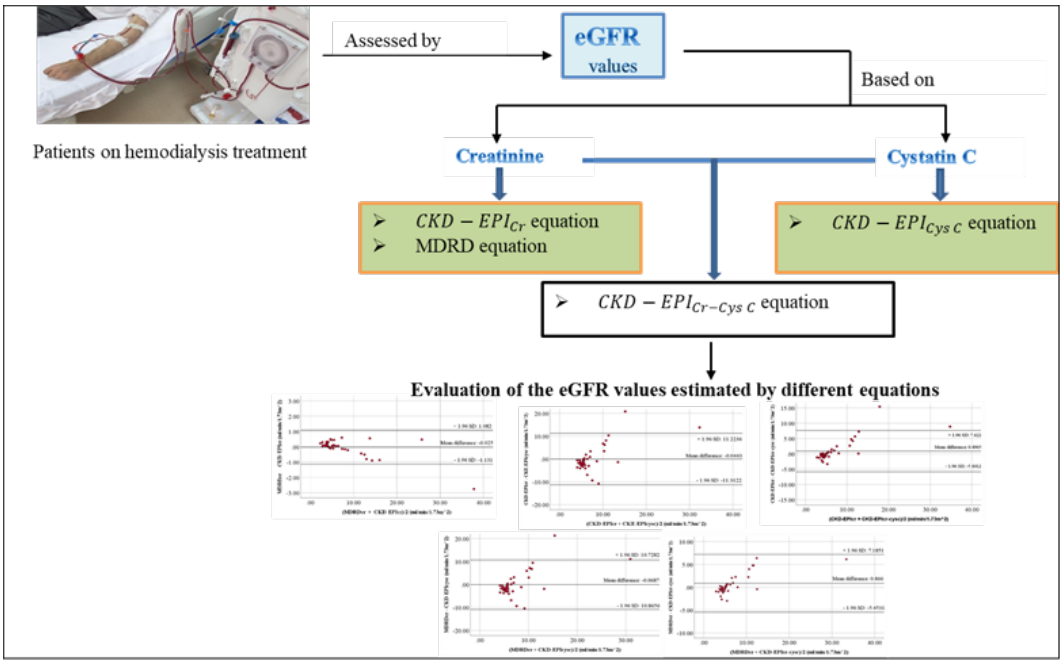
RESEARCH ARTICLE

Correlation of eGFR values estimated by different equations based on creatinine and cystatin-C in patients on hemodialysis treatment at Teaching Hospital Karapitiya, Galle, Sri Lanka

Udani A. Wanniarachchi, Sachinthi S. Amarasiri*, Anoja P. Attanayake



Highlights

- Different eGFR equations are developed to assess kidney impairment based on creatinine and cystatin-C.
- Based on the findings, CKD-EPI_{Cr} and MDRD equations are suitable to use in routine clinical settings.
- It is recommended to consider CKD-$EPI_{Cr\text{-}Cys\ C}$ as a confirmatory tool where necessary.
- The selected eGFR equations can be applied irrespective of the age and gender of the patients.

Ceylon Journal of Science 52 (4) 2023: 439-444
DOI: http://doi.org/10.4038/cjs.v52i4.8093

RESEARCH ARTICLE

Correlation of eGFR values estimated by different equations based on creatinine and cystatin-C in patients on hemodialysis treatment at Teaching Hospital Karapitiya, Galle, Sri Lanka

Udani A. Wanniarachchi[1], Sachinthi S. Amarasiri[1,*], Anoja P. Attanayake[2]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka.
[2]Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Received: 24.01.2023; Accepted: 27.09.2023

Abstract: The present study was to compare eGFR values estimated from different equations based on creatinine and cystatin-C in patients on hemodialysis treatment. eGFR values were calculated using Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology equations based on Creatinine (CKD-EPI$_{Cr}$) and cystatin-C values alone (CKD-EPI$_{Cys\ C}$) and by their combination (CKD-EPI$_{Cr\text{-}Cys\ C}$), using 40 pre-dialysis samples. No significant differences in renal function parameters and eGFR were observed, either between male (55%) and female (45%) patients or within different age groups ($p>0.05$). Certain levels of agreement ($p>0.05$) were observed between the equations, except for the values derived from CKD-EPI$_{Cys\ C}$ and CKD-EPI$_{Cr\text{-}Cys\ C}$ ($p = 0.022$). Significant positive correlations were observed between all selected equations. The highest correlation was observed between CKD-EPI$_{Cr}$ and MDRD ($r = 0.998$, $p = 0.000$), whereas the lowest correlation was found between MDRD and CKD-EPI$_{Cys\ C}$ ($r = 0.552$, $p = 0.000$). The present findings revealed that MDRD and CKD-EPI$_{Cr}$ equations are suitable for the calculation of eGFR in routine local clinical settings for the assessment of progression of kidney impairment in patients with CKD on hemodialysis treatment. However, the use of CKD-EPI$_{Cr\text{-}Cys\ C}$ rather than the CKD-EPI$_{Cys\ C}$ is recommended as a confirmatory tool where necessary.

