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Evaluation of Cystatin C and Performance of Cystatin C Based Equations for Calculating GFR in Management of Chronic Kidney Disease Patients

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ABSTRACT

Background: Glomerular Filtration Rate (GFR) is the best overall index for the assessment of kidney function in both health and disease state. Several studies suggested that CKD-EPI Creatinine-Cystatin C equation 2012 (published by CKD-EPI group) performed better than equation based on either of these markers alone and gives more accurate estimation of GFR.
Methods: The study was performed on 109 CKD patients. Serum creatinine and cystatin c levels were measured in same patients. GFR was estimated using 5 equation (Larsson, hoek, le bricon, filler, CKD-EPI Cystatin c) that are based on serum cystatin c, and three equations (Cockcroft – Gault, MDRD and CKD-EPI Creatinine) based on serum creatinine. We evaluated the new CKD-EPI Creatinine-Cystatin C equation 2012 (published by CKD-EPI group) with other equations in a population of north Indian in classifying CKD across body mass index, diabetes, and hypertension status.
Results: The ROC curve analysis showed significant ($p<0.001$) diagnostic accuracy of all GFR estimating equation with highest being of Cystatin C (AUC=0.986) followed by Larsson and Hoek (AUC=0.974), Creatinine and MDRD (AUC=0.961), CG (AUC=0.916), Filler (AUC=0.842) and Lebricon (AUC=0.736) the least. Our data showed that serum cystatin C correlated better with GFR than did creatinine.
Conclusion: Cystatin C is a potential marker of kidney function in patients of chronic kidney disease. The equations containing both serum creatinine and serum Cystatin C are more accurate than other equations for estimating GFR. Cystatin C along with creatinine may be used for routine diagnostic use in chronic kidney disease patients.

Keywords: *Chronic Kidney Disease (CKD), Glomerular Filtration Rate (GFR), Serum creatinine, cystatin c, Cockcroft- gault equation, MDRD*

INTRODUCTION

Chronic Kidney Disease is a major public health problem worldwide with dramatically rising incidence and prevalence associated with poor outcome and high cost. [1-2] Diabetes and hypertension are leading causes of kidney failure. In type 2 diabetes patients, there is an increasing incidence of diabetic nephropathy. With the use of suitable biomarker, we can identify the patients at an early stage. So by early

diagnosis and treatment, kidney failure can be either prevented or postponed.

Glomerular Filtration Rate (GFR) and Proteinuria measurements are key element to estimate the global function of the kidney.[3] Methods based upon plasma clearance of creatinine, ^{51}Cr-EDTA, or iohexol are still considered the gold standard for measuring GFR. In the present article, we will focus on the estimation of GFR because measurement of GFR require specialized technical personnel over a period of several hours and timed urine collection which is imprecise and inconvenient. Currently in clinical practice, serum creatinine is the most widely used endogenous marker for the assessment of kidney function but creatinine is not an ideal marker of kidney function because its production is influenced by factors such as age, gender, muscle mass, physical activity and diet.[4] The sensitivity of serum creatinine in the detection of CKD is poor due to its tubular secretion and extra renal elimination via the gut, serum creatinine concentration in the body may remain within the reference range until about 50% kidney function has been lost.[5] So by using serum creatinine, patients with early stage kidney disease may go undetected. Therefore, new atternative markers for the detection of CKD at both early as well as late stage are needed. For this, cystatin c a noval serum marker has been approved by the FDA for clinical use.

It is a small 13 kDa endogenous protein belonging to the cystatin superfamily of cysteine protenase inhibitor. It is produced by all nucleated cell in the body and freely filtered through glomerular membrane, completely absorbed and catabolised by kidney tubules.[6-7] Its production rate is not affected by inflammatory processes, age, sex, and nutritional status.[8-9]

Several authors have proposed Creatinine and Cystatin C based equation to improve GFR estimation. Studies have been suggested that eGFR obtained from different equations differed widely.[10] In 2012, CKD-EPI group published the combined equation (CKD –EPI creatinine and cystatin c equation).[11] We had searched out so many publications regarding this and finally found that combination of serum Creatinine and Cystatin c equation performed better than equation based on either of these markers alone and may be useful as a confirmatory test for CKD.[11] Therefore, in present study we considered the CKD-EPI combined equation as a standard equation for GFR estimation.

