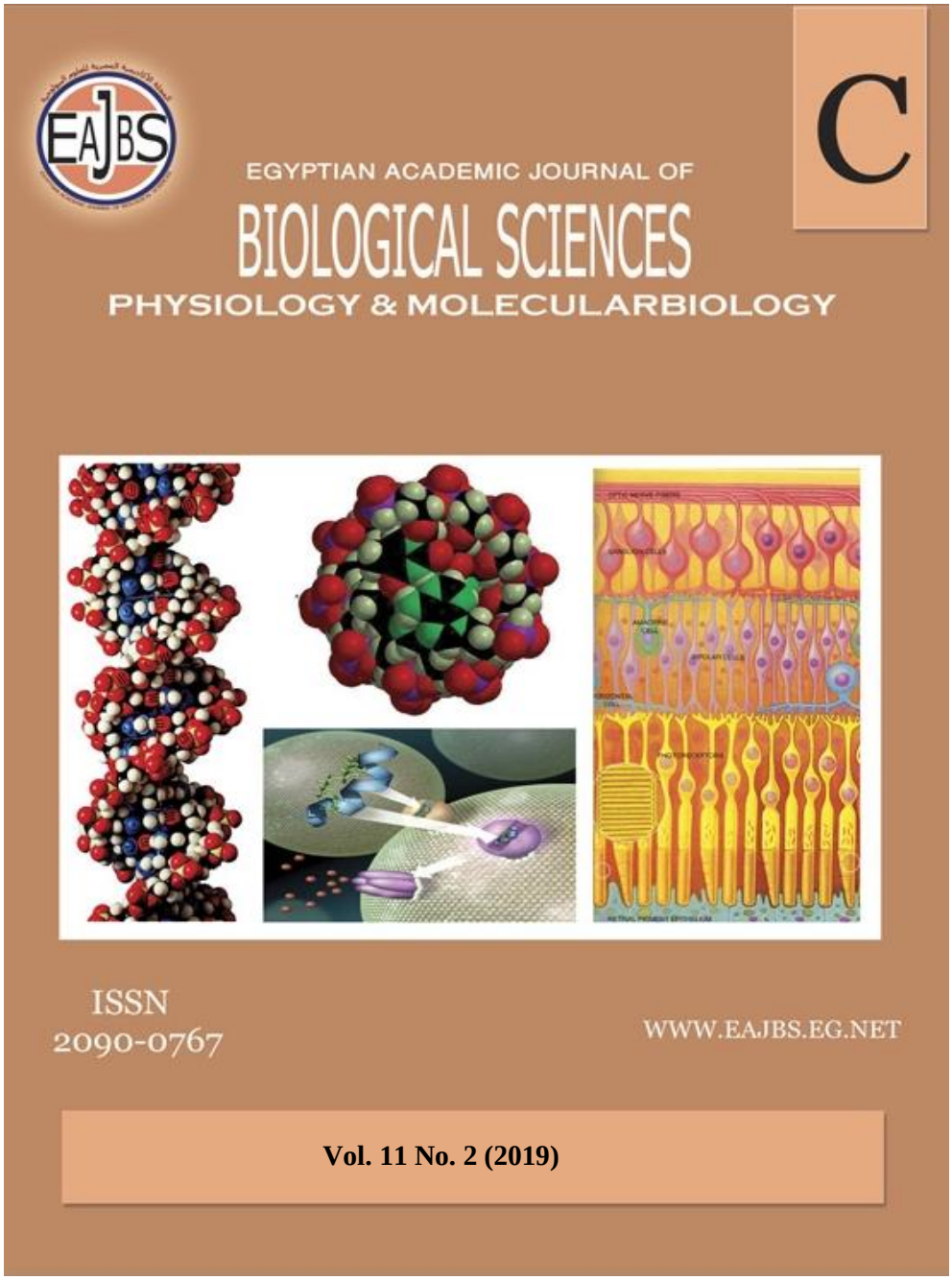

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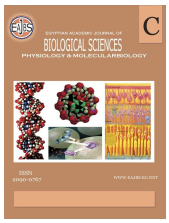
Citation: *Egypt. Acad. J. Biolog. Sci. (C. Physiology and Molecular biology) Vol. 11(2 1) pp.111-122(2019)*

Egypt. Acad. J. Biolog. Sci., 11(2): 111- 122 (2019)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
http://eajbsc.journals.ekb.eg



Evaluation of three Glomerular Filtration Rate (GFR) Equations Discriminates Stages of Chronic Kidney Disease (CKD) in Sudanese Patients

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ARTICLE INFO
Article History
Received:20/4/2019
Accepted:27/5/2019

***Keywords*:**
Plasma cystatin C, Plasma creatinine, Glomerular filtration rate, CKD, ROC analysis

ABSTRACT

Background: Chronic kidney disease (CKD), represent a diagnostic challenge. CKD is an asymptomatic disease, tests for screening and early diagnoses are critical in nephrology.

