Creatinine, cystatin, and combined-based equations in assessment of renal functions in type 2 diabetic Egyptian patients

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Background
Diabetic nephropathy is the principal single cause of end-stage renal disease. The most important parameter in the clinical evaluation of kidney function is the glomerular filtration rate (GFR), which is generally accepted as the best overall index of kidney function; GFR remains the cornerstone of the clinical evaluation of overall kidney function. Our study was performed to compare between estimated GFR equations based on serum creatinine and/or cystatin C performance in relation to measured GFR using radionuclide study and degree of proteinuria.
Patients and methods
In our cross-sectional study, 80 adult type 2 diabetic patients, with diabetic nephropathy and proteinuria more than 300 mg/24 h, were included after application of inclusion and exclusion criteria, and subjected to history taking, clinical examination, and laboratory investigation including serum creatinine, cystatin C, 24-h urinary protein/creatinine clearance, and renal isotope technetium-99m-diethylene triamine pentaacetic acid scanning.
Results
There was a linear correlation between serum creatinine and cystatin C (r=0.867, P=0.000). Cystatin C was better correlated (r=–0.781, P=0.000) with isotopically measured GFR than creatinine (r=–0.106, P=0.348). Cystatin C was better than creatinine in all estimated GFR equations tested in our study [Modification of Diet in Renal Disease, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Cr 2009, CKD-EPI Cr-Cys 2012, CKD-EPI Cys 2012]. The best performance among all equations tested when compared with isotopically measured GFR was the CKD-EPI Cr-Cyst 2012 (r=0.816, P=0.000). Cystatin C showed a significant negative correlation with hemoglobin level, a finding that could not be established with serum creatinine; there was no significant association of creatinine or cystatin with the level of proteinuria.
Conclusion
In patients with early overt diabetic nephropathy, serum cystatin C showed a significantly stronger correlation than creatinine with isotopically measured GFR, and among the studied equations for GFR estimation the CKD-EPI Cr-Cyst 2012 equation performed best.

Keywords:
cystatin C, diabetic nephropathy, estimated glomerular filtration rate equations, glomerular filtration rate, type 2 diabetes

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Introduction

Diabetic nephropathy is the most common disorder leading to end-stage renal disease (ESRD) in adults. The mortality rate of patients with diabetic nephropathy is high [1,2].

Glomerular filtration rate (GFR) is defined as the clearance of a substance in the plasma, which is exclusively metabolized by the kidneys and freely filtered by the glomeruli.

Current gold-standard methods for determining GFR use the clearance of exogenous radioisotopes such as Cr-EDTA or nonradiolabeled markers such as inulin [3]. Serum creatinine is considered relatively specific, but not very sensitive, as its levels significantly increase only when more than 50% of the GFR is reduced. To overcome the limitations of using creatinine alone, equations to estimate glomerular filtration rate (eGFR) based on serum creatinine have been developed that include variables such as age, sex, race, and measurements of body size [3].

Cystatin C is produced at a constant rate with a half-life of 2 h, freely filtered by the renal glomeruli, metabolized by proximal tubule, and identified as a promising marker of renal failure. Its concentration is almost totally dependent on GFR, and is independent of height, sex, age, and muscle mass [4].

Aim

There are no clear data from local Egyptian studies comparing serum cystatin C and serum creatinine levels in type 2 diabetic patients with overt diabetic nephropathy (proteinuria>300 mg/24 h). Hence, this study was conducted to determine whether serum cystatin C is a better marker of GFR when compared with serum creatinine in Egyptian type 2 diabetic patients with established diabetic nephropathy and elevated plasma creatinine up to 3 mg/dl.

Patients and methods

This study included 80 patients; the patients were chosen from Kasr El-Aini Hospital with type II diabetic nephropathy known to have renal impairment. After taking a written consent, and approval was obtained from the local ethical committee every patient in this study was subjected to the following: history taking with special stress on diabetes mellitus, the drug(s) used for its treatment, and other complication(s) if present; thorough examination; and laboratory tests including complete blood count, thyroid-stimulating hormone, urine analysis, 24 h urinary proteins, creatinine clearance, alanine aminotransferase, aspartate aminotransferase, albumin, uric acid, lipid profile, serum creatinine, and cystatin C.