Keywords: Chronic Kidney Disease; Correlation; Creatinine; Cystatin-C; eGFR equations; Hemodialysis

INTRODUCTION

Chronic kidney disease (CKD) has become an emerging global health burden due to its high prevalence, associated healthcare cost, and independent risk of cardiovascular disease. The global prevalence of CKD is around 13.4%, with a majority of patients categorized in the CKD stages 3-5 (Hill, *et al*., 2016; Lv and Zhang, 2019). The majority of the cases reported within the geographically discrete areas in the dry zone of Sri Lanka are claimed as chronic kidney disease of unknown etiology (CKDu), which has no association with the known risk factors of CKD such as hypertension, diabetes mellitus, or chronic glomerulonephritis (Rajapakse, *et al*., 2016; He, *et al*., 2017).

One of the main criteria for defining CKD is based on the value of the glomerular filtration rate (GFR). According to the guidelines of "Kidney Disease Improving Global Outcomes (KDIGO)," CKD is defined as a condition of decreased GFR <60 ml/min/1.73m^2 for three months or more, irrespective of the clinical diagnosis (Inker, *et al*., 2014). Therefore, the precise evaluation of GFR is crucial for the accurate diagnosis, drug administration, prognostic assessment, and more importantly for the evaluation of the progression of kidney impairment in patients with CKD (Inker, *et al*., 2012).

The direct measurement of GFR has become impractical in the clinical setting due to complicated, expensive, time-consuming procedures that are being employed (Zou, *et al*., 2020; Rule and Lieske, 2011). Therefore, the current practice is to calculate an estimated GFR value (eGFR) based on endogenous biomarkers of renal function.

Among the different equations for calculating eGFR, the equations based on serum creatinine are more common (Ferguson *et al*., 2015). Creatinine is a waste product produced in the muscles during the breakdown of creatine (Pasala and Carmody, 2017). The serum creatinine level is considered an important parameter of kidney function that reflects glomerular filtration. It is widely considered the universal parameter of kidney function during the diagnosis and management of kidney diseases (Toffaletti, 2018). However, the estimation of GFR based on serum creatinine remains relatively imprecise due to the association of various non-GFR determinants such as age, gender, race, diet, and muscle mass (Inker *et al*., 2012; Ferguson *et al*., 2015). Although creatinine-based eGFR equations have been developed to model age and gender, these demographic factors do not fully account for non-GFR determinants of creatinine, particularly muscle mass (Rule & Lieske, 2011). Therefore, imprecise estimation of GFR might cause misclassification of patients with an eGFR value <60 ml/min/1.73m^2 as having CKD leading to unnecessary diagnostic and therapeutic interventions (Inker *et al*., 2012).

Cystatin C is a novel biomarker of kidney function that is used in the calculation of eGFR. It is a non-glycated protein produced in human nucleated cells (Ferguson *et al.,* 2015). Cystatin-C is freely filtered through the glomerulus, virtually reabsorbed completely, and metabolized by proximal tubular cells (Maheshwari *et al*., 2015). The non-GFR determinants of cystatin-C are less defined and are

**Corresponding Author's Email: amssamarasiri@gmail.com*
https://orcid.org/0000-0003-1136-1197

less or not affected by demographic factors such as age, gender, race, and muscle mass (Munikrishnappa, 2009; Rule & Lieske, 2011). Therefore, numerous studies have shown better precision in eGFR values derived from serum cystatin-C-based eGFR than equations based on creatinine alone (Munikrishnappa, 2009; Inker *et al*., 2012).

However, the eGFR equations, employed in the current clinical practice have been validated only in Caucasians and African-Americans and therefore the applicability of those equations for the Sri Lankan population is a matter of concern. Since previous literature signifies the fact that race is an important determinant of the reliability of these equations, a proper scientific comparison between the eGFR values obtained from different equations might be beneficial in the assessment of the progression of kidney impairment in patients with CKD in Sri Lanka.

The decisions on dialysis treatments in the late-stage CKD patients are mainly made based on the eGFR values together with the clinical presentation in the current health care setting in Sri Lanka. Therefore, an accurate estimation of GFR is crucial, especially for CKD patients on dialysis treatments. Hence, the present study was designed to evaluate the correlation of eGFR values estimated by different equations based on creatinine and cystatin-C, in patients with CKD on hemodialysis treatment in a Sri Lankan clinical setting.

