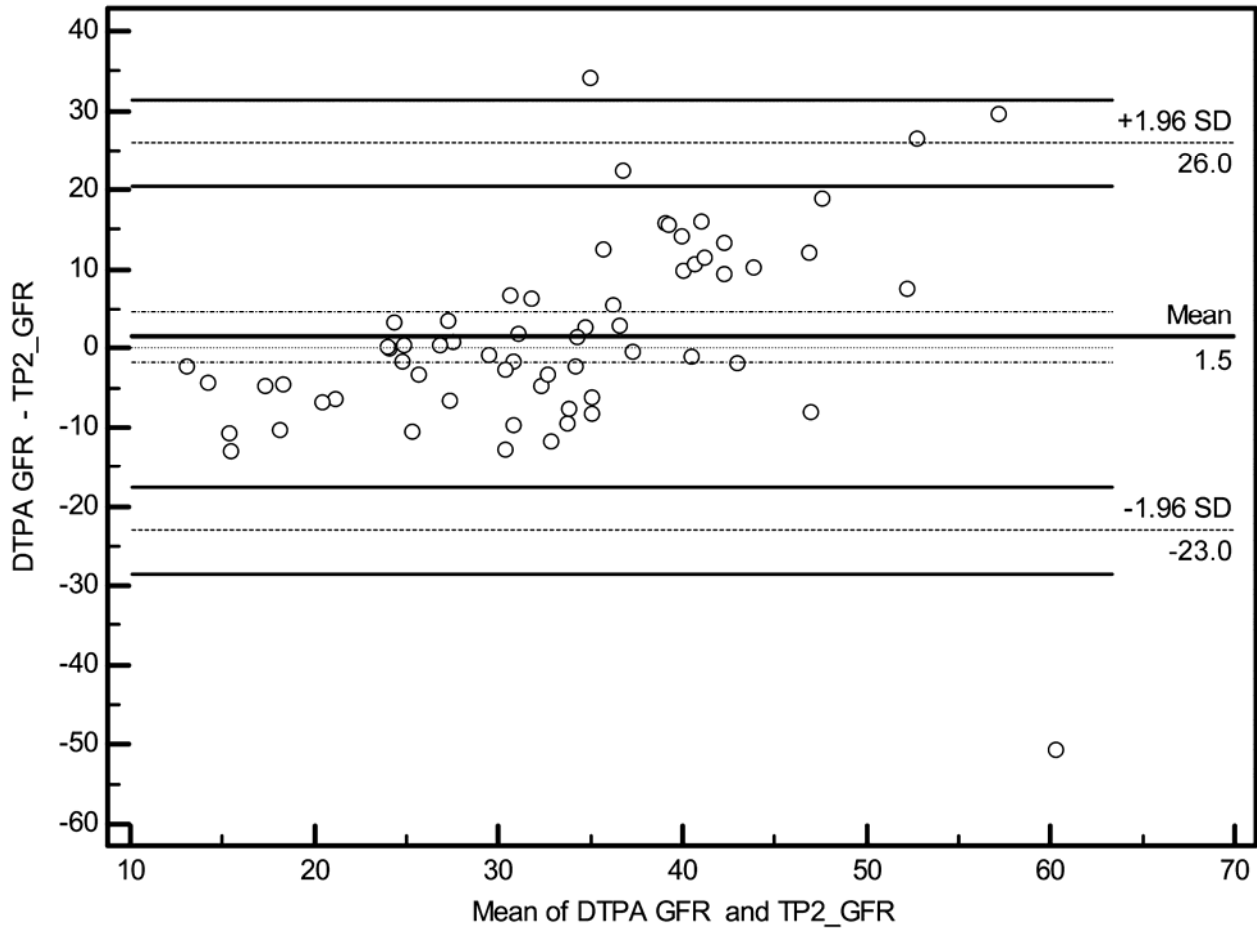


Figure 6a. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and BTP GFR values, B2M(Urine) \leq 0.2 (mg/l), at a confidence level of 95%. BTP, beta-trace protein; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentacetic acid; B2M, β 2 microglobulin.

