Cystatin C as a marker of GFR in comparison with serum creatinine and formulas depending on serum creatinine in adult Egyptian patients with chronic kidney disease.

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Abstract: Background and aim of the study: There is no literature available on the performance of cystatin C in adult Egyptian patients with Chronic Kidney Disease (CKD). Our study was aimed to compare the diagnostic performance of serum Cystatin C, serum creatinine,cystatin C-based formula and creatinine-based formulas with measured glomerular filtration rate (GFR) in adult Egyptian patients with CKD. **Methods:** The study was conducted on 80 patients were known as CKD[42 of them where males (52.3%) and 38 females (46.7%)] with mean age 56.58 ± 13.06 years, attending the Nephrology Department,Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. Serum cystatin C was measured with *Human Cystatin C ELISA – Biovendor*. TheeGFR was calculated using Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD) and simple cystatin C formulas. GFR was measured using ^{99m}TC - diethylenetriaminepenta acetic acid (DTPA) renal scan method. **Results:** There was significant correlation between serum Cystatin C and measured GFR (r=-0.8792; p<0.0001) than between serum creatinine and measured GFR (r=-0.5861). There was significant correlation between Cystatin C –based formula in the studied CKD patients and the measured GFR in the same patients(r= 0.899; p< 0.0001) better than the correlation between measured GFR in the studied CKD patients and GFR calculated from the MDRD formula (r = 0.788; P < 0.0001) and C&G formula (r = 0.683; P < 0.0001) in the same patients.The receiver operating characteristic curve(ROC) analysis showed that serum cystatin C had bigger AUC and higher sensitivity(AUC:0.902;sensitivity:97.6%) than serum creatinine (AUC: 0.711; sensitivity: 72.6%). Also the cystatin C-based formula and MDRD, had bigger AUC (0.875; 0.930 respectively) and higher sensitivity (97.5%; 90.5% respectively) than the C&G formula (0.872; 81.0%), but no statistically significant differences between the formulas was found**. Conclusion:** The present study suggest that serum Cystatin C is a good alternative marker to serum creatinine in CKD patients and that Cystatin C-based formula, which requires just one variable (serum cystatin C), achieved a diagnostic performance that was at least comparable if not better than the creatinine-based formulas using more variables.

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1- Introduction

Chronic kidney disease (CKD) is an important public health problem classified into stages according to the level of GFR.Therefore, estimation of the GFR is essential for the evaluation of patients with CKD and is useful tool to screen for CKD also in high-risk groups as persons with diabetes mellitus. GFR estimation allows us to detect early impairment of kidney function, prevent further deterioration and complications, correct the dosage of drugs cleared by the kidney so as to avoid potential drug toxicity, and manage CKD patients. Recently, the National Kidney Disease Education Program (NKDEP) recommended reporting GFR values above 60 mL/min/1.73 m^2 not as an exact number but simply as >60 mL/min/1.73 m^2, and contrary for the values of 60 mL/min/1.73 m^2 and below the exact numerical estimate should be reported[1]. For clinicians the GFR below 60 mL/min/1.73 m^2 is very important. The values indicate the presence of CKD and represent an increased risk of impaired kidney function, progression to kidney failure, and premature death caused by cardiovascular events of patients with CKD [2, 3].

Serum creatinine level,the most commonly used surrogate measure for glomerular filtration rate (GFR), does not increase until renal function decreases to 50% of it's normal value. It's excretion rate varies with age, sex,physical exercise and lean body mass. The population variance of serum creatinine level is large making it a poor measure for comparison with reference range[4]. Creatinine clearance is often inaccurate, but it is widely used in clinical practice.The gold standard tests such as 51cr - labeled EDTA,99mTc labeled DPTA or Iohexol are too cumbersome to use in clinical setting[5].

Over the last decades several different markers for estimation of GFR have been proposed. Despite all known disadvantages, serum creatinine and predictive equations, such as the Cockcroft-Gault (C&G) formula and abbreviated modification of diet in renal disease (MDRD) formula, have become the most commonly used marker to estimate GFR in clinical practice as in most studies [6-8]. Furthermore, estimation of GFR derived from MDRD formula is recommended in annual evaluation of all patients with type 2 diabetes mellitus (DM2) [9]. Unfortunately, both these formulas are also limited by lack of validation in the full range of GFR to which they are applied [10].

Recently, serum cystatin C low-molecular-weight protease inhibitor, that is freely filtered across the glomerular membrane and then reabsorbed and metabolized in the proximal tubule, was proposed as a new endogenous marker of GFR [11,12]. The previous reports have suggested that serum cystatin C is a better indicator of GFR than serum creatinine in patients with spine injury, liver cirrhosis, diabetes, mild to moderate impaired kidney function, and in elderly patients [13-17].