Objectives: To assess the potential role of a three glomerular filtration rate (GFR) calculation equations in discriminating between stages of chronic kidney disease (CKD) in Sudanese patients.

Methods: The study was carried out at Khartoum State, Sudan from July 2017 to August 2017. Three hundred and seventy-five (375) CKD patients aged 18–90, their GFR have been estimated by three equations as follows: combined creatinine (cr) and cystatin C (cysC) equation (GFRcr-cysC), Modification of Diet in Renal Disease (MDRD) equation (GFRcr) and CystatinC-based equation (GFRcysC). The discriminating ability between stages was tested by ROC analysis.

Results: The area under the receiver operating (AUC) curve values of the discriminated stages was tested by ROC analysis as the follows: between stage 1 and 2 were: GFRcr-cysC; 0.98 ($p > 0.001$), GFRcr; 0.77 ($p < 0.001$) and GFRcysC; 0.82 ($p < 0.01$). Between stage 2 and 3 were: GFRcr-cysC; 0.98 ($p > 0.001$), GFR cr; 0.82 ($p < 0.001$) and GFRcysC; 0.89 ($p < 0.001$). Between stage 3 and 4 were: GFRcr-cysC; 0.96 ($p > 0.001$), GFRcr; 0.92 ($p < 0.001$) and GFRcysC; 0.67 ($p < 0.001$). Between stage 4 and 5 were: GFRcr-cys; 0.99 ($p > 0.001$), GFRcr; 0.95 ($p < 0.001$) and GFRcysC; 0.65 ($p < 0.001$). GFRcr-cysC had the highest AUC of all.

Conclusion: Taking together, the study demonstrated that GFRcr-cysC equation is a valuable marker for early detection of CKD.

INTRODUCTION

The diagnosis and staging of Chronic Kidney Disease (CKD) based on estimates of GFR from plasma creatinine (cr) and cystatin C (cysC) concentrations represent a major step in the diagnosis of CKD and in following its course but has significant limitations. The main limitations of this methodology are its low discriminatory power in establishing the presence or absence of early CKD in individuals and its unsatisfactory performance in predicting the course of CKD.

The direction of Research in this field is currently towards identifying new biomarkers that either is superior indicators of GFR or indicate early injury of the renal tissue. The last group of biomarkers has the potential of leading to improved early detection of CKD (Ahmed *et al*, 2017). CKD is an asymptomatic disease, tests for screening and early diagnoses are critical in nephrology. The tests that best detect abnormalities in kidney function and defined stages of CKD are those that measure GFR (Taal, 2011).

Epidemiology: Although, several epidemiologic studies were conducted to estimate the disease burden in different parts of these countries, the largest being the EGIPT-CKD (Egypt Information, Prevention, and Treatment of CKD) Project. Preliminary data for about 1,000 participants showed the prevalence of proteinuria to be as high as 21%, including 3.9% with elevated serum cr levels (Barsoum, 2012).

Risk Factors: CKD is affected by various risk factors, including demographic variables (age, gender and ethnicity), hereditary factors, primary renal disease, cardiovascular disease and nephrotoxins act. (Taal, 2011; Parveen *et al*, 2016).

However, Abd ElHafeez *et al,* (2018) reported that the main risk factors of CKD in Africa is attributed to hypertension and diabetes. The poor data quality restricts the validity of the findings and draws attention to the importance of designing future robust studies.

Estimation of the GFR:

The GFR can be estimated from the plasma concentration of filtration markers (such as cr or urea) (Nankivell, 2001 and Burtis, 2012). Although, estimating cr is not costly there are limitations of using cr in calculating GFR such as muscle mass, age, gender, ethnic race, and diet. Moreover, these factors result in a complication of its equations that calculate GFR. However, cr is not sensitive in CKD early stages. On the other hand, cysC is not affected by those factors mentioned above, so that, its equations that calculate GFR are simple. Moreover, cysC is sensitive in diagnoses early stages of CKD (Taal, 2011). But in clinical practice, cysC is economically costly especially during a routine check-up in those living in areas where little access to primary health care is available (Filler, 2005).

Janice *et al*, (2017) reported that there is a good correlation between cr and cysC this fact gives the one an idea to propose a new simple model for calculating GFR in early stages in CKD the model used the predictive value of cysC from estimated cr.

The receiver operating characteristic (ROC) curve: This test provides a way of assessing whether a particular type of test provides useful information, and can be used to compare two different tests and to select an optimal cut-off value for a test (Aviva, 2009)

MATERIALS AND METHODS

The aim of this study was to assess the potential role of a three glomerular filtration rate (GFR) calculation equations in discriminating between stages of CKD in Sudanese patients.

Enrolment of Patients:

A total of Three hundred and seventy-five (375) patients were included for final analysis. They were collected from three different regions of Khartoum State, Sudan of the following Health Institutions renal centers:

1- Hospital A: Dr. Selma Center for renal disease, University of Khartoum (Khartoum)
2- Hospital B: Military hospital (Omdurman)
3- Hospital C: Ahmed Gasim Hospital (Bahri, Khartoum North)

The study was carried out at Ahmed Gasim the teaching hospital of the

University of Alzaiem Alazhari, Khartoum, Sudan, on 375 subjects some of them diagnosed with CKD when visited the Nephrology Outpatient Department (OPD) between July/August 2017.