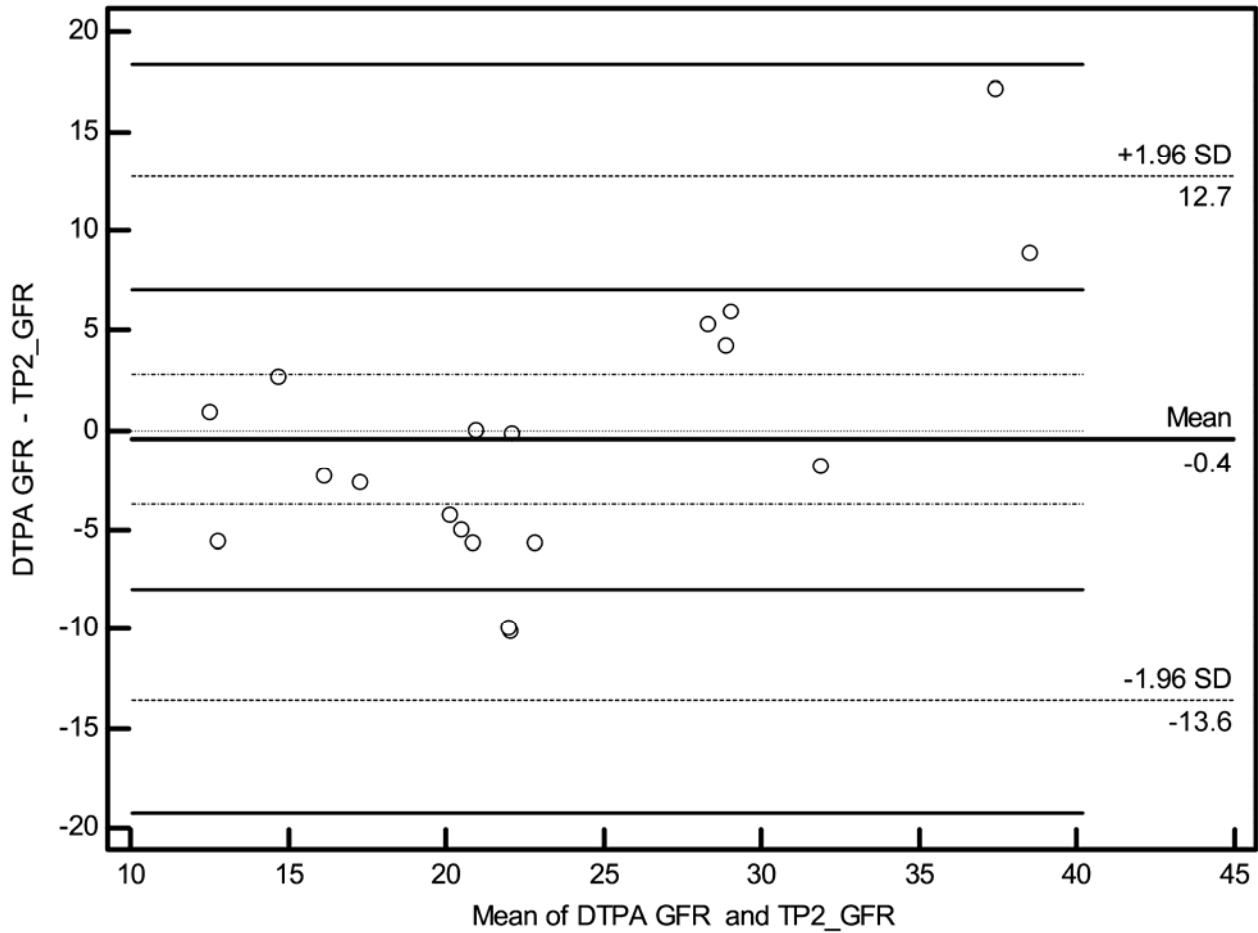


Figure 6b. Scatter plot drawn using Bland-Altman analysis showing difference values between 99m-Tc DTPA GFR and BTP GFR values, B2M(Urine) >0.2 (mg/l), at a confidence level of 95%. BTP, beta-trace protein; GFR, glomerular filtration rate; 99m-Tc DTPA, technetium-99m diethylenetriamine pentacetic acid; B2M, β 2 microglobulin.

Table 1. Mean, SD, minimum, and maximum values of NAG, B2M, Cys C, BTP, creatinine, urea, Na, phosphorus, FT4 and TSH; GFR values calculated with BTP levels; GFR values calculated with Cys C; and GFR values calculated with (ml/min/1.73m²) calculated with Tc-99m DTPA

	N	min	max	mean	± SD
Tc-99m DTPA GFR	84	9	72	31.91	14.28
URINE B2M	84	0.2	28.2	2.58	5.86
URINE CysC	84	0.23	1.45	0.35	0.29
SERUM B2M	84	0.8	15.7	6.60	3.69
SERUM CysC	84	0.21	4.28	1.92	0.81
S.Creatinine	83	1.02	79.72	3.16	8.58
S. Na	84	118	145	135.76	4.77
S. Phosphorus	84	2.15	32.39	3.95	3.19
S. BUN	84	12	225	36.04	25.77
U. Creatinine	84	1.5	795.3	95.65	93.63
U. Na	84	8	193	58.70	33.32
U. Phosphorus	84	2.31	69.63	27.65	16.66
U. T. Protein	84	1.4	578.4	51.80	93.03
Urine NAG (IU/L)	84	0.30	49.25	12.06	8.57
BTP (ng/mL)	84	49	4880	2108.51	920.57
Serum FT4	84	0.5	1.5	1.24	0.21
TSH	84	0.2	33.760	1.86	3.71

B2M, β 2 microglobulin; BTP, beta-trace protein; Cys C, cystatin C; GFR, glomerular filtration rate; NAG, N-acetyl-b-D-glucosaminidase; Tc-99m DTPA, technetium-99m diethylenetriamine pentaacetic acid.