According to the previous report on the accuracy of combined equation as a GFR estimates, we planned a study to compare the performance of the three creatinine based equations [Cock-croft Gault (CG),[12] Modification of Diet in Renal Disease (MDRD),[13] CKD-EPI Creatinine 2009 (creatinine)],[14] and five cystatin c based equations [Larsson,[15] Hoek,[16] Lebricon,[17] Filler,[18] CKD-EPI Cystatin C 2012],[19] with the combined equation and to identify the equation which is more closer to combined equation.

MATERIALS & METHODS

Study population:

The present study was conducted on chronic kidney disease diagnosed patients aged between 18–70 years. Patients on glucocorticoids therapy, history of thyroid dysfunction and other major disorders interfering with study as decided by treating physician were excluded from the study. All patients have signed the written informed consent for their inclusion in this study. Ethical clearance for the same was obtained from the institutional ethics committee. The approval number is CDRI/IEC/2015/ A13.The study was conducted according to good clinical practice guidelines and principles of declaration of Helsinki.

Creatinine and cystatin c assay:

The serum sample was collected and then stored at -20˚c deep freezer (Celfrost) until analysis. Serum creatinine was measured by Jaffe's method using Semi-Automated Clinical Chemistry analyzer CHEM TOUCH (TRANSASIA Bio-medical Ltd,

India, Erba Manheim). Serum cystatin C measurements were performed by using the ELISA kit. The kit was stored at 4˚c.

GFR estimating equation:

Combined Creatinine–Cystatin C equation:

This equation was developed in 2012. This equation is more precise than equations using only creatinine or cystatin C. It may be useful for confirmation of eGFRcr<60 ml/min/ 1.73 m2.

Table 1: Creatinine–Cystatin C Equation (CKD-EPI 2012) [11]

Sex	Creatinine	Cystatin	Equation for estimating GFR
Female	≤0.7	≤0.8	$130\times(Scr/0.7)^{-0.248}\times(Scys/0.8)^{-0.375}\times0.995^{Age}$ [× 1.08 if black]
		>0.8	$130\times(Scr/0.7)^{-0.248}\times(Scys/0.8)^{-0.711}\times0.995^{Age}$ [× 1.08 if black]
Female	>0.7	≤0.8	$130\times(Scr/0.7)^{-0.601}\times(Scys/0.8)^{-0.375}\times0.995^{Age}$ [× 1.08 if black]
		>0.8	$130\times(Scr/0.7)^{-0.601}\times(Scys/0.8)^{-0.711}\times0.995^{Age}$ [× 1.08 if black]
Male	≤0.9	≤0.8	$135\times(Scr/0.9)^{-0.207}\times(Scys/0.8)^{-0.375}\times0.995^{Age}$ [× 1.08 if black]
		>0.8	$135\times(Scr/0.9)^{-0.207}\times(Scys/0.8)^{-0.711}\times0.995^{Age}$ [× 1.08 if black]
Male	>0.9	>0.8	$135\times(Scr/0.9)^{-0.601}\times(Scys/0.8)^{-0.375}\times0.995^{Age}$ [× 1.08 if black]
		>0.8	$135\times(Scr/0.9)^{-0.601}\times(Scys/0.8)^{-0.711}\times0.995^{Age}$ [× 1.08 if black]

Creatinine Based Estimation of GFR:

The three formulae (Cockcroft-gault, MDRD, CKD-EPI) studied to predict GFR from serum creatinine

Cockcroft-gault equation [12]: The CG equation is as follows:

For men: CrCl (ml/min) = {[(140 – Age in(yr)] × Weight (kg)}/SCr(mg/dl) × 72

Where CrCl is creatinine clearance and S_{Cr} is serum creatinine. For women, the above equation should be multiplied by 0.85.[1]

MDRD (Modification of Diet in Renal Disease) Equation [13]:

The four variable version of the MDRD equation (ml/min per 1.73 m2) is as follows:

$GFR = 186 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times 0.742$ (if patient is female) × 1.212 (if patient is black) [2]

CKD-EPI Equation:

The CKD-EPI equation was developed in 2009 using a diverse population estimate GFR from serum creatinine, age, sex and race.