METHODOLOGY

Retained blood samples of the pre-dialysis patients, received at the Chemical Pathology Laboratory at Teaching Hospital Karapitiya were used for this laboratory-based study.

A sample size of 85 was supposed to be used in the investigations based on the calculation; N = [(Zα+Zβ)/C] 2 + 3, considering an expected correlation coefficient of 0.30 (Hulley *et al.*, 2013). However, considering the reduced number of samples received at the Chemical Pathology Laboratory of Teaching Hospital Karapitiya within the stipulated study period, the proposed sample size (85) was reduced and a convenient sample of 40 was used in the present study. Accordingly, pre-dialysis blood samples of both male and female CKD patients (between 18 – 80 years) undergoing hemodialysis treatment at Teaching Hospital, Karapitiya during the study period were included in the study. Ethical approval for the study was granted by the Ethics Review Committee of the Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka.

The baseline characteristics of the selected patients were collected using the request forms. Serum concentrations of creatinine and blood urea nitrogen (BUN) were estimated according to the procedures of Biorex Diagnostics (UK) and SPECTRUM Diagnostic (Egypt), by spectrophotometric methods. The cystatin-C assay was performed using the ELISA kit of Elabscience Biotechnology lnc (USA). Alternative quality control procedures such as duplicate testing and testing with retained samples were employed in both assays to have précised results. Furthermore, the results were compared with the values, generated by the Chemical Pathology Laboratory of Teaching Hospital, Karapitiya, Galle, Sri Lanka for each of the relevant samples. The eGFR values were calculated using the following formulas:

MDRD equation - (2006)

$eGFR = 175 \times (S_{Cr})^{-1.154} \times (age)^{-0.203}$ [x 0.742 if female]

Creatinine based CKD-EPI equation ($CKD\text{-}EPI_{Cr}$) – (2009)

$eGFR = 141 \times \min(S_{Cr}/\kappa,1)^{\alpha} \times \max(S_{Cr}/\kappa,1)^{-1.209} \times 0.993^{Age}$ [x 1.018 if female]

Cystatin-C based CKD-EPI equation ($CKD\text{-}EPI_{Cys\ C}$) - (2012)

$eGFR = 133 \times \min(S_{cysC}/0.8,1)^{-0.499} \times \max(S_{cysC}/0.8,1)^{-1.328} \times 0.996^{Age}$ [x 0.932 if female]

Creatinine and cystatin-C based CKD-EPI equation ($CKD\text{-}EPI_{Cr\text{-}Cys\ C}$) - (2012)

$eGFR = 135 \times \min(S_{Cr}/\kappa,1)^{\alpha} \times \max(S_{Cr}/\kappa,1)^{-0.601} \times \min(S_{cysC}/0.8,1)^{-0.375} \times \max(S_{cysC}/0.8,1)^{-0.711} \times 0.995^{Age}$ [× 0.969 if female]

* For MDRD: Scr = mg/dL.

*For $CKD\text{-}EPI_{Cr}$: k: 0.7 (female) or 0.9 (male); α: -0.329 (female) or -0.411(male); min indicates the minimum of S_{Cr} / k or 1; max indicates the maximum of S_{Cr} / k or 1.

*For $CKD\text{-}EPI_{CysC}$: min indicates a minimum of S_{cysC}/0.8 or 1; max indicates a maximum of S_{cysC}/0.8 or 1.

*For $CKD\text{-}EPI_{Cr\text{-}CysC}$: κ = 0.7 (females) or 0.9 (males); α = -0.248 (females) or -0.207 (males); $\min(S_{Cr}/\kappa$ or 1) indicates the minimum of S_{Cr}/κ or 1; $\max(S_{Cr}/\kappa$ or 1) indicates the maximum of S_{Cr}/κ or 1; $\min(S_{cysC}/0.8, 1)$ indicates the minimum of $S_{cys\ C}/0.8$ or 1; $\max(S_{cysC}/0.8, 1)$ indicates the maximum of $S_{cysC}/0.8$ or 1.

Statistical analysis was performed using SPSS version 25.0. Paired *t*-test and Pearson's correlation were used respectively for the pairwise comparison of means and for the assessment of the correlation between the eGFR values derived from different equations. The degree of agreement between paired measurements was analyzed by the Bland Altman method. The correlation of eGFR with age and gender was assessed using Pearson's correlation and Pearson and Chi-square analysis respectively. The findings were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The present study was designed to compare eGFR values estimated from different equations based on creatinine and cystatin-C values in patients on hemodialysis treatment. eGFR values were calculated using MDRD, $CKD\text{-}EPI_{Cr}$, $CKD\text{-}EPI_{Cys\ C,}$ and $CKD\text{-}EPI_{Cr\text{-}Cys\ C}$ equations. The findings revealed certain levels of agreement ($p>0.05$) between the eGFR values derived from different equations, except for the values derived from $CKD\text{-}EPI_{Cys\ C}$ and $CKD\text{-}EPI_{Cr\text{-}Cys\ C}$ ($p=0.022$). Significant positive correlations were observed between all selected equations with the highest correlation between $CKD\text{-}EPI_{Cr}$ and MDRD equations. The findings are discussed in detail below.