Exclusion criteria

Patients with active urinary tract infections, type I diabetes mellitus, decompensated heart failure, acute kidney injury, uncontrolled thyroid disease, serum creatinine above 3 mg/dl, renal impairment because of other causes, uncontrolled blood pressure (>140/90), and chronic liver disease (Child B–C) were excluded from the study.

Estimation of glomerular filtration rate

Renal isotope scanning: This was performed for the estimation of GFR, according to the technetium-99m-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) radionuclide study [5].

Patient preparation (hydration): The patient was made to drink 300–500 ml of water, and was voided before the beginning of the study.

Instrumentation (gamma camera): The large field of view gamma camera (Phillips) was used. The patient was positioned before viewing with the gamma camera. Low-energy all-purpose parallel whole collimator was used. Acquisition parameters were as follows: a computer setup of a preprogrammed study and 64×64 matrixes for a 20-cm field of view.

Patient position: The patient was made to lie in a supine position for posterior imaging.

Radiopharmaceutical: A measure of 3–5 μCi of ^{99m}Tc-DTPA was given intravenously in a bolus form.

Computer acquisition: The following acquisition settings were used: 1 s frames×60, and then 30 s frames for 5 min.

Image processing was carried out by drawing the region of interest on the computer around the kidneys and background. The GFR (global and differential) is calculated by a closed computer program based on Gate's method (normal range: 80–130 ml) [6].

Estimation of cystatin C serum concentration

Sampling: A volume of 2 ml of whole-blood sample was taken through peripheral vein from each patient under complete resting conditions and pooled into a dry tube.

Fasting status is not mandatory for performing the test. Samples were centrifuged at 3400 rpm, and resulting sera were separated and the procedure was initiated.

Kit description: A kit manufactured by Dade Behring Diagnostics was used for the analysis.

Assay: The procedure was as follows: pipette 100 μl of each diluted standard concentration, diluted quality controls, diluted samples, and dilution buffer, preferably in duplicate, into the appropriate wells. Incubate the plate at room temperature for 30 min, with shaking at about 300 rpm on the orbital microplate shaker.

Wash the wells three times with the wash solution. Invert the plate and blot it against paper towels to remove the remaining wash solution.

Add 100 μl of conjugate solution into each well.

Incubate the plate at room temperature for 30 min, with shaking at about 300 rpm on the orbital shaker. The incubation time must be increased to 90 min when performed without shaking. Wash the wells

with the wash solution three times. Invert the plate and blot it.

Add 10 µl of substrate solution. Protect it from light.

Incubate the plate for 10 min at room temperature.

Stop the color development by adding 100 µl of stop solution.

Determine optical density in the plate by reading absorbencies at 450 nm.

Measurements: All results of cystatin C serum level were calculated in mg/l; the normal range of serum cystatin C is 0.6–1.2 mg/l.

The following equation by Filler *et al.*, 2003, was used to measure GFR from cystatin C: log eGFR=1.962 +[1.123×log {1/cystatin C (mg/l)}].

Estimation of serum creatinine

Serum creatinine was measured by the kinetic colorimetric method. This method is based on the following principle: creatinine reacts with alkaline picrate to give a color complex that can be read at 510 nm. The rate of color development is proportional to the creatinine concentration in the sample using kinetic photometric equipment.

Equation used to estimate glomerular filtration rate

Cockcroft–Gault equation:

140−age (years)×weight (kg)/72×serum creatinine (mg/dl) (×0.85 in female).

Ab-MDRD:

Male: 186×serum creatinine−1.154×age−0.203 (×1.212 for Black).

Female: correction factor=0.742.

CKD-EPI Cr 2009 equation:

141×minimum (serum creatinine/*k*, 1)×*a*×maximum (serum creatinine/*k*, 1)×1.209×0.993 age (×81.018 if female) (×1.159 if Black),

where *k* is 0.7 for female and 0.9 for male; *a* is −0.329 for female and −0.411 for male.