Ethical Approval:

The study protocol was approved by the research ethics board (REB), Khartoum State Ministry of Health, Sudan (No: WS/WK/AGTG/44/A- Date: 2017/8/10th). Informed consents were obtained from all the study participants.

Diagnosis of CKD:

A diagnosis of CKD was made by the nephrologist based on the National Kidney Foundation Kidney/Disease Outcome Quality Initiative guidelines, according to those individuals with an eGFR <60 mL/min/1.73 m^2 for 3 months are classified as having CKD (Levey *et al*., 2003 and KDOQI, 2008). CKD subjects aged between 18-90 years were included in the study. Individuals with skeletal muscle atrophy, malnutrition, heart failure, ketoacidosis, hypothyroidism or hyperthyroidism, malignant tumor, and acute inflammatory conditions were excluded from this study.

Blood Sampling:

A sample of 5 ml venous blood was collected from each subject and drawn into a heparinized plasma vacationer. Blood is drawn into green top heparin tubes, inverted 8-10 times immediately after the blood sample has been taken. Centrifugation conditions: ≤1300 g for 10 minutes at 18–25°C then transferred into a plain container and stored at – 20 degrees Celsius until analysis.

Laboratory Assessment:

Biochemical parameters analyzed were plasma cr and plasma cysC. cr was estimated by kinetic colorimetric compensated Jaffe method (Jaffe *et al.,* 1986), as reported by the manufacturer, ROCHE COBAS c311 clinical chemistry automated analyzer (Roche Diagnostics, Swiss), which was standardized to the spectrometry reference, and cysC was estimated by measured ELISA enzymatic activity measurements in the wavelength range between 400 and 750 nm using kinetic measurement methods. (Convergys® EL-Reader 96X, US). (Png *et al*., 2011).

GFR Estimates:

Patient samples were classified according to the proposed KDIGO classification of chronic kidney disease into 5 stages (Levey *et al*., 2011). After the determination of plasma concentrations, glomerular filtration rate was estimated by using the following three equations: 1- GFR cr-cysC: the equation used combined cr and cysC as following: $eGFR = 169 \times cr^{-0.608} \times cysC^{-0.63} \times Age^{-0.157} (Female \times 0.83)$ (Ma, *et al*. 2007)). 2- GFR cr: Modification of Diet in Renal Disease (MDRD) equation, GFR is calculated from demographic data and a single plasma cr result (in mg/dL) $(eGFR = 186 \times (\text{plasma creatinine})^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if black})$ (Levey *et al*. 1999)). 3- GFR cysC: In order to estimate GFR based solely on plasma cysC levels the following equation was implemented:

$(eGFR = 86.7 / cysC) - 4.2$ (MacIsaac *et al*. 2006)).

Where, eGFR (estimated glomerular filtration rate) = ml/min/1.732 m2

Plasma cr = Standardized plasma creatinine in mg/dl

Age = years

Plasma cysC = Standardized plasma Cystatin C in mg/L

Statistical Analysis:

Statistical analysis was done using SPSS. Data were represented as mean ± standard deviation. One way anova test was used for comparison between the groups. The ability of the studied tests and formulae to discriminate stages of CKD was evaluated using the area under the ROC curve (AUC). The sensitivity, specificity, differential positive rate

(DPR), Diagnostic accuracy (DA), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were calculated according to the following formulae:

Sensitivity = a/(a+c)
Specificity = d/(b+d)
DPR = sensitivity + specificity -100
DA = a+d\a+b+c+d
PPV = a/(a+b)
NPV = c/(c+d)
PLR = sensitivity /(1-specificity)
NLR = (1- sensitivity) /specificity

Where: a = true positive cases, c = false negative cases, d = true negative cases, b = false positive cases.

Statistical significance was considered as $p < 0.001$.

RESULTS

The aim of this study was to assess the potential role of a three GFR calculation equations in discriminating between stages of CKD in Sudanese patients.

Demographic and clinical characteristics of the study population:

Demographic variables including age, gender and ethnicity are summarized in Table 1. *P-values* are presented to indicate any significant differences between the groups. An analysis of variance showed that among the 375 individuals recruited, there was a significant difference between the three renal centers for age p =0. 002. However, there were no significant differences between the renal centers in gender p =0.202 across the three hospitals. 51.47% of the total percentage was male, and the mean age was 51.66 ± 14.39 years as shown in Table 1. All patients ethnicity fall under the Non-African-American Category.

Table 1 Demographic variable by the center.

