Comparison of Creatinine, Cystatin C and Combined Creatinine-Cystatin C for Renal Function Assessment in Patients with Diabetes

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ABSTRACT

Objective: To compare the estimated glomerular filtration rate (eGFR) assessed through the CKD-EPI equations based on creatinine, Cystatin C and creatinine-Cystatin C levels for estimating kidney function among patients with diabetes.

Study Design: Cross-sectional analytical study.

Duration and Place of Study: Nephrology Department, Armed Forces Institute of Urology, Rawalpindi Pakistan, from Aug 2020 to Mar 2021.

Methodology: A total of 70 patients were recruited. Serum samples were collected for creatinine and Cystatin C levels and 24 hours of urine for creatinine clearance. The eGFR values were calculated using the creatinine, Cystatin C and combined creatinine-Cystatin C CKD-EPI equations and compared with 24 hours of urinary creatinine clearance.

Results: A total of 22 (31.4%) patients had early stage, while 48 (68.6%) had late-stage chronic kidney disease (CKD). The highest Spearman correlation coefficient was found for eGFR CKD-EPIcr-cys (rho=0.844), followed by CKD-EPIcys (rho= 0.835) and CKD-EPIcr (rho=0.709).

Conclusion: CKD-EPIcr-cys is the most accurate, recommended method of calculating eGFR.

Keywords: Chronic kidney disease, Diabetes mellitus, Estimated glomerular filtration rate, Glomerular filtration rate.

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INTRODUCTION

Chronic kidney disease (CKD) is a global public health problem that often leads to end-stage renal disease, cardiovascular disease and premature death.[1] The kidney disease quality outcome initiative (K/DOQI) defines CKD as kidney damage or decreased kidney function quantified by glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for three months or more.[2] CKD is ranked as the 12th major cause of mortalities worldwide.[3] In the developed world, the most important causative factors for CKD are diabetes mellitus and high blood pressure.[4] They have been reported to account for around 40-60% of the cases in South Asia.[5] It is imperative to detect kidney dysfunctions as early as possible, especially in patients with diabetes, as interventions at this stage have shown to slow down CKD progression to end-stage renal and cardiovascular disease.[6]

Chronic kidney failure usually evolves over several years with long latent periods with no clinical symptoms. Therefore, the diagnosis, evaluation and treatment are mainly based on biomarkers that assess kidney function.[7] GFR is the ideal method for determining kidney function. Although serum creatinine measurement is specific, it has significantly underestimated the high-normal range GFR levels.[8] Using eGFR calculations are also used to estimate GFR (eGFR). However, eGFR calculations can show bias in patients with less muscle tissue and those older people having lesser dietary intake. Cystatin C is a biomarker suggested as an alternative and adjunct to serum creatinine levels for calculating eGFR. Cystatin C is secreted by nuclear-bearing cells. As a result, it has been suggested as a significantly better eGFR indicator than creatinine, especially in the geriatric population.[9]

The biomarker, Cystatin C, either alone or in combination with creatinine in the eGFR calculation formula, is believed to have better sensitivity and specificity and has been reported to perform better than GFR estimates measured with either creatinine or Cystatin C alone.[9] There is a paucity of studies that evaluate the reliability and accuracy of the equations used for disease detection and progression in patients with diabetes in Pakistan. Therefore, the present study aimed

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to compare the GFR estimates calculated through the CKD-EPI calculations using creatinine, cystatin C and creatinine-cystatin C values for asses-sing kidney function among patients with diabetes in Pakistan. This study also assesses the correlation of the three GFR estimates with the standard 24 hours creatinine clearance.

METHODOLOGY

This cross-sectional analytical study was conducted at the Department of Nephrology, Armed Forces Institute of Urology Rawalpindi Pakistan, from August 2020 to March 2021. Ethical approval was taken from the Ethical Review Committee of AFIU (Nephro-ADM-TRG-1/IRB/2020/102). The WHO sample size calculator was used for estimating the required sample for this study. The prevalence of diabetes in people with chronic kidney disease (CKD) was used from the study by Koye *et al*.[10] A reported prevalence of 30.9% in China and 53% in Singapore were used, with the 5% level of significance and 90% power of the study, the sample size of 50 was calculated.

Fifty patients with CKD were selected for this study using non-probability convenience sampling.

Inclusion Criteria: Patients of either gender, with age 18 to 65 years with CKD and diabetes mellitus were included in the study.

Exclusion Criteria: Patients who had any form of cancer, thyroid disease, tuberculosis or had been on steroids therapy during the last one year were excluded from the study.

Written, informed consent was taken from all the participants. Each patient was then given written instructions to collect a 24-hour urine sample. After each patient collected their 24-hours urine sample, serum creatinine and Cystatin C values were then assessed. Three millimetres (3 ml) blood sample was collected from the patients' veins. The blood sample was then separated by placing the sample tubes in a centrifuge at 3500 rpm for 180 seconds. Serum creatinine assay using spectrophotometric techniques was then conducted using the modified Jaffe principle on a fully automated chemistry analyzer, ADVIA® 1800. The analysis took four hours to complete.