Aim of the study

To compare the diagnostic performance of serum CystatinC, serum creatnine, cystatin C-based formula and creatinine-based formulas with measured glomerular filtration rate (GFR) in adult Egyptian patients with CKD.

2- Subjects and methods

This study was conducted on 80 patients were known as CKD[42 of them where males (52.3%) and 38 females (46.7%)] with mean age 56.58 ± 13.06 years, attending the Nephrology Department, Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. The patients with cardiac failure,malignancy and liver cirrhosis were excluded from the study. Written consent was obtained from all the study participants.

All patients subjected tofull history taking and clinical examination.Blood sample(10 cc) was drawn from each patient.Venous blood is collected and divided in tubes as follows:tubes in which blood samples were centrifuged and serum aliquoted and stored at –70°C until cystatin C measured,tubes in which blood samples were centrifuged and serum aliquoted were routine investigations where done, tubes containing EDTA for blood picture and tubes containing citrate for prothrombin time and concentration and INR.

Routine investigations as complete blood picture,kidney function tests:[Serum urea,creatinine, sodium and potassium and uric acid],liver function tests: [Alanine transaminase (ALT), Aspartate transaminase (AST), Prothromibin time (PT) and concentration (PC) and international normalized ratio (INR) and serum albumin,ESR, random blood sugar and urine analysis were done.

GFR was measured using ^{99m}TC - diethylenetriaminepenta acetic acid (DTPA) renal scan method.

Serum Cystatin C (*Human Cystatin C ELISA – Biovendor) Assay procedure:*

Reagents prepared, Standards, controls and samples are diluted as follows: Each concentration is diluted 400x in two steps (10x and 40x). 100µ were pipetted of each standard, control and sample into appropriate wells. The plate is incubated at room temperature for 30 minutes; shaked at about 300 rpm on the orbital microplateshaker.The well are washed 3 times with wash solutions, remaining washing solution was removed.100µ of conjugate solution was added in each well. Plate incubated at room temperature for 30 minutes; shaked at about 300 rpm on the orbital microplateshaker. The wells are washed 3 times with wash solutions, remaining washing solution was removed.100µ of substrate solution was added and protected from light by covering plate with aluminum foil. Incubation at room temperature for 20 minutes. Adding 100µ of stop solution stopped the color development. Optical density was determined in the plate by reading absorbances at 450nm***. (Yang X, 2006)***[18].

GFR was calculated according to C&G, MDRD, and cystatin C based formulas:

***C& G formula*:**

GFR (ml/min/1.73m^2) = [(140-age) × weight (kg)](/ 72 × S.Cr (mg/dl**).** For women, multiply with 0.85[19].

MDRD formula.

GFR=1 7 5 × s.c r (m g / d L)− 1. 1 5 4× a g e (y e a r s)− 0. 2 0 3.

The correction factor of 0.742 was used for women[20]. The C&G formula was standardized for a 1.73 m^2 body surface area (according to the DuBois and DuBois method). The MDRD formula already standardized for a 1.73 m^{2}body surface area.

***Simple cystatin C formula*:**

100/s.cystatinC(mg/L)[21].

IV-Statistical Analysis

The results were expressed as means ± standard deviation of the means (SD) or percentage. Pearson's correlation coefficient was used for defining the correlation between measured GFR and serum creatinine, serum cystatin C, the GFR calculated from the serum creatinine-based formulas, and the GFR calculated from the cystatin C formula. In order to determine the diagnostic accuracy of the serum cystatin and Cysatin C-based formula in comparison with the other markers of GFR, receiver-operating characteristic (ROC) plots were constructed and

analysed. The area under the curve (AUC) describes the test's overall performance and is used to compare different tests. Sensitivity and specificity were calculated. The measured GFR was used as the gold standard and the cut-off value was set at 60 mL/min/1.73 m^2 for CKD as defined by the National Kidney Foundation [22]. The analysis was performed using Statistical Analysis System, version 6.03, on an IBM at personal computer and MedCalc for windows (version 12.7.5). P value <0.05 was considered significant.

3- Results.

The Demographic features of the studied CKD patients are shown in Table 1

The mean age of the studied 80 CKD patients was 56.58 ± 13.06 (range: 24-68. years).The mean value of BMI was 26.82 ± 7.47

The laboratory profile of the studied CKD patients is shown in Table 2:

The mean value of s.creatinine, within the studied CKD patients was 1.97 ± 0.32(mg/dl), while for BUN, the mean value within the studied CKD patients was 38.4 ± 15.33(mg/dl).