Overall	Hospital A	Hospital B	Hospital C	Total	Test Statistic	*p*-value
Variable	(n=92)	(n=213)	(n=70)	(n=375)	ANOVA	
Age mean ± (SD)	47.29 (16.66)	52.66 (12.02)	54.37 (16.65)	51.66 (14.39)	F=6.152 df=2, 372	0. 002
Gender % Male	25.9	53.9	20.2	51.47	F=0.700 df=2, 292	0. 497
Ethnicity	Non-African-American Category					

CKD prevalence in the study population:

Patient samples were classified according to the proposed KDIGO classification of chronic kidney disease (Levey *et al*., 2011); number of the patients with stage three had the highest percentage value 101 (26.9%) as presented in Table 2 and Figure 2.

Overall, 58 (15.5%) of the patients had normal eGFR, whereas three-quarters of the analyzed subjects had CKD as the following: 66 (17.6%) had mild decrease excretory renal function (eGFR: 60-89 ml/min/1.73m^2), about 101 (26.9%) of the patients had a moderate decrease eGFR of 59-30 ml/min/1.73m^2, and 81 (21.6%) showed severe decrease in eGFR (29-15 mL/min/1.73 m^2). And about fifth 69 (18.4%) of the patients had a substantially renal failure <15 mL/min/1.73m^2 (Table 2 and Figure1).

Table 2 Number of CKD stages by hospitals.

eGFR Stage (ml/min/1.73m2)	Hospital			
	Hospital A	**Hospital B**	**Hospital C**	Total
Stage 1 (> 90)	24	17	17	58
Stage 2 (60-89)	14	34	18	66
Stage 3 (59-30)	18	59	24	101
Stage 4 (29-15)	9	61	11	81
Stage 5 (<15)	26	38	5	69
Total	91	209	75	375

eGFR, estimated glomerular filtration rate (calculated using the MDRD formula); KDIGO, Kidney Disease, Improving Global Outcomes.

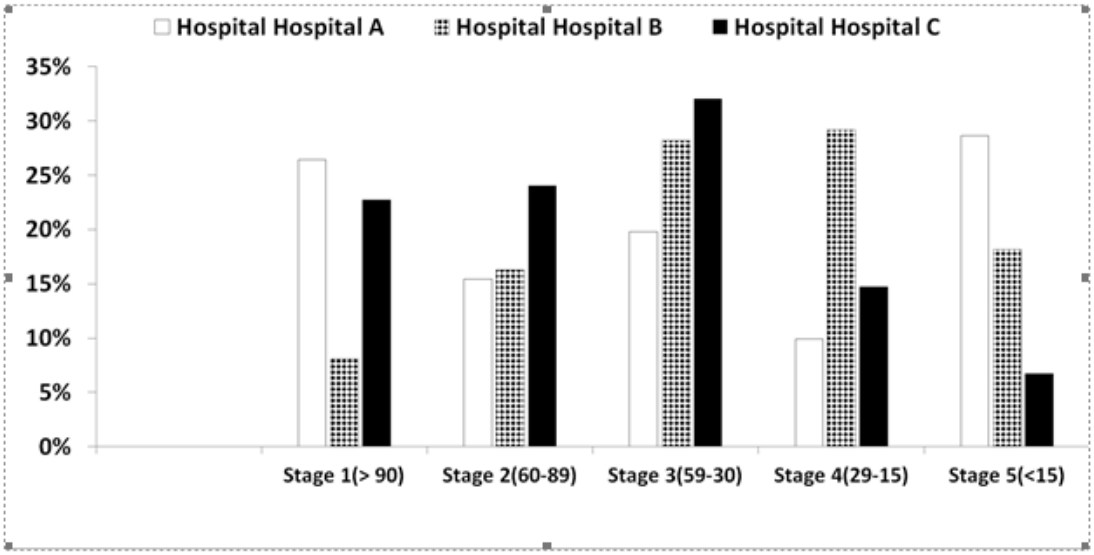

Fig. 1 Percentage of CKD stages by hospitals.

ROC analysis of the studied markers and their diagnostic criteria:

Discriminating between stage 1 and stage 2 of CKD:

GFR cr–cysC: The discriminating ability of the studied markers, GFR cr–cysC in detecting early CKD was assessed by plotting the ROC curves, which were drawn by the sensitivity and (1-specificity) at different cut-off levels (Table 3 and Figure 2.). The AUC was 0.983 indicating the success of using GFR cr–cysC as a diagnostic marker for stage 1 and stage 2 of CKD. The nearest point to the upper left corner of the Figure 2 represent the optimal threshold (90.15 mL/min/1.73 m^2), the sensitivity was (100 %), the specificity was (97.3 %) and the DA, positive and negative predictive values, and DPR were (98.65 %), (100 %) and (97%) respectively. As for the likelihood ratios (LR) positive and negative, the PLR was 37.04 and the NLR was zero (Table 3). The maximum detected DPR was at (0.973).