Table 2: Creatinine Equation (CKD-EPI 2009)[14]

Sex	Creatinine	Equation for estimating GFR
Female	≤0.7	$144\times(Scr/0.7)^{-0.329}\times0.993^{Age}$ [× 1.159 if black]
Female	>0.7	$144\times(Scr/0.7)^{-1.209}\times0.993^{Age}$ [× 1.159 if black]
Male	≤0.9	$141\times(Scr/0.9)^{-0.411}\times0.993^{Age}$ [× 1.159 if black]
Male	>0.9	$141\times(Scr/0.9^{-1.209}\times0.993^{Age}$ [× 1.159 if black]

Cystatin C based estimation of GFR:

GFR estimated using five equations that were based on serum cystatin C are:

1. $GFR_{Larsson} = 99.43 \times ScytC^{-1.5837}$[15]
2. $GFR_{Hoek} = -4.32+(80.35\times1/cytC)$[16]
3. $GFR_{Lebricon} = 78/ScystC +4$[17]
4. $GFR_{Filler} = 91.62\times ScytC^{-1.123}$[18]
5. CKD-EPI Cystatin C (2012) =

Table 3: Cystatin C Equation (CKD-EPI 2012)[19]

Sex	Cystatin C	Equation for estimating GFR
Female or male	≤0.8	$133\times(Scys/0.8)^{-0.499}\times0.996^{Age}$ [× 0.932 if female]
Female or male	>0.8	$133\times(Scys/0.8)^{-1.328}\times0.996^{Age}$ [× 0.932 if female]

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error (SE). The association of eGFR obtained from different equation with combined equation was analysed by ANOVA followed by Dunnett's test, Pearson correlation, receiver operating characteristic (ROC) curve, X2 test and

concordance correlation analysis. A P value <0.05 was considered statistically significant.

RESULT

A total of 109 patients participated in this study. The basic characteristics of subjects at presentation is summarized in Table 4. The age of subjects ranged from 20-70 yrs with mean (± SE) 44.62 ± 1.17 yrs and median 45 yrs. Among subjects, 41 (37.6%) were females and 68 (62.4%) were males. Further, the height, weight, BMI, Creatinine and Cystatin C of subjects ranged from 132-179 cm, 34-96 kg, 15-43 kg/m2, 0.6-8.1 mg/dl and 0.5-6.8 mg/l respectively mean (± SE) 158.39 ± 0.82 cm, 61.83 ± 1.23 kg, 24.63 ± 0.47 kg/m2, 1.99 ± 0.15 mg/dl and 2.02 ± 0.13 mg/l respectively. Correlating the observed baseline Creatinine and Cystatin C levels, Pearson correlation analysis showed a significant and positive (direct) correlation between Creatinine and Cystatin C (r=0.95, p<0.001) suggesting these can be used interchangeably (coefficient of determination: R2=90.0%) (Fig. 1).

Table 4: Basic characteristics (Mean ± SE) of subjects

Basic characteristics	No. of subjects (n=109) (%)
Age (yrs)	44.62 ± 1.17
Sex: Female Male	 41 (37.6) 68 (62.4)
Height (cm)	158.39 ± 0.82
Weight (kg)	61.83 ± 1.23
BMI (kg/m^2)	24.63 ± 0.47
Creatinine (mg/dl)	1.99 ±0.15
Cystatin C (mg/l)	2.02 ± 0.13

Table 5: Comparison (p value) of estimated mean GFR of combined with other GFR estimating equations by Dunnett's test & Pearson correlation analysis.