The mean value of serum albumin within the studied CKD patients was 3.41 ± 0.42(g/dl).

The mean value of serum cystatin C within the studied CKD patients was 1.64 ± 0.27(mg/l)**.**

The mean value of measured GFR in the studied CKD patients was 35.46 ± 8.57 (mL/min/1.73 m^2).

The mean values for the C&G, MDRD and Cystatin C-based formulas in the studied CKD patients are shown in table 3.

The mean value of CG in the studied CKD patients was 43.16 ± 15.83(mL/min/1.73 m^2).

The mean value of MDRD in the the studied CKD patients was 33.14 ± 11.72(mL/min/1.73 m^2).

The mean value of cystatin C- based formula in the studied CKD patients was 36.54 ± 7.50 (mL/min/1.73 m^2).

Correlation between serum Cystatin C and serum creatnine with measured GFR and estimated GFR

There was significant correlation between serum Cystatin C and measured GFR (r=-0.8792; p<0.0001) than between serum creatinine and measured GFR (r=-0.5861). Serum creatinine correlates with $eGFR_{CG\&MDRD}$ (r=-0.8647; p<0.0001 and r=-0.9213; p<0.0001) better than the correlation of cystatin C with $eGFR_{CG\&MDRD}$ (r=-0.6120 and r=-0.5467). The ROC analysis showed that serum cystatin C (AUC: 0.902; sensitivity: 97.6%; specificity:78.9%) had bigger AUC and higher sensitivity than serum creatinine (AUC: 0.711; sensitivity: 72.6%; specificity: 65.8%). (Fig.1;Table 4)

For the correlation between serum Cystatin C and creatinine with age and BMI, there was no significant correlation with serum cystatin C (r=0.0260, and r=-0.0843, respectively; p>0.05) and serum creatinine showed significant correlation with age (r=-0.6225; p<0.01) and BMI (r=-0.5137; p=0.0223).

Correlation between the Cystatin C –based formula and measured GFR.

There was significant correlation between Cystatin C –based formula in the studied CKD patients and the measured GFR in the same patients (r= 0.899; p< 0.0001).

Correlation between the creatinine –based formulas and measured GFR

There was Statistically significant correlation between measured GFR in the studied CKD patients and GFR calculated from the MDRD formula (r = 0.788; P< 0.0001) and C&G formula (r = 0.683; P < 0.0001) in the same patients. The Correlation between the Cystatin C –based formula and the C&G, and the MDRD in the studied CKD patients. There was a significant correlation between Cystatin C –based formula and CG (r= 0.671; p< 0.01). There was a significant correlation between Cystatin C –based formula and MDRD (r= 0.613; p< 0.01).

The ROC and AUC analysis of creatinine-based formulas (C&G and MDRD) and cystatin C-based-formula in the studied CKD patients.

The ROC curve analysis (cut-off for GFR 60 mL/min/1.73 m^2) showed that the cystatin C-based formula and MDRD, had bigger AUC(0.875;0.930 respictively) and higher sensitivity(97.5%; 90.5% respictively) than the C&G formula(0.872;81.0%), but no statistically significant differences between the formulas was found (Figure 2

Diagnostic accuracy (AUC, sensitivity, and specificity) at the cut-off value for GFR 60 mL/min/1.73 m^2 of the different creatinine-based formulas, and the cystatin C-based formula are presented in table 5.

The Cystatin C –based formula showed the nearest mean value (36.54 ± 7.50 mL/min/1.73 m^2) to the measured GFR mean value in the studied patients (35.46 ± 8.57 mL/min/1.73 m^2) with a difference of only 1.08 mL/min/1.73 m^2, compared with 2.32 mL/min/1.73 m^2 with MDRD and with 7.7 mL/min/1.73 m^2 with CG.

The MDRD formula underestimated the GFR with -2.32 mL/min/1.73 m^2. While the CG showed maximum lack of precision with an overestimation of GFR with 7.7 mL/min/1.73 m^2, and the most accurate formula for our patients was the cystatin C-based formula with only an overestimation of 1.08 mL/min/1.73 m^2.

Table 1: Demographic features of the studied CKD patients.

Variable	CKD patients (n= 80)
Age (yrs)	
Range	24-68
Mean ± SD	56.58 ± 13.06
Sex	
Male	42 (52.3%)
Female	38(47.7%)
Weight (Kg)	71.48 ± 17.26
Height (m)	1.67 ± 0.03
BMI (kg/m^2)	26.82 ± 7.47

Values are the mean ± SD or (n)= number tested or (%) =percent.