GFR cr:

Figure 2 Represent the ROC curve of GFR cr as a diagnostic marker for stage 1 and stage 2 of CKD population. The AUC was 0.77 indicating the validity of using GFR cr as a diagnostic marker for CKD. The nearest point to the upper left corner of the Figure 2 refers to the optimal cur-off value (83.75 mL/min/1.73 m^2, Table 3). The sensitivity, specificity, DA, DPR, PPV and NPV were (62.2 %), (83.8 %), (77.02 %), (0.459), (100%) and (97%) respectively.

whereas the PLR was 3.84 and NLR was 0.45 (Table 3).

GFR cysC:

The AUC of GFR cysC 0.821, indicating the successes of using GFR cysC as a diagnostic marker for stage 1 and stage 2 of CKD (Figure 2). The nearest point to the upper left corner refers to the optimal cut-off value (97.25 mL/min/1.73 m^2) and large AUC. The sensitivity, specificity, DA, DPR, positive and negative predictive values were (81.1 %), (73 %), (78 %), (0.541), (71%) and (96%) respectively whereas the PLR was 3.0 and NLR was 0.26 (Table 3).

Discriminating between stage 2 and stage 3 of CKD:

GFR cr–cysC:

After ROC curve analysis, we revealed the optimal cut-off value which is the nearest point to the upper left corner that satisfies maximum sensitivity and maximum specificity. For the GFR cr–cysC (Fig. 3) an optimum cut-off level of (59.75 mL/min/1.73 m^2) and AUC 0.98 at which the sensitivity was 97.1% and specificity 96.3%. DA, DPR, positive and negative predictive values were (96%), (0.935), (99 %), and (89%) respectively. As for the LR positive and negative, the PLR was 26.24 and the NLR was 33.21 (Table 3).

GFR cr:

ROC analysis revealed an optimal cut-off value of (41.95 mL/min/1.73 m^2) at and AUC 0.81.7 which the sensitivity (85.7%) and specificity (69.5%) were obtained. The DA, DPR, PPV and NPV were (52%), (0.703), (100 %), and (38%) respectively whereas the PLR was 2.81 and NLR was 33.21 (Table 3 and Figure 3).

GFR cysC:

At (62.6 mL/min/1.73 m^2) as a cut-off value and AUC 0.889, the sensitivity was 88.6% and specificity was 81.7%. The DA, DPR, PPV and NPV were (93%), (0.703), (99 %), and (83%) respectively whereas the PLR was 4.84 and NLR was 0.14 (Table 3 and Figure 3).

Discriminating Between Stage 3 and Stage 4 of CKD:

GFR cr–cysC:

Figure 4 showed the AUC was 0.962 indicating the success of using GFR cr–cysC as a diagnostic marker for stage 3 and stage 4 of CKD. The nearest point to the upper left corner represents the optimal threshold (30.05 mL/min/1.73 m^2), the sensitivity was (96.5 %), the specificity was (98.4 %) and the DA, DPR, PPV and NPV values were (97%), (0.949), (95%), and (99%) respectively. As for the LR positive and negative, the PLR was 60.31 and the NLR was 0.04 (Table 3).

GFR cr:

Figure 4 Represent the ROC curve of GFR cr as a diagnostic marker for stage 3 and stage 4 of CKD population. The AUC was 0.924 indicating the validity of using GFR cr as a diagnostic marker for CKD. The nearest point to the upper left corner refers to the optimal cut-off value (19.35 mL/min/1.73 m^2, Table 3). The sensitivity, specificity, DA, DPR, PPV and NPV values were (96 %), (0.739), (92 %) and (99%) respectively. whereas the PLR was 8.61 and NLR was 0.18 (Table 3).

GFR cysC:

The AUC of GFR cysC 0.667, indicating the successes of using GFR cysC as a diagnostic marker for stage 3 and stage 4 of CKD (Fig. 4). The nearest point to the upper left corner refers to the optimal cut-off value (49.95 mL/min/1.73 m^2) .The sensitivity, specificity, DA, DPR, PPV and NPV values were (51.8%), (83.9%), (91%), (0.356), (95%) and (89%) respectively whereas the PLR was 3.22 and NLR was 0.57 (Table 3).

Discriminating Between Stage 4 and Stage 5 of CKD:

GFR cr–cysC:

ROC curve analysis for GFR cr–cysC revealed that, the optimal cut-off value which is the nearest point to

the upper left corner that satisfies maximum sensitivity and maximum specificity for the stage 3 and stage 4 of CKD. (Figure 5.), the optimum cut-off level was (14.6 mL/min/1.73 m^2, Table 3) and AUC 0.987 at which the sensitivity was 97% and specificity 96%. DA, DPR, PPV and NPV values were (97%), (0.93), (92%) and (98%) respectively. As for the LR positive and negative, the PLR was 24.25 and the NLR was 0.03 (Table 3).

GFR cr:

ROC analysis revealed an optimal cut-off value of (6.1 mL/min/1.73 m^2, Table 3) and AUC 0.951 at which the sensitivity (84.8%) and specificity (92%) were obtained. The DA, DPR, PPV and NPV were (73%), (0.768), (50%) and (100%) respectively whereas the PLR was 10.6 and NLR was 0.17 (Fig. 5).