CKD-EPI Cr-Cyst 2012 equation:

135×minimum (serum creatinine/*k*, 1)×*a*×maximum (serum creatinine/*k*, 1)×0.601×minimum (serum creatinine/0.8, 1)−0.375×maximum (serum creatinine/0.8, 1)−0.711×0.995 age (×0.969 if female) (×1.08 if Black).

where *k* is 0.7 for female and 0.9 for male; *a* is −0.248 for female and −0.207 for male. Min indicate the minimum of Scr/k or 1 and max indicate the maximum of Scr/K or 1

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences version 24 software package program. To assess the performance of formulae, eGFR results were compared with isotopic GFR by means of two-tailed, paired, and unpaired *t*-tests (confirmed by nonparametric equivalents for non-normal distributions as appropriate), and by Levene's test for equality of variance. Results are expressed as the mean±2 SD. Correlations between variables are expressed by Pearson's coefficient. Regression analysis was performed to check the predictive power of serum creatinine and serum cystatin C compared with isotopic GFR. *P* value less than 0.05 was taken to indicate statistical significance.

Results

Our study was performed to compare between eGFR equations based on serum creatinine and/or cystatin C performance in relation to measured GFR using radionuclide study and degree of proteinuria. In our cross-sectional study, a linear correlation between serum creatinine and cystatin C (r=0.867 and P=0.000) was shown. Cystatin C was better correlated (r=−0.781, P=0.000) with isotopically measured GFR than creatinine (r=−0.106, P=0.348). In addition, the performance of cystatin C was better than that of creatinine in all eGFR equations tested in our study [Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Cr 2009, CKD-EPI Cr-Cys 2012, CKD-EPI Cys 2012].

The best performance among all equation tested when compared with isotopically measured GFR was the CKD-EPI Cr-Cyst 2012 (r=0.816, P=0.000). Cystatin C showed a significant negative correlation with hemoglobin level, which could not be established with serum creatinine; there was no significant association of either creatinine or cystatin with the level of proteinuria.

From the above-mentioned results, we concluded that, among patients with early overt diabetic nephropathy,

serum cystatin C showed a significantly stronger correlation than serum creatinine with isotopically measured GFR, and among studied equations for GFR estimation the best performance was the CKD-EPI combined creatinine–cystatin 2012 equation.

Discussion

GFR prediction is widely used to screen for chronic kidney disease, especially in high-risk groups such as persons with diabetes. Strong evidence supports the need for early detection of diabetic nephropathy, when timely intervention can improve long-term outcome [7].

Our results showed a linear correlation between serum creatinine and cystatin C (r=0.867 and P=0.000). Randers *et al.* [8] studied serum cystatin C, serum and urine creatinine, and GFR by ^{99m}Tc-DTPA clearance technique in 76 patients with various kidney diseases and normal serum creatinine, and 61 dialysis patients. They found a significant linear relationship between serum level of cystatin C and creatinine in those with GFR higher than 30 ml/min [8].

Furthermore, the small size of cystatin C may make it dialyzable and possibly a marker for middle molecular size toxin removal, as Al-Malki *et al.* [9] postulated. They showed that the mean level of serum cystatin C in those on chronic dialysis was influenced by the method and intensity of dialysis. They mentioned that it may be able to have some role in monitoring the adequacy of dialysis.

In our study, the results showed that cystatin C (r=–0.781, P=0.000) is better correlated with isotopically measured GFR than creatinine (r=–0.106, P=0.348). Also the performance of Cystatin C was better than creatinine in all eGFR equations tested in our study (MDRD, CKD-EPI Cr 2009, CKD-EPI Cr-Cys 2012, CKD-EPI Cys 2012). The performance of cystatin C-based equations in our cohort is consistent with that of others who have demonstrated the superiority of cystatin C over other methods of estimating GFR [10,11].

The methodology of development of a reliable equation for GFR estimation is a complex process influenced by many factors such as number of studied population, ethnicity, age, stage of chronic kidney disease, presence and degree of proteinuria, and associated comorbid conditions. In our study, we compared the performance of the two serological markers creatinine and cystatin in the most widely accepted and validated equations, recommended by the National Kidney Foundation. The Cockcroft–Gault equation, one of the most popular and usable equations, was published in 1976 and was widely adopted for estimation of creatinine clearance from serum creatinine levels.

There are some concerns and limitations; the high-risk patients who were used to develop the Cockcroft–Gault equation had lower muscle mass (creatinine excretion) compared with healthier individuals in the general population.