Then, a semi-automated NephstarTM system was used to assess Cystatin C levels based on the immunoelaphometric technique. The glomerular filtration rate (GFR) was then assessed using creatinine clearance based on the serum creatinine levels and 24-hours urine samples. In order to categorize the patients according to CKD staging, the following criteria set by Takahashi *et al*,[11] was used: stage-1 (GFR >90 ml/min/1.73m^2), stage-2 (GFR 60-89 ml/min/1.73m^2), stage- 3a (GFR 45-59 ml/min/1.73m2), stage-3b (GFR 30-44 ml/min/1.73m^2), stage-4 (GFR 15-29 ml/min/1.73m^2) and stage-5 (GFR <15 ml/min/1.73m^2). Furthermore, patients having a GFR >60 ml/min/1.73m^2 were categorized as early-stage CKD, while those with a GFR <60 ml/min/1.73m^2 were categorized as late-stage CKD.

In order to calculate the estimated glomerular filtration rate (eGFR), the CKD-EPI equation system was used. The CKD-EPI equations are further divided as: based on creatinine (eGFRcr), based on Cystatin C (eGFRcys), or based on both creatinine and Cystatin C (eGFRcr-cys).

Statistical Package for Social Sciences (SPSS) version 26.0 was used for the data analysis. Before analyzing the data, the Shapiro-Wilk normality test was run to assess if the data were normally distributed. Mean and standard deviation were computed for normally distributed -quantitative data. Median values (IQR) were computed if the variable was not normally distributed. Frequencies and percentages were computed for categorical data. In order to assess any correlation between the eGFR and GFR values, Spearman's correlation coefficient was calculated. The *p*-value of <0.05 was considered statistically significant.

RESULTS

A total of 70 patients were recruited for this study. Out of these 70 patients, 50 (71.4%) were males and 20 (28.6%) were females. The mean age of the patients was 50.34 ± 15.57 years.

The data were categorized into four age groups: group-1 (16-25 years); group-2 (26-40 years); group-3 (41-60 years), and group-4 (>60 years). The median and interquartile range (IQR) values of biochemical indicators have been shown in Table-I.

Table-I: Values of eGFR Indicators (n=70).

Parameter	Median (Inter Quartile Range)
Serum Creatinine (mmol/l)	1.60 (1.2)
Cystatin C (mmol/l)	1.45 (0.8)
Creatinine Clearance (ml/min)	45 (34.9)
eGFRcr CKD-EPI (ml/min/1.73m^2)	42 (29.5)
eGFRcys CKD-EPI (ml/min/1.73m^2)	38 (29.8)
eGFRcr-cys CKD-EPI (ml/min/1.73m^2)	42.5 (30.3)

There were 22 (31.4%) patients with early-stage CKD, while 48 (68.6%) patients had late-stage CKD. The CKD stage-wise median and IQR of serum crea-

tinine, Cystatin C and eGFR values calculated through different equations have been illustrated in Table-II.

Table-II Comparisons of different equations with creatinine clearance for different stages of chronic kidney disease (n=50).

Stage	n	Creatinine Median (IQR)	Cystatin C Median (IQR)	Median (IQR) eGFR Using Different Equations				
				CrCl	eGFRcr CKD-EPI	eGFRcys CKD-EPI	eGFRcr-cys CKD-EPI	*p*-value
1	5	0.9 (0.3)	0.88 (0.4)	97 (28.5)	103 (67)	(68)	91 (35.5)	0.523
2	17	1.2 (0.2)	0.90 (0.4)	74 (14.3)	64 (30)	61 (31.5)	69 (17.5)	0.833
3a	15	1.4 (0.4)	1.45 (0.4)	46 (5.8)	50 (17)	39 (13)	44 (12)	0.197
3b	21	2.3 (0.9)	1.50 (0.5)	40 (7.3)	31 (16.5)	27 (20.5)	34 (7)	0.051
4	11	2.5 (1.6)	2.40 (0.8)	25 (6.0)	29 (11)	20 (7)	25 (7)	<0.001
5	1	-	-	-	-	-	-	-

Spearman's correlation coefficient values (RHO) comparing the GFR (CrCl) and eGFR values calculated through the three different CKD-EPI equations have been shown in Table-III.

Table-III: Correlational Analysis between GFR (CrCl) and eGFR Values

Equation	Correlation Coefficient (r)	*p*-value
CKD-EPIcr	0.709	<0.001
CKD-EPIcys	0.835	<0.001
CKD-EPIcr-cys	0.844	<0.001

Spearman's correlation coefficient (rho) values comparing the GFR values (CrCl) and eGFR values calculated through different equations were conducted separately for early and late-stage CKD patients. The analysis has been illustrated in Tables-IV and V. The strongest correlation was found between the GFR (CrCl and eGFRcr-cys CKD-EPI at both early (r=0.486, p=0.002) and late-stage CKD (r=748, p<0.001).

Table-IV: Correlational coefficients of CKD-EPI EGFR values with crcl in early CKD (n=70).