A total of 40 samples received from CKD patients on hemodialysis treatment were studied comprising 22 (55%) males and 18 females (45%). The average age of the participants was 50 ±14 years with the majority between 39-59 years (52.5%).

During the study, significant differences were not observed for parameters of renal function, including creatinine, cystatin-C, and BUN, between the three age groups selected, as well as between the male and female participants ($p>0.05$). The findings are shown in Figure 1.

The evaluation of Pearson's correlation between the different equations of eGFR, based on creatinine and cystatin-C values revealed a significant positive correlation between the results as shown in Table 1.

The current KDIGO guidelines recommend the use of CKD-EPI$_{Cr}$ in routine clinical practice where CKD-EPI$_{Cys\ C}$ is recommended for specific circumstances (Hu, et al.,

Table 1: Correlation between different equations of eGFR.

Pairwise comparison of eGFR equations	Pearson's correlation (r)	p value
MDRD – CKD-EPI$_{Cr}$	0.998	0.000
MDRD – CKD-EPI$_{CysC}$	0.552	0.000
MDRD – CKD-EPI$_{Cr\text{-}CysC}$	0.906	0.000
CKD-EPI$_{Cr}$ – CKD-EPI$_{CysC}$	0.575	0.000
CKD-EPI$_{Cr}$ – CKD-EPI$_{Cr\text{-}CysC}$	0.921	0.000
CKD-EPI$_{CysC}$ – CKD-EPI$_{Cr\text{-}CysC}$	0.825	0.000

MDRD: creatinine-based Modification of Diet in Renal Disease study equation, CKD-EPI$_{Cr}$: creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation, CKD-EPI$_{CysC}$: Cystatin-C-based Chronic Kidney Disease Epidemiology Collaboration equation, CKD-EPI$_{Cr\text{-}CysC}$: creatinine and cystatin-C combined Chronic Kidney Disease Epidemiology Collaboration equation.