GFR estimating equations	Mean GFR	Mean Diff.	q value	p value	95% CI	Pearson Correlation analysis wrt combined equation ***-p>0.001
Creatinine [14]	55.50 ± 3.34	4.95	0.97	>0.05	8.56-18.47	0.95***
CG [12]	60.45 ± 3.53	1.70	0.33	>0.05	11.82-15.21	0.89***
MDRD [13]	53.83 ± 4.24	3.29	0.65	>0.05	10.22-16.81	0.93***
Cystatin C [19]	58.79 ± 3.59	2.33	0.46	>0.05	11.18-15.84	0.97***
Larsson [15]	53.17 ± 3.34	8.67	1.70	>0.05	4.85-22.18	0.92***
Hoek [16]	64.17 ± 4.83	3.32	0.65	>0.05	10.19-16.84	0.96***
Lebricon [17]	52.22 ± 2.85	3.38	0.66	>0.05	10.14-16.89	0.96***
Filler [18]	58.88 ± 2.76	7.43	1.46	>0.05	6.08-20.95	0.95***

Table 6: Diagnostic accuracy of different GFR estimating equations w.r.t. to Combined using ROC curve analysis (n=109)

GFR estimating equations	Sensitivity (95% CI)	Specificity (95% CI)	+PV	-PV	AUC	Z value	p value
Creatinine	92.11 (78.6-98.2)	100 (94.9-100.0)	100.0	95.9	0.961	20.28	<0.001
CG	97.37 (86.1-99.6)	85.92 (75.6-93.0)	78.7	98.4	0.916	12.79	<0.001
MDRD	92.11 (78.6-98.2)	100.00 (94.9-100.0)	100.0	95.9	0.961	20.28	<0.001

Cystatin C	100.00 (90.7-100.0)	97.18 (90.2-99.6)	95.0	100.0	0.986	35.57	<0.001
Larsson	94.74 (82.2-99.2)	100.00 (94.9-100.0)	100.0	97.3	0.974	25.45	<0.001
Hoek	94.74 (82.2-99.2)	100.00 (94.9-100.0)	100.0	97.3	0.974	25.45	<0.001
Lebricon	52.63 (35.8-69.0)	100.00 (94.9-100.0)	100.0	79.8	0.763	5.19	<0.001
Filler	68.42 (51.3-82.5)	100.00 (94.9-100.0)	100.0	85.5	0.842	7.90	<0.001

+PV: positive predictive value, -PV: negative predictive value, AUC: Area under the curve

Table 7: Inter reliability of different GFR estimating equations with Combined using concordance correlation analysis (n=109)

GFR estimating equations	Concordance correlation coefficient (95% CI)	Pearson ρ (Precision)	Bias correction factor C_b (Accuracy)
Creatinine	0.94 (0.91-0.96)	0.95	0.99
CG	0.86 (0.81-0.90)	0.89	0.97
MDRD	0.93 (0.90-0.95)	0.93	0.99
Cystatin C	0.97 (0.96-0.98)	0.97	1.00
Larsson	0.85 (0.80-0.88)	0.92	0.92
Hoek	0.94 (0.91-0.96)	0.96	0.98
sLebricon	0.93 (0.91-0.95)	0.95	0.98
Filler	0.93 (0.90-0.95)	0.95	0.98

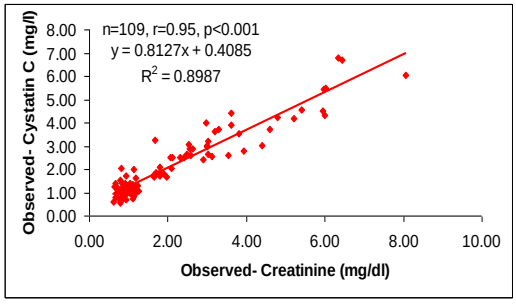


Fig. 1. Correlation between observed Creatinine and Cystatin C.