Table 2: Laboratory profile of the studied CKD patients.

Variable	CKD patiets (n= 80)
Albumin(g/dl)	3.41 ± 0.42
BUN(mg/dl)	38.4 ± 15.33
S. creatinine (mg/dl)	1.97 ± 0.32
Measured GFR(mL/min/1.73 m^2)	35.46 ± 8.57
S.Cystatin C(mg/l)	1.64 ± 0.27

Values are the mean ± SD or (n) = number tested.

Table 3: The mean values for the Creatnine-based formulas of the studied CKD patients compared with Cystatin C –based formula in the same patients.

Variable	CKD patients(n= 80)
C&G(mL/min/1.73 m^2)	43.16 ± 15.83
MDRD(mL/min/1.73 m^2)	33.14 ± 11.72
Cystatin C –based formula (mL/min/1.73 m^2)	36.54 ± 7.50

Values are the mean ± SD or (n) = number tested.

Table 4: Diagnostic accuracy (AUC, sensitivity, specificity) and comparison of ROC curves of serum cystatin C and creatinine as markers of GFR.

Variable	AUC	Senstivity %	Specificity %	*P* value
Serum Cystatin C	0.902	88.1%	78.9%	0.0008
Serum Creatnine	0.711	72.6%	65.8%	

P value calculated according to serum creatnine.
AUC: Area under curve.

Table 5: Diagnostic accuracy (AUC, sensitivity, specificity) and comparison of ROC curves at cut-off value for GFR 60 mL/min/1.73 m^2 of calculated clearance from the C&G formula, the MDRD formula, and the simple cystatin C formula. The GFR determined with ^{99m}TC-DTPA was used as the gold standard.

Equation	AUC	Sensitivity %	Specificity %	*P** value	*P*** value
C&G formula	0.872	81.0%	78.9%	0.1924	0.1479
MDRD formula	0.930	90.5%	78.9%	0.9591	
Cystatin C-based formula	0.875	97.5%	76.3%		

*P** value calculated according to cystatin C-based formula.
*P*** value calculated according to MDRD formula.
AUC: Area under the curve.

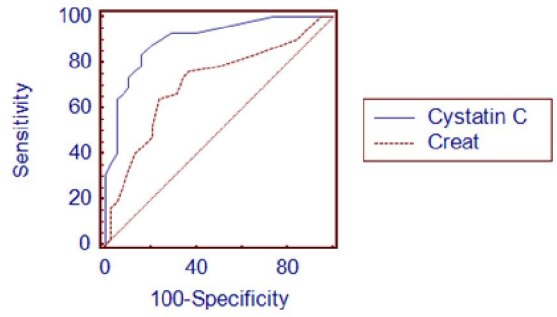

Fig.1: Reciever operating curve analysis of diagnostic accuracy of serum cystatin C and creatnine as markers of GFR.

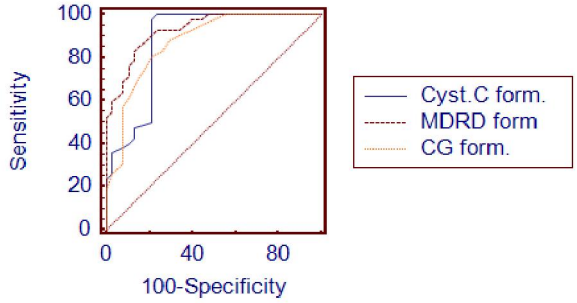

Fig.2: Reciever operating curve analysis of diagnostic accuracy of calculated clearance from the cystatin C formula, the MDRD formula, C&G formula. The GFR determined with ^{99m}TC-DTPA was used as the gold standard and cut-off value was set at 60 mL/min/1.73 m^2.

4- Discussion

The Kidney Disease Outcomes Quality Initiative (K/DOQI) CKD guidelines have established a five-stage classification of patients with CKD that is based solely on kidney function. The guidelines state that the stage of kidney disease should be determined for each CKD patient and that a clinical action plan should be developed on the basis of the stage of disease[23]. Thus inaccurate estimation of kidney function may be responsible for misclassification of some patients and lead to inappropriate evaluation or treatment of these patents [24].

The formulas that are most widely used to estimate kidney function and that are recommended in adults by K/DOQI guidelines[23]are the Cockcroft-Gault (CG) formula[19]and simplified Modification of Diet in Renal Disease (MDRD) formula[20]. However,

the formulas have some well-known limitations [25]. Therefore, new alternatives like creatinine-based CKD-EPI equation, cystatin C-based formulas, and equation that use both serum creatinine and serum cystatin C were developed [23, 26- 35].