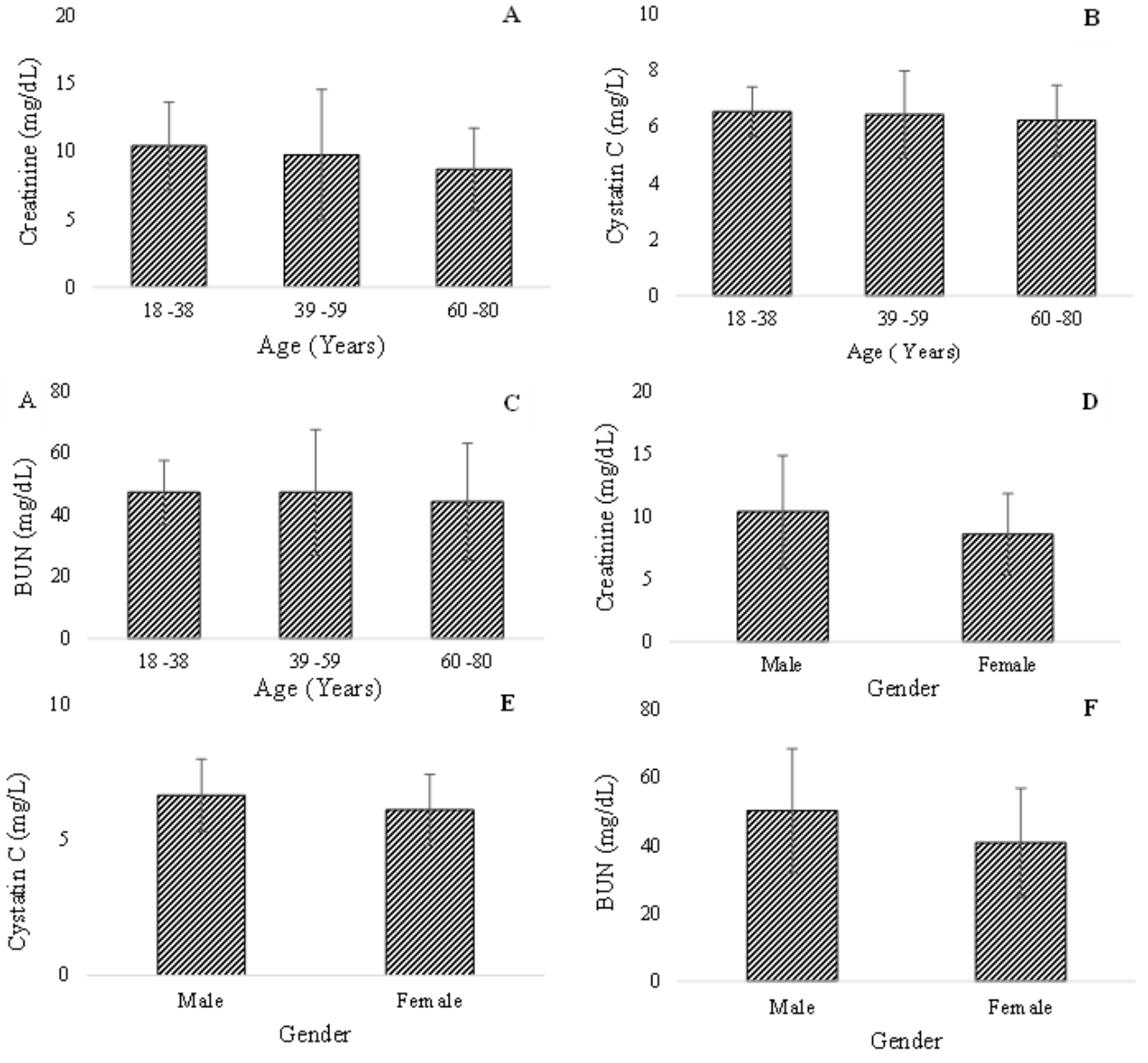


Figure 1: Changes in renal function parameters with age (A-C) and gender (D-F). The results are expressed as mean ± SD. BUN: blood urea nitrogen.

2020). Hence, CKD-EPI$_{Cr}$ equation was considered as the reference method in the present study. The findings revealed a significant high positive correlation in the eGFR values derived from CKD-EPI$_{Cr}$ with MDRD (r=0.998, p= 0.000) and CKD-EPI$_{Cr\text{-}CysC}$ (r=0.921, p= 0.000) equations. However, the correlation was relatively poor in CKD-EPI$_{Cr}$ with the values derived from CKD-EPI$_{Cys\ C}$. These findings are further substantiated by the findings of Hu *et al*., (2020) showing the same pattern of correlation in the eGFR calculated based on the eGFR equations.

Analysis of the Bland Altman plots for eGFR calculated based on different equations revealed lower limits of agreement for CKD-EPI$_{Cr}$ - MDRD (2.214 ml/min/1.73m^2), and CKD-EPI$_{Cr}$ - CKD-EPI$_{Cr\text{-}CysC}$ (13.463 ml/min/1.73m^2) pairs compared to the CKD-EPI$_{Cr}$ - CKD-EPI$_{CysC}$ (22.536 ml/min/1.73m^2) and MDRD - CKD-EPI$_{Cys\ C}$ (21.594 ml/min/1.73m^2) pairs in the present study. It is further supported by the scattering of the Bland Altman plots (Figure 2). These findings signified the applicability of creatinine-based eGFR equations and combined creatinine and cystatin-C-based CKD-EPI equation on the intended population.

Further, a statistically significant value was observed for the mean difference of the equations CKD-EPI$_{Cys\ C}$ and CKD-EPI$_{Cr\text{-}Cys\ C}$ (p = 0.022), indicating an absence of agreement between the two of these equations. These findings query the applicability of either CKD-EPI$_{Cys\ C}$ or CKD-EPI$_{Cr\text{-}Cys\ C}$ equations in the management of CKD patients in Sri Lanka.