GFR cysC:

At (46.35 mL/min/1.73 m^2) as a cut off value and AUC 0.648, the sensitivity was 45.5% and specificity was 88%. The DA, DPR, PPV and NPV were (1%), (0.335), (1%) and (0.8%) respectively whereas the PLR was 3.79 and NLR was 0.62 (Table 3 and Figure 5).

Table 3 ROC analyses of the CKD stages

Stages		GFRcr–cysC	GFRcr	GFRcysC
stage 1 and stage 2	AUC	o.983	0.77	0.821
	SE	0.017	0.055	0.05
	p-value	0.001	0.001	0.001
	0.99 C. I.	0.950-1	0.662-0.877	0.723-0.919
	cutoff	90.15	83.75	97.25
	DPR	0.973	0.459	0.541
	DA	0.986	0.77	0.78
	sensitivity	1	0.622	0.811
	specificity	0.973	0.838	0.73
	PPV	1	1	0.71
	NPV	097.	0.69	0.96
	PLR	37.04	3.84	3.00
	NLR	0.0	0.45	0.26
stage 2 and stage 3	AUC	0.98	0.817	0.889
	SE	0.012	0.045	0.031
	p-value	0.001	0.001	0.001
	0.99 C. I.	0.957-1	0.73-0.905	0.829
	cutoff	59.75	41.95	62.6
	DPR	0.963	0.552	0.703
	DA	0.96	0.52	0.93
	sensitivity	0.971	0.857	0.886
	specificity	0.963	0.695	0.817
	PPV	0.99	1	0.99
	NPV	0.89	0.38	0.83
	PLR	0.26.24	2.81	4.84
	NLR	33.21	0.21	0.14

AUC: area under the curve DPR: differential positive rate DA: diagnostic accuracy PPV: positive predictive value NPV: negative predictive value PLR: likelihood ratio positive NLR: likelihood ratio negative

Table 3 (*Continue*) ROC analyses of the CKD stages

Stages		GFRcr–cysC	GFRcr	GFRcysC
stage 3 and stage 4	AUC	0.962	0.924	0.667
	SE	0.02	0.023	0.044
	p-value	0.001	0.001	0.001
	0.99 C. I.	0.922-1	0.88-0.969	0.58-0.755
	cutoff	30.05	19.35	49.95
	DPR	0.949	0.739	0.356
	DA	0.97	0.96	0.91
	sensitivity	0.965	0.835	0.518
	specificity	0.984	903	0.839
	PPV	0.95	0.92	0.95
	NPV	0.99	0.99	0.89
	PLR	60.31	8.61	3.22
	NLR	0.04	0.18	0.57
stage 4 and stage 5	AUC	0.987	0.951	0.648
	SE	0.011	0.02	0.061
	p-value	0.001	0.001	0.029
	0.99 C. I.	0.950	0.898	0.493-0.804
	DPR	0.93	0.768	0.335
	DA	0.97	0.73	0.1
	cutoff	14.6	6.1	46.35
	sensitivity	0.97	0.848	0.455
	specificity	0.96	0.92	0.88
	PPV	0.92	0.5	1
	NPV	0.98	1	0.8
	PLR	0.24.25	10.6	3.79
	NLR	0.03	0.17	0.62

AUC: area under the curve DPR: differential positive rate DA: diagnostic accuracy PPV: positive predictive value NPV: negative predictive value PLR: likelihood ratio positive NLR: likelihood ratio negative.

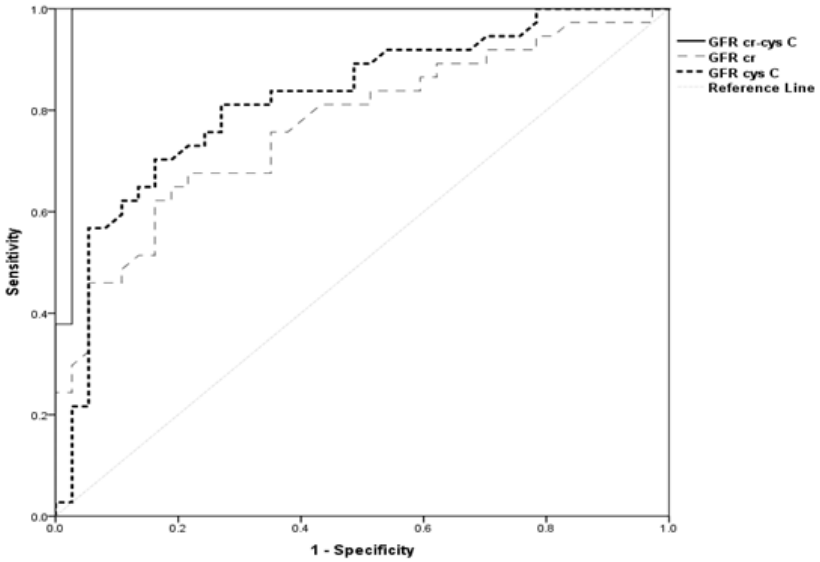

Fig. 2 ROC curve of GFR cr–cysC, GFR cr and GFR cysC equations to discriminate between stage 1 and stage 2 of CKD patients. AUC values were: GFR cr–cysC; 0.983 ($p > 0.001$), GFR cr; 0.770 ($p < 0.001$), GFR cysC; 0.821 ($p < 0.001$). GFR cr–cysC had the highest AUC.