It has been demonstrated that the (CG) formula can overestimates GFR at low renal function levels[36-38]and underestimate high GFR values[39]. Other GFR overestimation biases were demonstrated for overweight patients, young and females [37] subjects.

The MDRD clearly underestimates GFR in subjects with normal renal function [40,41]. Levey et al, emphasized the need for caution in applying the MDRD formula to individuals with a Scr. within the normal range because it has not been validated in people without renal disease[42]. That the MDRD underestimates GFR was also recently demonstrated in renal failure[38,40] in females and in overweight patients, while the opposite is true of lean subjects[43].

Many studies comparing the MDRD and CG equations using GFR measured by reference to Iohexol confirmed these findings: CG was relatively more accurate in subjects with mild or no renal insufficiency [44] while the MDRD performed better in kidney transplanted patients with renal failure.

A substantial body of evidence has developed over the past several years which supports the use of Cystatin C as an alternative and more sensitive endogenous marker for the estimation of GFR than serum creatinine and serum creatinine based GFR estimations[45-51].

In our study, we evaluated the performances of the serum CystatinC, serum creatinine, Cystatin C-based formula and creatinine-based formulas(CG and MDRD) as markers of GFR in a cohort of 80 Egyption CKD patients. An important characteristic of our study is that,included patients whose s.creatinine ranged from 0.6 to 3.0 mg/dl, thus the performances of CG and MDRD formulas and Cystatin C-based formula could be assessed over a wide range of kidney function.

Furthermore, because all patients included in the study are Egyptians, the performance of the MDRD and CG could be assessed in a group of patients whose anthropometric characteristics are different from Europeans and Americans.

Our present study showed that serum cystatin C is the most useful endogenous marker of GFR. We also have shown that the simple cystatin C-based formula achieved at least a compahable if not better a diagnostic performance than the creatinine-based formulas.

The present study showed that,the correlation of serum cystatin C with measured GFR was better than the correlation of serum creatinine with measured GFR. These results suggest that cystatin C is a good marker of renal function in patients with renal impairment, as has been reported in non-diabetic patients[52-56], patients with renal transplant[57] and in healthy patients[58].

Cystatin C is proposed to reflect GFR independent of age and BMI[59-61]. Also in the present study, similar findings have been observed. Our present study showed that,there was no significant correlation of serum cystatin C with age and BMI (r=0.0260,and r=-0.0843, respectively; $p>0.05$) and serum creatinine showed significant correlation with age (r=-0.6225; $p<0.01$) and BMI (r=-0.5137; $p=0.0223$). Serum creatinine correlated well with eGFR (C&G and MDRD) than serum cystatin C, which may be due to the eGFR being calculated using serum creatinine levels.

There is also evidence that confirms the influence of creatinine with BMI. In the study by O'Riodan et al. among 53 geriatric outpatients aged >70 years, cystatin C was considerably more accurate than creatinine in estimating GFR, with values greater than reference range having a 97% sensitivity in detecting GFR <60 ml/min/1.73m^2 compared with a sensitivity of only 37% for creatinine[62].

Similarly, the present study also revealed that cystatin C was found to be more accurate than creatinine in estimating GFR with 97.5% sensitivity, compared with a sensitivity of 72% for creatinine in all age groups of patients. An increased serum cystatin C concentration may provide a clinically important indication of a decreased GFR, even if serum creatinine concentration remains unchanged[63]. This fact confirms that cystatin C is less dependent on age groups.

Some authors even concluded that the cystatin C formula is complementary to the serum creatinine-based equations or can be used in place of the serum creatinine-based equations [64].

Similarly in our present study,the correlation between the "gold standard", measured GFR and the cystatin C-based formula was better than the correlation between the measured GFR and GFR calculated with the MDRD and C&G formulas. According to our results, the cystatin C-based formula and MDRD formula had bigger AUC and higher sensitivity than C&G formula, but no statistically significant difference in diagnostic accuracy between the cystatin C-based formula and creatinine-based formulas was found.

In our study, the MDRD formula underestimated the GFR with -2.32 mL/min/1.73 m^2. While the CG showed maximum lack of precision with an overestimation of GFR with 7.7 mL/min/1.73 m^2, and the most accurate formula for our patients was the cystatin C-based formula with only an overestimation of 1.08 mL/min/1.73 m^2.