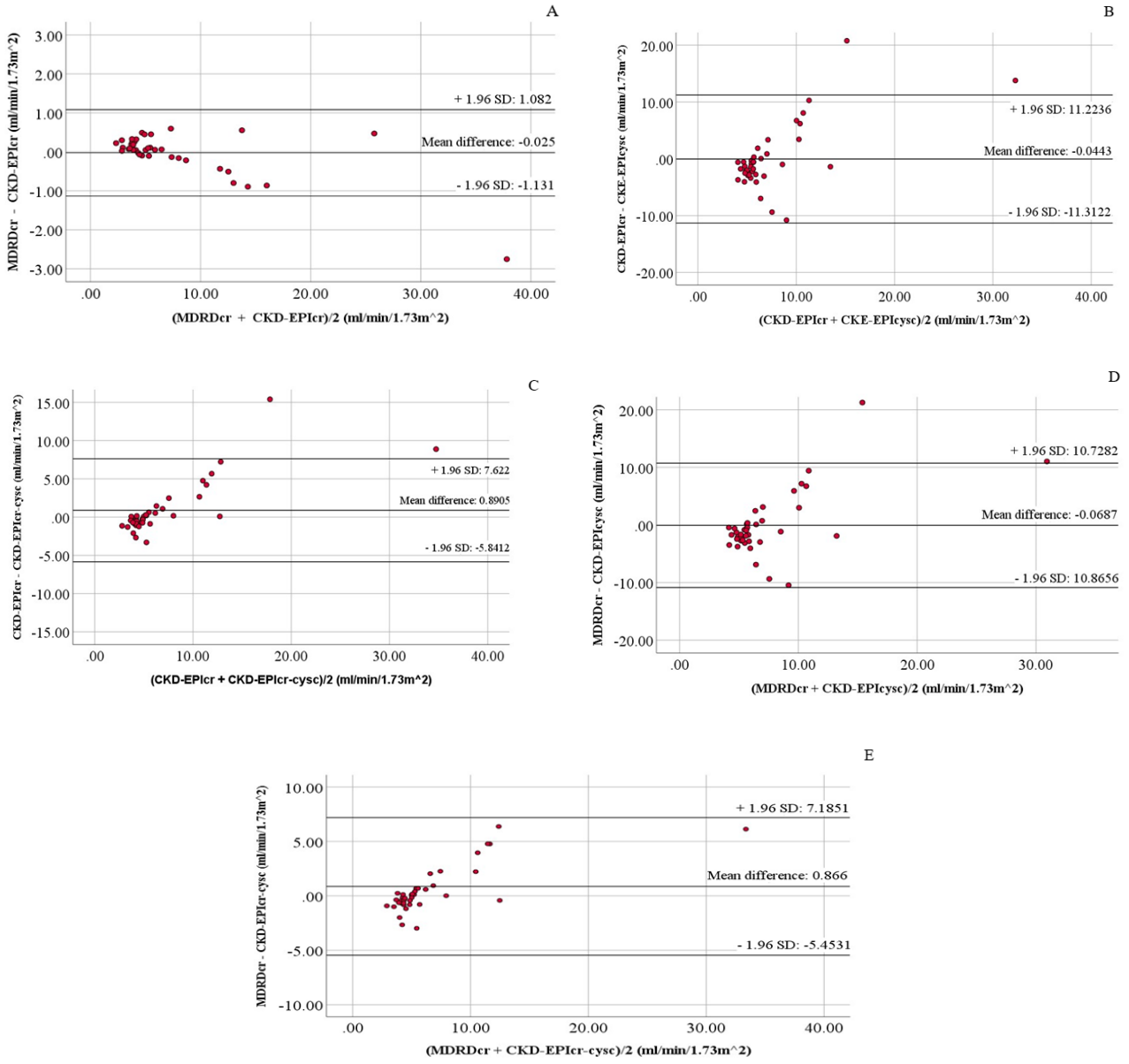


Figure 2: Bland Altman plots for the assessment of degree of agreement between CKD-EPI$_{Cr}$ and MDRD (A), CKD-EPI$_{Cr}$ and CKD-EPI$_{CysC}$ (B), CKD-EPI$_{Cr}$ and CKD-EPI$_{Cr\text{-}CysC}$ (C), CKD-EPI$_{CysC}$ and MDRD (D), and CKD-EPI$_{Cr\text{-}CysC}$ and MDRD (E) equations. The difference between the eGFR values of two separate equations are plotted against the average eGFR values of the same pair.

A relatively higher significant positive correlations were observed for eGFR calculated based on CKD-EPI_{Cr} - CKD-$EPI_{Cr\text{-}Cys\ C}$ ($r = 0.921$, $p = 0.000$) and MDRD - CKD-$EPI_{Cr\text{-}Cys\ C}$ ($r = 0.906$, $p = 0.000$) pairs when compared to the CKD-EPI_{Cr} - CKD-$EPI_{Cys\ C}$ ($r = 0.575$, $p = 0.000$) and MDRD - CKD-$EPI_{Cys\ C}$ ($r = 0.552$, $p = 0.000$). These findings suggest potential improvement in the correlation of eGFR values by CKD-$EPI_{Cr\text{-}Cys\ C}$ by summation of serum cystatin-C with creatinine rather than using it alone. These findings are further supported by previous reports (Hu *et al*., 2020; Inker *et al*., 2012).

The advantages of using the combined eGFR equation rather than the equations based on creatinine or cystatin-C alone have been highlighted in several studies (Inker *et al*., 2012). According to the explanations, it is hypothesized that errors due to non-GFR determinants of both creatinine and cystatin C would be minimized during the summation of serum cystatin-C with creatinine, leading to an improvement in the accuracy of the results. The CKD-EPI working groups also have explained the above factor, indicating that the errors due to non-GFR determinants of serum creatinine and cystatin-C are independent and smaller in the combined equation. This was further strengthened by the findings of Khalid *et al*. (2020), which revealed a strong correlation between the creatinine-cystatin-C combined eGFR equation, which will be useful in the clinical diagnosis of patients with CKD.