Table 2. P and r values provided by correlation comparisons between the GFR values calculated with Tc-99m DTPA and BTP, B2M, Cys C, Creatinine

N=89		BTP (mg/l)	B2M (mg/l) (serum)	Cys C (mg/l) (serum)	Creatinine (mg/dl)(serum)
DTPA GFR	r=	-0.090	-0.762	-0.797	-0.033
	p=	0.417	<0.0001	<0.0001	0.769

BTP, beta-trace protein; Cys C, cystatin C; GFR, glomerular filtration rate; 99mTc-DTPA, technetium-99m diethylenetriamine pentaacetic acid.

Table 3. P and r values provided by correlation comparisons between Tc-99m DTPA GFR values and BTP GFR and Cys C GFR in Group 1, 2, and 3

DTPA GFR		BTP GFR	Cys C GFR
Group1 (0-15 ml/min/1.73 m ²)	r	-0.200	-0.683
	P	0.606	0.042
	N	9	9
Group2 (15-30 ml/min/1.73 m ²)	r	0.671**	0.821**
	P	0.000	0.000
	N	33	33
Group2 (30-60 ml/min/1.73 m ²)	r	0.201	0.764**
	P	0.227	0.000
	N	38	38

*=statistically significant

BTP, beta-trace protein; Cys C, cystatin C; GFR, glomerular filtration rate; Tc-99m DTPA, technetium-99m diethylenetriamine pentaacetic acid.

Table 4. Limits of agreement, SD, and mean difference values between Tc-99m DTPA GFR and Cys C GFR and BTP GFR with Bland–Altman analysis

	Limits of Agreement		Standard deviation	Mean difference
	d±1.96*Standard deviation (within 95% confidence interval)			
Cys C GFR - DTPA GFR	-26.5	3.6	7.7	-11.4
BTP GFR - DTPA GFR	-21.4	23.4	11.4	1.0

BTP, beta-trace protein; Cys C, cystatin C; GFR, glomerular filtration rate; Tc-99m DTPA, technetium-99m diethylenetriamine pentaacetic acid.

Table 5. Limits of agreement, SD, and mean difference values between Tc-99m DTPA GFR and Cys C GFR and BTP GFR in Group 1, 2, and 3 with Bland–Altman analysis

	Limits of Agreement $d \pm 1.96 * \text{Standard Deviation}$ (confidence interval of 95%)		Standard deviation	Mean difference
Cys C GFR - DTPA GFR (Group 1)	-24.8	6.2	7.9	9.3
Cys C GFR - DTPA GFR (Group 2)	-23.8	2.3	6.5	-10.8
Cys C GFR - DTPA GFR (Group 3)	-29.2	3.3	8.3	-12.9
BTP GFR - DTPA GFR (Group 1)	-14.9	3.2	4.6	-5.9
BTP GFR - DTPA GFR (Group 2)	-13.0	4.8	4.5	-4.1
BTP GFR - DTPA GFR (Group 3)	-19.5	30.9	12.9	5.7

BTP, beta-trace protein; Cys C, cystatin C; GFR, glomerular filtration rate; Tc-99m DTPA, technetium-99m diethylenetriamine pentaacetic acid

Table 6. P and r values provided by correlation comparisons between Tc-99m DTPA GFR values and BTP GFR and Cys C GFR in NAG \leq 6.1(IU/L) and NAG $>$ 6.1(IU/L)

		NAG \leq 6.1(IU/L) (N=19)	NAG $>$ 6.1(IU/L) (N=63)	p
		DTPA GFR	DTPA GFR	
Cys C GFR	r=	0.957	0.887	0.0001
	p=	<0.0001	<0.0001	
BTP GFR	r=	0.801	0.694	0.0001
	p=	<0.0001	<0.0001	

BTP, beta-trace protein; Cys C, cystatin C; GFR, glomerular filtration rate; Tc-99m DTPA, technetium-99m diethylenetriamine pentaacetic acid; NAG, N-acetyl-b-D-glucosaminidase

Table 7. P and r values provided by correlation comparisons between Tc-99m DTPA GFR values and BTP GFR and Cys C GFR in B2M(Urine) \leq 0.2 (mg/l) and B2M(Urine) $>$ 0.2 (mg/l)

		B2M(Urine) \leq 0.2 (mg/l) (N=19)	B2M(Urine) $>$ 0.2 (mg/l) (N=63)	p
		DTPA GFR	DTPA GFR	
Cys C GFR (Hoek)	r= p=	0.892 <0.0001	0.839 <0.01	0.0001
BTP GFR 2	r= p=	0.626 <0.0001	0.722 <0.0001	0.02

BTP, beta-trace protein; Cys C, cystatin C; GFR, glomerular filtration rate; Tc-99m DTPA, technetium-99m diethylenetriamine pentaacetic acid; B2M, β 2 microglobulin