As in other studies, the two most important biases of the CG and the MDRD formulas go in the opposite direction, i.e. they respectively overestimate and underestimate the GFR in healthy and overweight subjects, particularly among females. A certainly normal Scr (0.85mg/dl) gives rise to a GFR estimation of 87ml/min/1.73m^2 by the MDRD formula (which is likely to prompt an erroneous diagnosis of 'mild renal failure'), while the CG formula would suggest 'hyperfiltration[36,39,44,65].

Conclusion

The results of the present study suggest that Cystatin C is a good alternative marker to creatinine in CKD patients and that Cystatin C-based formula, which requires just one variable (serum cystatin C), achieved a diagnostic performance that was at least comparable if not better than the creatinine-based formulas using more variables.

References

1. Myers G L, Miller W G, Coresh J *et al.,* "Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program," *Clinical Chemistry 2006*;52(1):5–18.
2. Go A S,Chertow G M, FanD, McCulloch C E, and Hsu C Y, "Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization," *The New England Journal of Medicine 2004;*351(13):1296–1305.
3. Stevens LA, Coresh J., Greene T., and Levey AS, "Assessing kidney function—measured and estimated glomerular filtration rate," *The New England Journal of Medicine 2006*;354(23):2473–2483.
4. Perrone RD, Madias NE, Levey AS: Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem 1992*;38:1933-1953.
5. Krutzen E, Back SE, Nilsson-Ehle I, Nilsson-Ehle P: Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. *J Lab Clin Med 1984*;104:955-961.
6. Levey AS, Berg RL, Gassman JJ, Hall PM, and Walker WG, "Creatinine filtration, secretion and excretion during progressive renal disease," *Kidney International 1989;*6(supplement 27):S73–S80.
7. National Kidney Foundation: K/DOQI Clinical practice guideline to define chronic kidney disease: evaluation, classification and stratification," *American Journal of Kidney Diseases 2002;*39 (supplement 1):S1–S266.
8. Froissart M, Rossert J, Jacquot C, Paillard M, and Houillier P, "Predictive performance of the modification of diet in renal disease and Cockcroft-Gault equations for estimating renal function," *Journal of the American Society of Nephrology 2005;*16(3):763–773.
9. American Diabetes Association Position Statement, "Standard of Medical Care in Diabetes," *Diabetes Care 2009;*32:33–34.
10. Poggio ED, Wang X, Greene T, Van Lente F, and Hall PM, "Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease," *Journal of the American Society of Nephrology 2005;*16(2):459–466.
11. Randers E and Erlandsen EJ, "Serum cystatin C as an endogenous marker of the renal function—a review," *Clinical Chemistry and Laboratory Medicine 1999;* 37(4):389–395.
12. Coll E, Botey A, Alvarez L *et al.,* "Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment," *American Journal of Kidney Diseases* 2000;36(1):29–34.
13. Dharnidharka VR, Kwon C, and Stevens G, "Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis," *American Journal of Kidney Diseases 2002*;40(2):221–226.
14. Willems D, Wolff F, Mekahli F, and Gillet C, "Cystatin C for early detection of renal impairment in diabetes," *Clinical Biochemistry* 2009;42(1-2):108–110.
15. Dhia RB, Hellara I, Harzallah O, *et al.,* "Evaluation of the renal function in type 2 diabetes: clearance calculation or cystatin C?" *Analyses de Biologie Clinique* 2012;70(3):287–294.
16. Hojs R, Bevc S, Antolinc B, Gorenjak M, and Puklavec L, "Serum cystatin C as an endogenous marker of renal function in the elderly," *International Journal of Clinical Pharmacology Research 2004*;24(2-3):49–54.
17. Hojs R, Bevc S, Ekart R, Gorenjak M, and Puklavec L, "Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function," *Nephrology Dialysis Transplantation* 2006;21(7):1855–1862.
18. Yang X, Wang H, Dong M, alteration and significance of serum cardiac troponin I and Cystatin C in preeclampsia; Clin Chem Acta 2006;374:168-169.
19. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nehru 1976*; 16:31–41.
20. Levey AS, Greene T, Kusek JW, *et al.,*: A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrology 2000*;11:155A**.**
21. Perkins BA, Nelson RG, Ostrander BEP *et al.,* "Detection of renal function decline in patients