The lack of a standardized serum creatinine assay has also been considered a problem with the Cockcroft–Gault equation, but this is not the case. Serum creatinine assay calibration has no influence on the coefficients of the Cockcroft–Gault equation, because the regression did not involve serum creatinine. Because the Cockcroft–Gault equation was developed using only white men, however, the model was not optimized to account for sex and race differences in muscle mass. Nonetheless, the Cockcroft–Gault equation is still used widely particularly for drug dosing, for which estimates in ml/min units are desired.

Because the Cockcroft–Gault equation has been used to determine recommended dosages for various medications, there is a consistent approach when using this equation to adjust medication dosage.

By contrast, the most widely used GFR-estimating equation today is the MDRD equation, which was published in 1999 and later simplified [12].

This equation used for automatically estimating GFR from serum creatinine for most laboratories was developed using patients who had CKD identified by elevated serum creatinine levels and who had a four-fold higher risk for progressing to ESRD than dying first [13].

Subsequently, several studies have shown that in 'low-risk' populations, such as living kidney donors or individuals with early diabetes, the MDRD equation systematically underestimated GFR, particularly in patients with high-normal serum creatinine levels [14].

This suggests that no one equation can accurately estimate GFR regardless of clinical presentation. Studies further showed that the use of an equation that is developed with mostly healthy individuals would

lead to a much lower prevalence of an eGFR (<60 ml/min/1.73 m^2) [15].

In a trial to reach a more precise equation, the CKD Epidemiology Collaboration (CKD-EPI) equation was published in 2009 and intended to be more generalized across various clinical settings compared with the MDRD equation. Weight, diabetes, and transplant were considered as potential variables, but the final equation uses the same variables as the MDRD equation [16].

The source studies that were used for the CKD-EPI equation can be broken down into two groups:

(1) High-risk populations such as patients with clinical CKD, characterized by an average (measured GFR) less than 90 ml/min/1.73 m^2.
(2) Low-risk populations such as potential kidney donors, characterized by an average (measured GFR) more than 90 ml/min/1.73 m^2.

The best performance among all equations tested when compared with isotopically measured GFR was the CKD-EPI Cr-Cyst 2012 (r=0.816, P=0.000).

The concept of using two serological markers in equations to estimate GFR was tested by investigators of the CKD-EPI who tried to prove the concept by comparing the performance of three equations (CKD-EPI): creatinine only, CKD-EPI Cys only and combined one. They conducted a cross-sectional analysis in diverse populations totaling 5352 participants from 13 studies.

In participants whose eGFR based on creatinine was 45–74 ml/min/1.73 m^2, the combined equation improved the classification of measured GFR as either less than 60 ml/min/1.73 m^2 or greater than or equal to 60 ml/min/1.73 m^2 [net reclassification index, 19.4% (P<0.001)] and correctly reclassified 16.9% of those with an eGFR of 45–59 ml/min/1.73 m^2 as having a GFR of 60 ml/min/1.73 m^2 or higher [16].

The same finding has been recently emphasized by Trimarchi *et al.* [17] in a cross-sectional study in 300 patients, and the results showed that creatinine CKD-EPI and combined CKD-EPI equations yielded the highest correlations with ^{99m}Tc-DTPA (r=0.839, P<0.0001 and r=0.831, P<0.0001), respectively.

Konrad *et al.* assessed the serum cystatin C concentration in 152 patients with type 1 and type 2 diabetes. GFR was estimated based on the cystatin C concentration according to the Grubb formula and compared with GFR estimated based on serum creatinine concentration according to MDRD:

(1) They correlated strongly in patients with GFR lower than 60 ml/min/1.73 m^2 (r=0.62, P<0.0001).
(2) In patients with GFR higher than 60 ml/min/1.73 m^2, the correlation was much weaker (r=0.24, P=0.019).

They concluded that in patients with impaired renal function cystatin C did not seem to have any advantage over serum creatinine in the estimation of GFR. The advantage of cystatin C over serum creatinine may be that it is found in early stages of diabetic kidney disease, when GFR is still normal or elevated. Cystatin C may be used for early prediction of renal function impairment in diabetic kidney disease [18].Considering evaluating kidney function and clinical outcomes, the use of cystatin C alone or in combination with creatinine increases the accuracy of estimating GFR, helps in proper CKD staging, and strengthens the association between the eGFR and the risks of death and ESRD across diverse populations [19].