However, based on the present findings and the potential drawbacks associated with the assessment of cystatin-C, including the involvement of complicated and time-consuming procedures, financial constraints, etc. the use of creatinine based eGFR equations (CKD-EPI_{Cr} and MDRD) could be beneficial for the routine clinical setting in Sri Lanka.

The eGFR values for different equations are summarized in Table 2, in the aspect of different age groups and gender.

The correlation of eGFR values derived from different equations with age was assessed using Person's correlation and the results are shown in Table 3. No significant correlation was observed in either equation of eGFR with the age in the present study ($p>0.05$). Similarly, none of the equations showed significant results during the comparison of different equations of eGFR with gender by independent sample t-test ($p>0.05$).

Table 3: Correlation of different eGFR equations with age of the study population.

	Pearson correlation (r)	p value
MDRD	0.025	0.877
CKD-EPI_{Cr}	-0.008	0.960
CKD-EPICys C	-0.060	0.714
CKD-EPICr-Cys C	-0.059	0.716

The correlation findings on eGFR values based on different eGFR equations with respect to age and gender support the fact that the equations could be applied disregarding the age and gender of the patient. However, considering the absence of previously published supportive reports and the minimum number of recruited participants in the present study, it is worth studying the association between eGFR values calculated based on different equations of creatinine and cystatin-C in a large cohort of CKD patients on hemodialysis treatment.

Among the different equations for the calculation of eGFR, the equations based on serum creatinine are more common (Ferguson, *et al*., 2015). Furthermore, equations based on cystatin-C alone and combined creatinine and cystatin-C-based equations are also widely used in current clinical practice. However, to date, no conclusions have been drawn on which equations should be used for an accurate estimation of GFR in the Sri Lankan population. The findings on the applicability of different eGFR equations in different study populations with different ethnicities further signify the requirement to evaluate the applicability of these equations for the Sri Lankan population to properly assess the progression in patients with kidney impairment of CKD patients on hemodialysis treatment.

The present study was conducted using a total of 40 samples received from CKD patients on hemodialysis treatment, to compare different equations of creatinine and cystatin C-based eGFR in patients on hemodialysis treatment. A reduction of the sample size to 40, considering the limited number of samples received at the Chemical Pathology Laboratory of Teaching Hospital Karapitiya, Galle, Sri Lanka is a limitation of this study. Moreover, the majority of the CKD patients enrolled in this study were from the age group 39 - 59 years and more importantly represent the working population of the country. However, it would be interesting if it is possible to analyze the effect of

Table 2: eGFR values from different equations.

	eGFR (mL/min/1.73m^2)			
	MDRD	CKD-EPI_{Cr}	CKD-EPI_{CysC}	CKD-$EPI_{Cr\text{-}CysC}$
Age groups				
18-38 years	6.14± 2.66	6.23± 2.82	7.23± 1.20	6.05± 1.72
39-59 years	7.76± 7.64	7.99± 8.31	7.78± 4.70	6.99± 5.81
60-80 years	7.51± 6.85	7.11± 6.79	6.94± 2.60	5.90± 2.02
Gender				
Male	8.15± 8.10	8.12± 8.57	7.42± 4.25	6.87± 5.54
Female	6.41± 4.17	6.51± 4.55	7.46± 2.93	6.05± 2.38

The results are expressed as mean ± SD.

occupation on the severity of the disease, which is another limitation of the study.

CONCLUSION

The present findings revealed that MDRD and CKD-EPI$_{Cr}$ equations may be suitable for the calculation of eGFR in routine local clinical settings for the assessment of the progression of kidney impairment of patients with CKD on hemodialysis treatment. Furthermore, based on the findings, it can be recommended to use the CKD-EPI$_{Cr\text{-}CysC}$ equation rather than the CKD-EPI$_{Cys}$ in specific circumstances as a confirmatory tool.

ACKNOWLEDGEMENT

The authors wish to acknowledge Prof. Bilesha Perera, Department of Community Medicine, Faculty of Medicine, University of Ruhuna, for the guidance given in statistical data analysis. Further, the laboratory staff of the Chemical Pathology Laboratory, Teaching Hospital, Karapitiya, Galle, Sri Lanka, and the Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna is acknowledged for the technical assistance provided.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ferguson, T.W., Komenda, P., & Tangri, N. (2015). Cystatin-C as a biomarker for estimating glomerular filtration rate. *Current Opinion in Nephrology and Hypertension,* **24**(3), 295-300. doi:10.1097/MNH.0000000000000115.