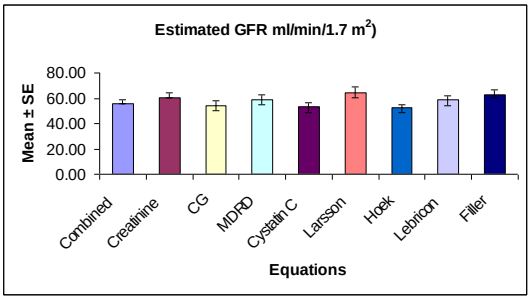
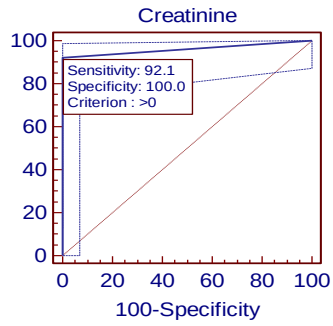
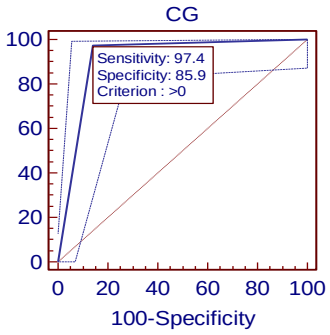
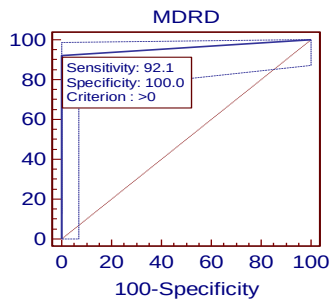
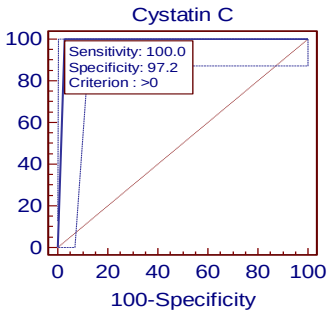


Fig. 2. Estimated mean GFR of different GFR estimating equations.









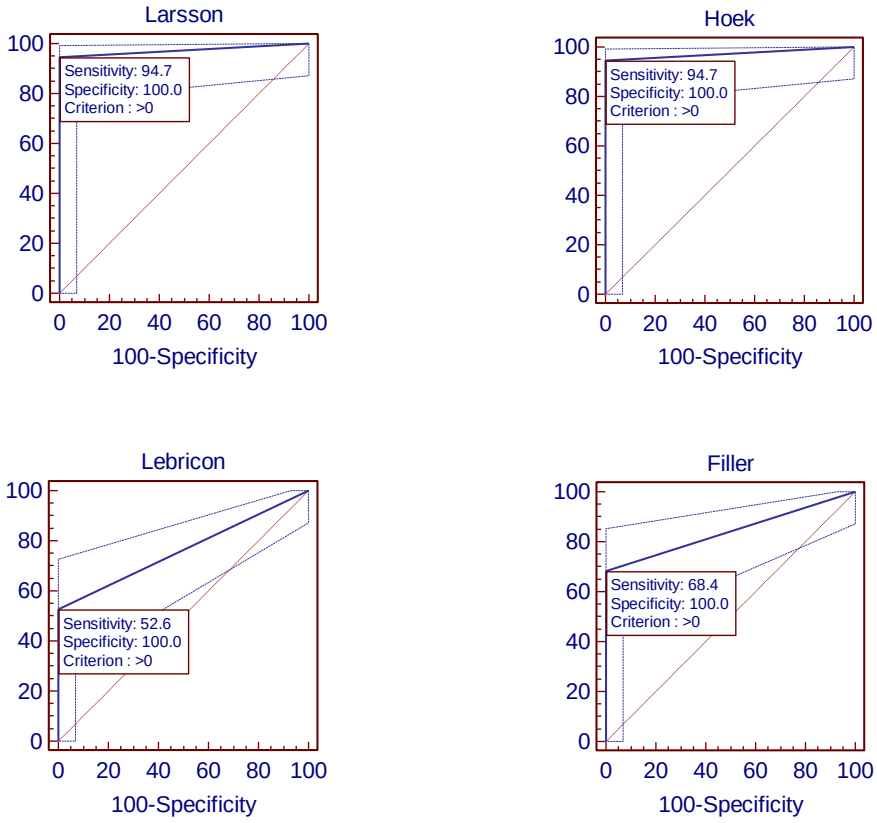


Fig. 3. Diagnostic accuracy of different GFR estimating equations w.r.t. to Combined using ROC curve analysis.