with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study," *J Am Soc Nephology* 2005;16,(5):1404–1412.
22. Froissart M, Rossert J, Jacquot C, Paillard M, Houillier P. Predictive performance of the modification of diet in renal disease and Cockcroft-Gault equations for estimating renal function. *Journal of the American Society of Nephrology*. 2005;16(3):763–773.
23. National Kidney Foundation: K/DOQI Clinical practice guide-line to define chronic kidney disease: Evaluation, classification and stratification. *Am J Kidney Dis, 2002;39:51-266.*
24. Froissart M, Fouqueray B, Houillier P, *et al.,* Classification of the stages of chronic renal disease: Limitations and pitfalls using Cockcroft's formula versus GFR measurement(abstract). *J Am Soc Nephrology, 2002;13:431A.*
25. Stevens LA, Coresh J, Greene T, and Levey AS, "Assessing kidney function—measured and estimated glomerular filtration rate," *The New England Journal of Medicine* 2006;354(23):2473–2483.
26. Hoek FJ, Kemperman FAW, and Krediet RT, "A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate," *Nephrology Dialysis Transplantation 2003;*18910):2024–2031.
27. Larsson A,MalmJ,Grubb A, and Hansson LO, "Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L," *Scandinavian Journal of Clinical and Laboratory Investigation* 2004;64(1):25–30.
28. Hojs R, Bevc S, Ekart R, Gorenjak M, and Puklavec L, "Serum crystatin C-based equation compared to serum creatinine-based equations for estimation of glomerular filtration rate in patient with chronic kidney disease," *Clinical Nephrology* 2008;70(1):10–17.
29. Tidman M,Sjöström P, and Jones I, "A comparison of GFR estimating formulae based upon s-cystatin C and s-creatinine and a combination of the two," *Nephrology Dialysis Transplantation2008*;23(1):154–160.
30. Chudleigh RA,OllertonRL,Dunseath G *et al.,* "Use of cystatin C-based estimations of glomerular filtration rate in patients with type 2 diabetes," *Diabetologia 2009;*52(7):1274–1278.
31. Ma YC, Zuo L, Chen JH *et al.,* "Improved GFR estimation by combined creatinine and cystatin C measurements," *Kidney International 2007;*72(12):1535–1542.
32. Stevens LA, Coresh J, Schmid CH *et al.,* "Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD," *American Journal of Kidney Diseases 2008*;51(3):395–406.
33. Perkins BA, Nelson RG, Ostrander BEP *et al.,* "Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study," *Journal of the American Society of Nephrology 2005*;16(5):1404–1412.
34. Hojs R, Bevc S, Ekart R, Gorenjak M, and Puklavec L, "Serum cystatin C-based formulas for prediction of glomerular filtration rate in patients with chronic kidney disease," *Nephron—Clinical Practice 2010*;114(2):c118–c126.
35. Stevens LA, Coresh J, Feldman HI *et al.,* "Evaluation of the modification of diet in renal disease study equation in a large diverse population," *Journal of the American Society of Nephrology 2007*;18(10):2749–2757.
36. Poggio ED, Wang X, Greene T, *et al.,* Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. *J Am Soc Nephrology, 2005*;16:459-466.
37. Froissart M, Rossert J, Jacauot C, *et al.,* Predictive performance of the modification of diet in renal disease and Cockcroft-Gault equations for estimating renal function. *J Am Soc Nephrology, 2005;16(3):763-773*.
38. Kuan Y, Hossain M, Surman J, *et al.,* GFR prediction using the MDRD and Cockcroft and Gault equations in patients with end-stage renal disease. *Nephrology Dial Transplant, 2005;20(11):2394-401.*
39. Rule AD, Gussak HM, Pond GR, *et al.,* Measured and estimated GFR in healthy potential kidney donors. *Am J Kidney Dis, 2004;43(1):112-9.*
40. Cirillo M, Anastasio P, De Santo NG: Relation of gender, age, and body mass index to errors in predicted kidney function. *Nephrology Dial Transplant, 2005;20(9):1791-8.*
41. Lin J, Knight EL, Hogan ML, *et al.,* A comparison of prediction equations for estimating glomerular filtration rate in adults without kidney disease.*J Am Soc Nephrology, 2003;14:2573-2580.*
42. Levey AS, Bosch JP, Lewis JB, *et al.,* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Inter Med. 1999;130(6):461-470*.
43. Bedhhu S, Samore MH, Roberts MS, Stoddard GJ, Pappas M, Cheung AK: Creatinine production, nutrition, and glomerular filtration rate estimation. *J Am Soc Nephrology, 2003;14(4):1000-5.*
44. Boston AG, Kronenberg F, Ritz E: Predictive performance of renal function equations for patients with chronic kidney disease and normal