We could not find a significant correlation between either serum creatinine (r=–0.04, P=0.7) or cystatin (r=–0.08, P=0.48) and level of proteinuria. Other studies have reported the same findings that there is no linear correlation between the degree of proteinuria and functional state of kidney [20].

Our results showed a significant negative correlation between serum cystatin and hemoglobin level (r=–0.591, P=0.000), which could not be seen with serum creatinine (r=–0.2, P=0.07). This finding might give cystatin an advantage over creatinine in predicting clinical and functional state of such high-risk patients. However, this finding has not been specifically addressed and studied for renal patients, but superiority of cystatin C to detect renal abnormality has been addressed in patients with beta thalassemia and sickle cell disease [21,22].

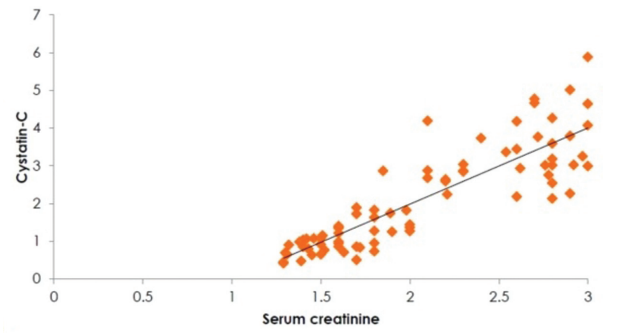
This finding warrants to be studied in more detail to confirm its consistency and reach an explanation for it.

In conclusion, in our cross-sectional study of cohort of Egyptian type 2 diabetic patients with overt diabetic nephropathy, cystatin C correlated well with serum creatinine. Nevertheless, serum cystatin C showed a significantly stronger correlation better than creatinine with isotopically measured GFR. Among studied

equations for GFR estimation, best performance was the CKD-EPI Cr-Cys 2012 equation. Also, serum cystatin C showed a significant negative correlation with hemoglobin level, which could not be established with serum creatinine. There was no significant association of either creatinine or cystatin with the level of proteinuria.

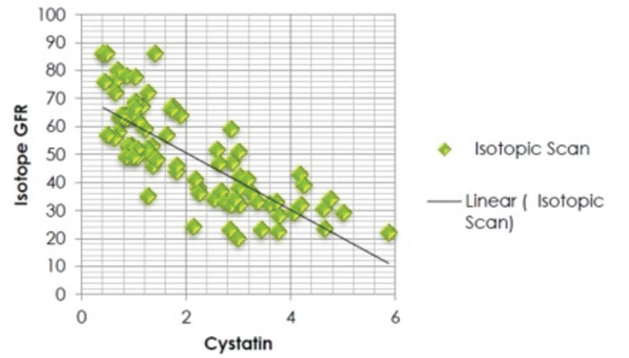
We recognized some limitations of our study: it was conducted on a small number of patients, only on patients with relatively advanced diabetic nephropathy, and it was a cross-sectional study. Our recommendation is to plan for large-scale prospective clinical trial to assess best eGFR equation used for such high-risk patients across different clinical spectrum of the disease and

Fig. 1



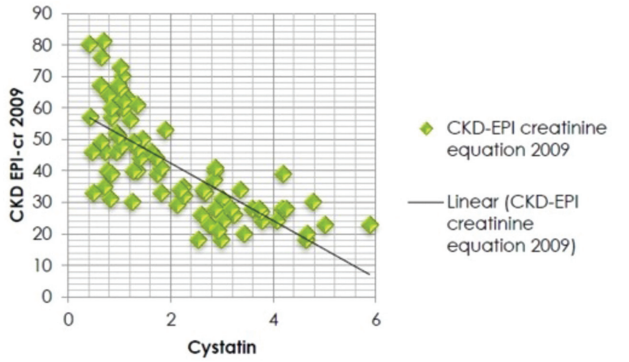
Correlation between serum creatinine and cystatin-C among the study population (r=0.867, P= 0.000)