Equation	Correlation Coefficient (r)	*p*-value
CKD-EPIcr	0.302	0.172
CKD-EPIcys	0.404	0.063
CKD-EPIcr-cys	0.486	0.022

Table-V: Correlational coefficients of CKD-EPI EGFR values with crcl in late CKD (n=70).

Equation	Correlation Coefficient (r)	*p*-value
CKD-EPIcr	0.501	<0.001
CKD-EPIcys	0.674	<0.001
CKD-EPIcr-cys	0.748	<0.001

DISCUSSION

Based upon the findings of present study, it is recommended that the eGFR estimations based on CKD-EPIcr-cys should be used for determining the severity of renal disease in patients with diabetes.

In patients with diabetes, several identified risk factors play a role in causing CKD.[12,13] The immutable factors include familial factors, male gender, age and the duration of diabetes. Other modifiable factors are poor glycemic control, high blood pressure, abnormal lipid profile, smoking, obese BMI, poor insulin resistance, lack of physical exercise, high salt diet, low birth weight, in-utero diabetes exposure and the presence of periodontitis. Low socioeconomic status is another significant risk factor as well.[14,15]

GFR assessment is a commonly used indicator for CKD, especially for high-risk patients with diabetes. Evidence suggests that early diabetic nephropathy can play a significant role in preventing long-term kidney damage.[13,16] Moreover, an accurate estimation of the GFR is required to evaluate renal function and the severity of renal disease so that appropriate treatment can be appropriate plan may be planned.[17] The overall correlational analysis found that the strongest correlation was found between GFR and eGFR (CKD-EPIcr-cyc) with a rho value of 0.844 (p< 0.001).

The correlation of eGFR levels calculated through three different CKD-EPI equations was compared with the GFR assessed through 24-hours Creatinine clearance. These correlations were studied separately for patients with early-stage and late-stage CKD. The results for patients with early-stage CKD found that the only correlation significant at the 0.05 level was between GFR and eGFR (CKD-EPIcr-cys) with the r value of 0.486 (p=0.022). The eGFR values calculated through the other two formulae were not significant at the 0.05 level. For late-stage CKD patients, all three correlations were found to be significant. However, the strongest correlation was found between GFR and eGFR (CKD-EPIcr-cyc) with the r-value of 0.748 (p<0.001).

Zou *et al*, conducted a meta-analysis of 35 different studies assessing the eGFR calculations done through the three CKD EPI equations.[16] In all the subgroups of patients with different levels of CKD, CKD-EPIcr-cys was found to be the most accurate estimate of GFR. CKD-EPIcys followed this, and the CKD-EPIcr equation provided the weakest estimate. Correlational

analysis revealed similar results, with CKD-EPIcr-cys having the strongest correlation with GFR. Compared to the diabetic patients in our study, these studies were conducted on the general population and patients with other diseases. Even then, the results from this systematic review corroborate the results of our study.

Khalid *et al*, compared the eGFR calculated through MDRD and the three CKD-EPI equations among 181 patients with CKD. While the eGFR (MDRD) was found to have a positive, strong correlation with GFR (CrCl) (r=0.867), it was still less than the correlation of GFR (CrCl) with all of the three CKD-EPI eGFR values. While the correlation of CKD-EPIcr was 0.880, that of CKD-EPIcys was slightly higher with the r-value of 0.896. However, the highest correlation was reported for CKD-EPIcr-cys (r=0.984).[9]

Elnokeety *et al*, also compared the eGFR values calculated through the three CKKD-EPI equations from the samples of 80 patients with diabetes. The strongest correlation was found between GFR (CrCl) and eGFR (CKD-EPIcr-cys) with the r-value of 0.816. Although this study was conducted on a sample of Egyptian patients with CKD and diabetes, the results were pretty similar to the present study.[2]

Chi *et al*, com-pared the accuracy of and eGFR calculated through MDRD and CKD-EPI equations against a reference GFR (CrCl) in 1296 Chinese patients with CKD. The eGFR was found to have the most negligible bias (-0.3 ml/min/1.73m2) with 83.7% results with 50% accuracy.[17] These results were also in line with the present study's findings.

Evidence suggests that renal tubular secretions of creatinine have reported ethnic variations. However, these variations are much less reported for Cystic C.[18] While the CKD-EPI equations have an adjustment approximation for black and non-black people, one for Asian populations does not exist. Therefore, using the CKD-EPI combined Cystatin and creatinine equation may cover this lack of adjustment. As shown by the results of our study and prior studies, the CKD-EPIcr-cys is the most accurate estimation of GFR and, thus, CKD disease status.

LIMITATIONS OF STUDY

This study was only a single-centre study conducted on a sample of only 70 patients. Future multi-centre studies should be conducted to study the different ethnic groups within Pakistan.

CONCLUSION

CKD-EPIcr-cys is the most accurate, recommended method of calculating eGFR

Conflict of Interest: None.

Author's Contribution

SU;, SS: Design analysis, interpretation, NMA:, MA:, AB:, AA:, FAS:, HS:, KMR:, BB: Interpretation.

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