DISCUSSION

The management of chronic kidney disease depends on GFR. There is tendency to overestimate or underestimate GFR depending on the equations used for calculating GFR. Inulin clearance and other methods using injected radioactive substances such as 52Cr-ethylenediaminetetraacetic acid, Tc-99m-DTPA are considered the true reference standards for determining GFR. Unfortunately, these tests are expensive and laborious. It is also difficult to convince ill patients and therefore are not suited to clinical practice (Lee et al, 2014). In our study, we did not measure inulin clearance which is limitation for our study. In this study we have measured the creatinin and cystatin c and measured GFR by combined equation. The Cystatin C has proven as established marker of chronic kidney disease and approved by US –FDA. The combined equations using creatinine and cystatin were considered the most accurate for GFR estimation and considered equivalent to GFR measured by inulin clearance. We have also evaluated performance of other equations against combined equation. We selected equations which are most commonly used in practice and applied to our own cohort of chronic kidney disease patients. This study also establishes Cystatin C as marker for kidney injury. Currently Cystatin C is not widely used to assess kidney function, having not been extensively validated in different patient groups and at different stages of chronic kidney disease. Compared with serum creatinine, Cystatin C is more expensive and there is also no standardized measurement of Cystatin C.

In our study we found the performance of Cystatin C based equations are more consistent similar to other Cystatin C based studies. The results are similar to meta-analysis performed by Inker et al in 2012, they concluded that combined Creatinine-

Cystatin C equation had greater precision and accuracy.

The estimated GFR of different equations is summarised in Table 5 and also shown in Fig. 2. Comparing the estimated mean GFR of different equations, ANOVA showed similar ($p>0.05$) GFR among the equations ($F=1.45$, $p=0.173$). Further, Dunnett's test showed that the estimated mean GFR of different equations did not differ significantly ($p>0.05$) with the estimated mean GFR of Combined i.e. found to be statistically the same. However, estimated mean GFR of CG was found to be the closest (MD=1.70) to Combined followed by Cystatin C (MD=2.33), MDRD (MD=3.29), Hoek (MD=3.32), Lebricon (MD=3.38), Creatinine (MD=4.95), Filler (MD=7.43) and Larsson (MD=8.67) the farthest. Pearson correlation analysis showed a significant ($p<0.001$) and positive correlation of different GFR estimating equations with Combined with highest being of Cystatin C ($r=0.97$) followed by Hoek and Lebricon ($r=0.96$), Creatinine and Filler ($r=0.95$), MDRD ($r=0.93$), Larsson ($r=0.92$) and CG ($r=0.89$) the least.

The diagnostic accuracy (sensitivity and specificity) of different GFR estimating equations w.r.t. Combined (gold standard) is summarised in Table 6. The ROC curve analysis showed significant ($p<0.001$) diagnostic accuracy of all GFR estimating equation with highest being of Cystatin C (AUC=0.986) followed by Larsson and Hoek (AUC=0.974), Creatinine and MDRD (AUC=0.961), CG (AUC=0.916), Filler (AUC=0.842) and Lebricon (AUC=0.736) the least.

Lastly, concordance correlation analysis was done between GFR estimating equation Combined and different GFR estimating equations and summarised in Table 7. The concordance correlation analysis showed a very high correlation between Combined and other GFR estimating equations suggesting higher inter reliability between the variables. Among equations, Cystatin C showed the highest precision with Combined ($\rho=0.97$) followed by Hoek ($\rho=0.96$), Creatinine, Lebricon and Filler ($\rho=0.95$), MDRD ($\rho=0.93$), Larsson ($\rho=0.92$), and CG ($\rho=0.89$) the least. Further, Cystatin C also showed highest accuracy with Combined (Cb=100.0%) followed by Creatinine and MDRD (Cb=99.0%), Hoek, Lebricon and Filler (Cb=98.0%), CG (Cb=97.0%), and Larsson (Cb=92.0%) the least.

CONCLUSION

In conclusion, our work shows that cystatin C is a potential marker of kidney function in patients of chronic kidney disease. The equations containing both serum creatinine and serum cystatin C are more accurate than other equations for estimating GFR.

Declaration by Authors

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