He, L., Wei, Q., Liu, J., Yi, M., Liu, Y., Liu, H., Sun, L., Peng, Y., Liu, F., Venkatachalam, M.A., & Dong, Z. (2017) AKI on CKD: heightened injury, suppressed repair, and the underlying mechanisms. *Kidney International,* **92**(5), 1071-1083. doi:10.1016/j.kint.2017.06.030.

Hill, N. R., Fatoba, S.T., Oke, J.L., Hirst, J.A., O'Callaghan, C.A., Lasserson, D.S., & Hobbs, F.D.R. (2016). Global prevalence of chronic kidney disease–a systematic review and meta-analysis. *PloS One,* **11**(7), e0158765. doi:10.1371/journal.pone.0158765.

Hu, J., Xu, X., Zhang, K., Li, Y., Zheng, J., Chen, W., & Wang, X. (2020). Comparison of estimated glomerular filtration rates in Chinese patients with chronic kidney disease among serum creatinine, cystatin-C-and creatinine-cystatin-C-based equations: A retrospective cross-sectional study. *Clinica Chimica Acta,* **505**, 34-42. doi:10.1016/j.cca.2020.01.033.

Hulley, S. B., Cummings, S. R., Browner, W. S., Grady, D., & Newman, T. B. (2013). Appendix 6C. *Designing clinical research: An epidemiologic approach*, ***79***.

Inker, L.A., Astor, B.C., Fox, C.H., Isakova, T., Lash, J.P., Peralta, C.A., Tamura, M.K., & Feldman, H.I. (2014). KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. *American Journal of Kidney Diseases,* **63**(5), 713-735. doi:10.1053/j.ajkd.2014.01.416.

Inker, L.A., Schmid, C.H., Tighiouart, H., Eckfeldt, J.H., Feldman, H.I., Greene, T., Kusek, J.W., Manzi, J., Lente, F.V., Zhang, Y.L., Coresh, J., & Levey, A.S. (2012). Estimating glomerular filtration rate from serum creatinine and cystatin-C. *New England Journal of Medicine,* **367**(1), 20-29. doi:10.1056/NEJMoa1114248.

Khalid, U.B., Haroon, Z.H., Aamir, M., Ain, Q.U., Mansoor, K., & Jaffar, S.R. (2020). Comparison of estimated glomerular filtration rate with both serum creatinine and cystatin-C (eGFRcr-cys) versus single analyte (eGFRcr or eGFRcys) using CKD-EPI and MDRD equations in tertiary care hospital settings. *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP,* **30**(7), 701-706. doi:10.29271/jcpsp.2020.07.701.

Lv, J.C., & Zhang, L.X. (2019). Prevalence and disease burden of chronic kidney disease. *Renal Fibrosis. Mechanisms and Therapies,* **1165**, 3-15. doi:10.1007/978-981-13-8871-2_1.

Maheshwari, K.U., Santhi, S., & Malar, R.J. (2015). Cystatin-C: An alternative dialysis adequacy marker in high flux hemodialysis. *Indian Journal of Nephrology,* **25**(3), 143. doi:10.4103/0971-4065.139489.

Munikrishnappa, D. (2009). Limitations of various formulae and other ways of assessing GFR in the elderly: is there a role for cystatin-C. *Nephrology*. Retrieved from https://www.asn- online.org/education/distancelearning/curricula/geriatrics/Chapter6.pdf

Pasala, S., & Carmody, J.B. (2017). How to use serum creatinine, cystatin-C and GFR. *Archives of Disease in Childhood-Education and Practice,* **102**(1), 37-43. doi:10.1136/archdischild-2016-311062.

Rajapakse, S., Shivanthan, M.C., & Selvarajah, M. (2016). Chronic kidney disease of unknown etiology in Sri Lanka. *International Journal of Occupational and Environmental Health,* **22**(3), 259-264. doi:10.1080/10773525.2016.1203097.

Rule, A.D., & Lieske, J.C. (2011). Cystatin-C is more than GFR, and this may be a good thing. *Journal of the American Society of Nephrology,* **22**(5), 795-802. doi:10.1681/ASN.2011030288.

Toffaletti, J.G. (2018). Clarifying the confusion of GFRs, creatinine, and cystatin-C. Retrieved from https://acutecaretesting.org/en/article/clarifying-the-confusion-of-gfrs-creatinine-and-cystatin-c.

Zou, L.X., Sun, L., Nicholas, S.B., Lu, Y., Sinha, S., & Hua, R. (2020). Comparison of bias and accuracy using cystatin-C and creatinine in CKD-EPI equations for GFR estimation. *European Journal of Internal Medicine,* **80**, 29-34. doi:10.1016/j.ejim.2020.04.044.