Fig. 2



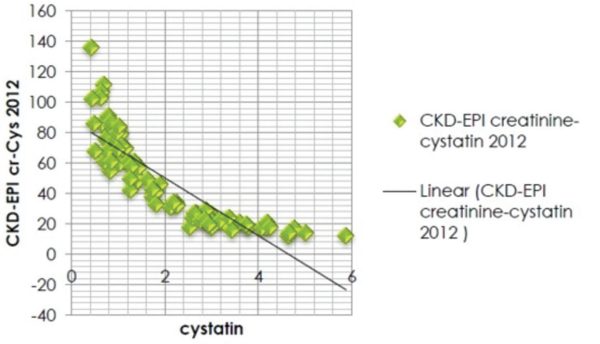
Demonstrate correlation between isotope scan (estimated GFR) and measured serum cystatin-c. (r =–0.781, P=0)

Fig. 3



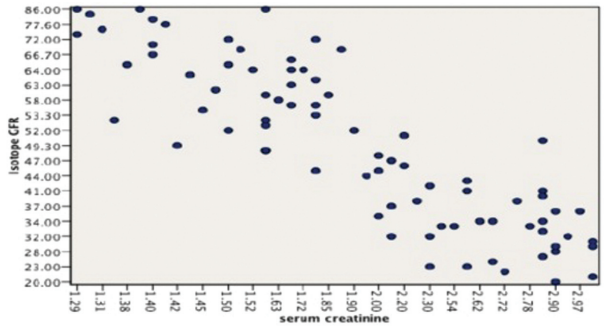
Demonstrate relation between CKD EPI-creatinine equation 2009 (estimated GFR) and measured serum cystatin-c. (r=–0.739, P=0)

Fig. 4



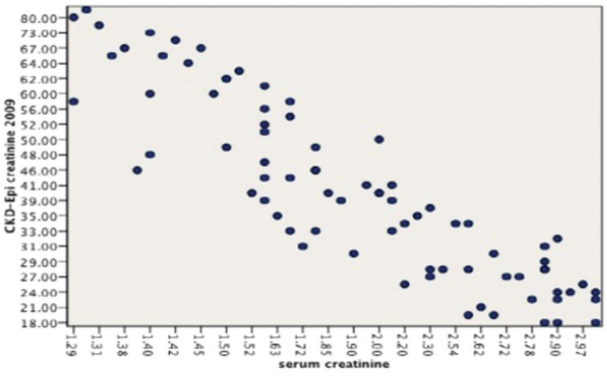
Demonstrate relation between CKD-EPI creatinine-cystatin 2012 equation (estimated GFR) and Cystatin-c (r=–0.862, P=0)

Fig. 5



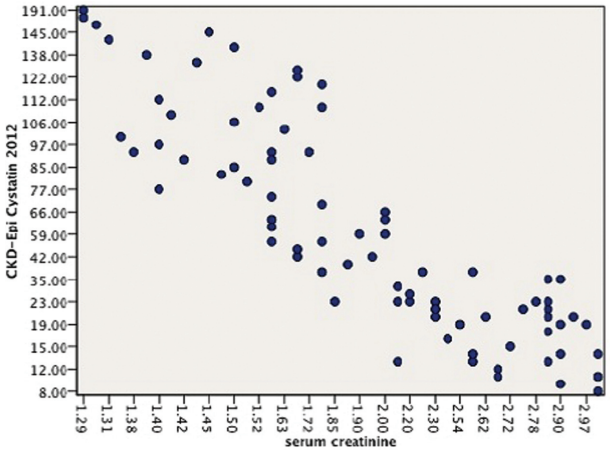
Demonstrate relation between isotope scan (estimated GFR) and measured serum creatinine. (r=–0.106, P=0.348)

Fig. 6



Demonstrate relation between CKD-EPI creatinine 2009 equation (estimated GFR) and measured serum creatinine.(r=0.253, P=0.023)

Fig. 7



Demonstrate relation between CKD-EPI creatinine-cystatin 2012 (estimated GFR) and measured serum creatinine. (r=–0.245, P=0.028)

prospective follow-up for evaluating the natural course of progression and effect timely effective interventions

As clinical perspectives of our work we suggest large clinical and epidemiological studies to evaluate the performance of cystatin C in different stages of diabetes for early detection of any decline and allowing early timely therapeutic interventions (Figs 1–7).

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Conflicts of interest
There are no conflicts of interest.

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