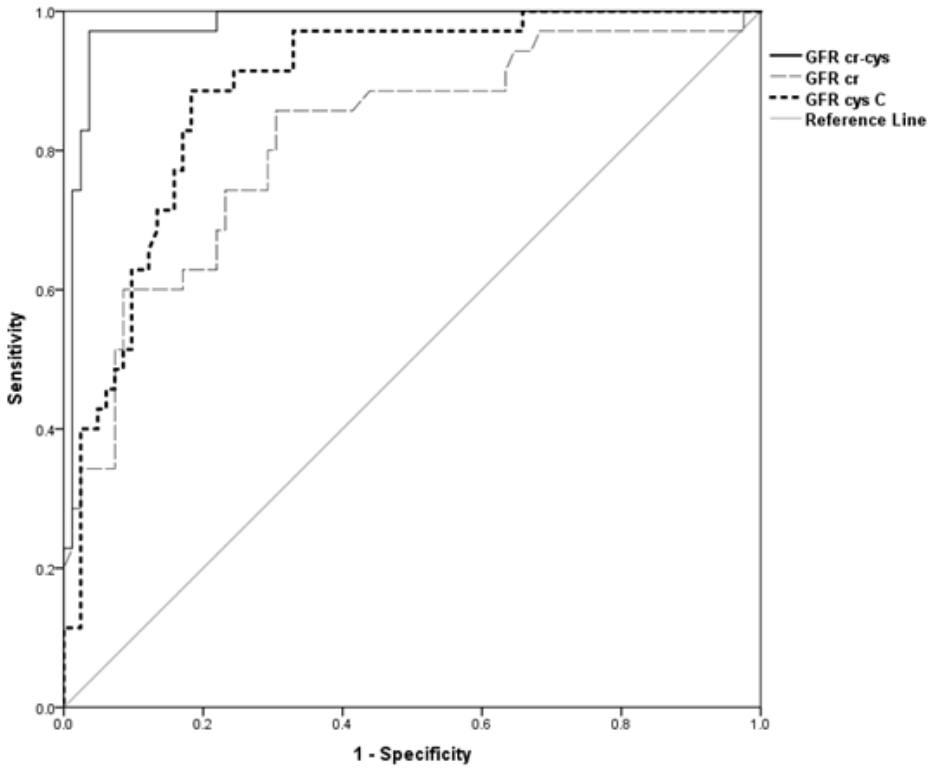

Fig. 3 ROC curve of GFR cr–cysC, GFR cr and GFR cysC equations to discriminate between stage 2 and stage 3 of CKD patients. AUC values were: GFR cr–cysC; 0.980 ($p > 0.001$), GFR cr; 0.817 ($p < 0.001$), GFR cysC; 0.889 ($p < 0.001$). GFR cr–cysC had the highest AUC.

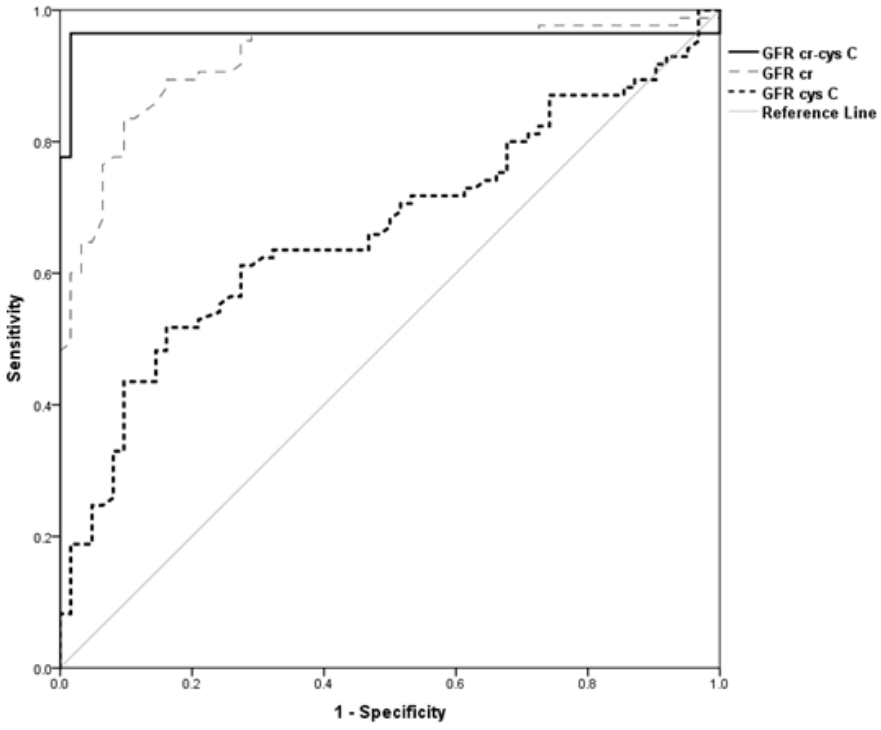

Fig. 4 ROC curve of GFR cr–cysC, GFR cr and GFR cysC equations to discriminate between stage 3 and stage 4 of CKD patients. AUC values were: GFR cr–cysC; 0.962 ($p > 0.001$), GFR cr; 0.924 ($p < 0.001$), GFR cysC; 0.667 ($p < 0.001$). GFR cr–cysC had the highest AUC.