serum creatinine levels. *J Am Soc Nephrology, 2002;13:2140-2144.*

45. F, Bökenkamp A, Hofmann W, Le Bricon T, Martínez-Brú C, Grubb A. Cystatin C as a marker of GFR- History, indications and future research Clinical *Biochemistry 2005;*38:1-8
46. Hojs R, Bevc S, Ekart R, Corenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function, *Nephrology Dialysis Transplant 2006;* 21: 1855–1862.
47. Grubb A, Nyman U, Bjo ¨ rk J, *et al*. Simple Cystatin C–Based Prediction Equations for Glomerular Filtration Rate Compared with MDRD Prediction Equation for Adults and the Schwartz and the Counahan–Barratt Prediction Equations for Children, *Clin. Chem 2005*;51: 1420 – 1431.
48. Ma YC, Zuo L, Chen JH, *et al*. Improved GFR estimation by combined creatinine and cystatin C measurements. *Kidney International* advance online publication, 26 September 2007.
49. P L, Triscornia S, Lucchesi D, *et al*. Cystatin C and Estimates of Renal Function: Searching for a Better Measure of Kidney Function in Diabetic Patients *Clin. Chem 2007*; 53: 480 – 488.
50. Beauvieux MC, Moigne FOL, L Asseur C, et al. New Predictive equations improve monitoring of kidney function in patients with diabetes, *Diabetes Care., 2007*; 30:1988-1994.
51. BS,Hojs R, Ekart R, *et al*. Simple Cystatin C Formula for Estimation of Glomerular Filtration Rate in Overweight Patients with Diabetes Mellitus Type 2 and Chronic Kidney Disease, *Experimental Diabetes Research 2012;2012:*8 pages.
52. Randers E, Erlandsen EJ, Pedersen OL, Hasling C, Danielsen H. Serum cystatin C as an endogenous parameter of the renal function in patients with normal to moderately impaired kidney function. *Clin Nephrol 2000*;54(3):203-209.
53. Randers E, Kristensen JH, Erlandsen EJ, Danielsen H. Serum cystatin C as a marker of the renal function. *Scand J Clin Lab Invest 1998*;58(7):585-592.
54. Tian S, Kusano E, Ohara T, Tabei K, Itoh Y, Kawai T, *et al.,* Cystatin C measurement and its practical use in patients with various renal diseases. *Clin Nephrol 1997;*48(2):104-108.
55. Herget-Rosenthal S, Trabold S, Pietruck F, Holtmann M, Philipp T, Kribben A. Cystatin C: efficacy as screening test for reduced glomerularfiltrationrate. *Am J Nephrol 2000*;20(2):97-102.
56. Coll E, Botey A, Alvarez L, Poch E, Quintó L, Saurina A, *et al.,* Serum cystatin C as a new marker for noninvasive estimation of glomerular filtrationrate and as a marker for early renal impairment. *Am J Kidney Dis 2000* Jul;36(1):29-34.
57. Risch L, Blumberg A, Huber A. Rapid and accurate assessment of glomerular filtration rate in patients with renal transplants using serum cystatin C. *Nephrol Dial Transplant 1999 Aug*;14(8):1991-1996.
58. Vinge E, Lindergård B, Nilsson-Ehle P, Grubb A. Relationships among serum cystatin C, serum creatinine, lean tissue mass and glomerular filtration rate in healthy adults.*Scand J Clin Lab Invest 1999 Dec*;59(8):587-592.
59. Grubb A, Simonsen O, Sturfelt G, Truedsson L, Thysell H. Serum concentration of cystatin C, factor D and α 2-microglobulin as a measure of glomerular filtration rate. *Acta Med Scand 1985*;218(5):499-503.
60. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function–a review. Clin Chem Lab Med 1999 Apr;37(4):389-395.
61. Keevil BG, Kilpatrick ES, Nichols SP, Maylor PW. Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. *Clin Chem 1998 Jul*;44(7):1535-1539.
62. O'Riordan SE, Webb MC, Stowe HJ, Simpson DE, Kandarpa M, Coakley AJ, et al. Cystatin C improves the detection of mild renal dysfunction in older patients. *Ann Clin Biochem 2003 Nov*;40(Pt 6):648-655.
63. Fliser D, Ritz E. Serum cystatin C concentration as a marker of renal dysfunction in the elderly.*Am J Kidney Dis 2001 Jan;*37(1):79-83.
64. Rule AD, Bergstralh EJ, Slezak JM, Bergert J, Larson TS.Glomerular filtration rate estimated by cystatin C among different clinical presentations. KidneyInternational*2006*;69(2):399–405.
65. Lewis J, Agodoa L, Cheek D, *et al.,* Comparison of cross-sectional renal function measurements in African Americans with hypertensive nephrosclerosis and of primary formulas to estimate glomerular filtration rate. *Am J Kidney Dis, 2001;38(4):744-53*.