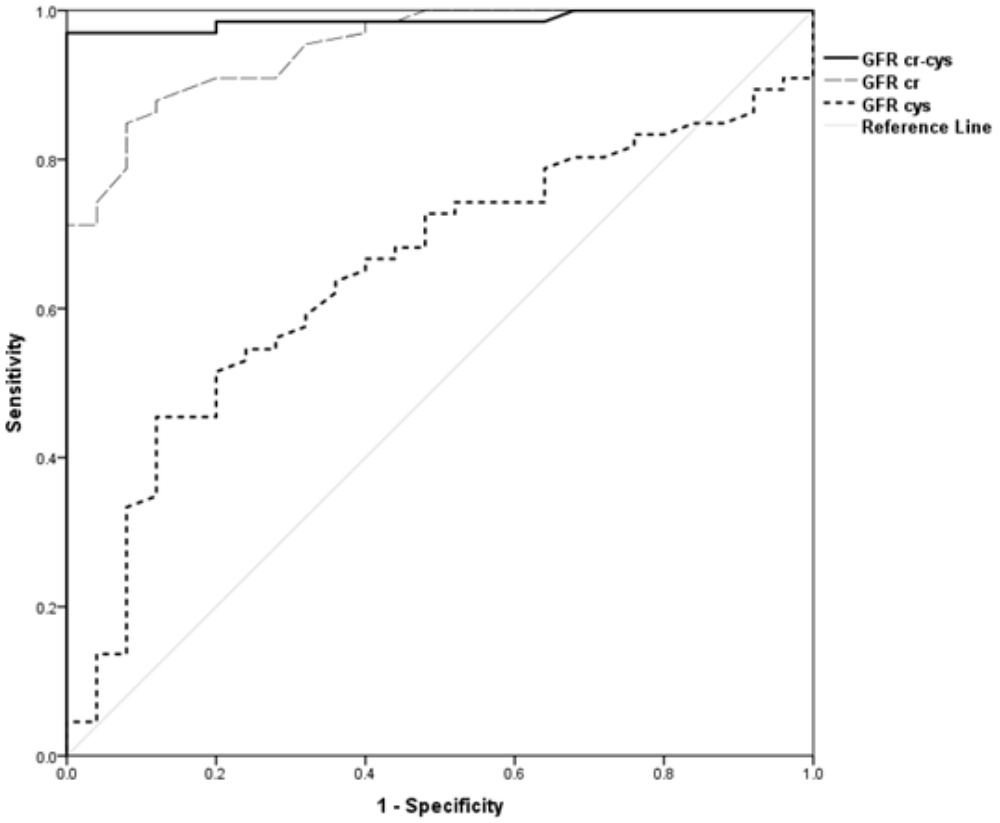

Fig. 5 ROC curve of GFR cr–cysC, GFR cr and GFR cysC equations to discriminate between stage 4 and stage 5 of CKD patients. AUC values were: GFR cr–cysC; 0.987 ($p > 0.001$), GFR cr; 0.951 ($p < 0.001$), GFR cysC; 0.648 ($p < 0.001$). GFR cr–cysC had the highest AUC.

DISCUSSION

Recent reports have stressed the need for new kidney markers that can enhance the accuracy of CKD detection for that, in the current study we evaluated three methods used in diagnosing the CKD. From the above, it is clear that the three methods significantly can be used to calculate the optimal cut-off value including mean ± SD, discriminant score and the ROC method (Table 3). The GFRcr–cysC method is the most accurate method within all stages with respect to the calculated DA and DPR. In addition, the ROC curve analysis is the only method can be used to evaluate a kidney marker as a whole according to its AUC (as shown in Table 3 and Figures 2, 3, 4 and 5) a finding that came in agreement with Jeffrey *et al*., (2015), who reported that combined eGFRcr-cysC performs best across all patient types for predicting measured GFR in comparison to the studied cr and cysC equations.

The predicted values of GFR based on separate GFRcysC and GFRcr equations was assessed with an evaluation of ROC curves, which are shown in Table 3, Figure 2 and Figure 3. The ROC curve using GFRcysC showed largest AUC values than that for GFRcr between stages 1 and 2 and between stage 2 and 3, this might reflect to some extent the GFRcysC preference over the GFRcr in this regard. On the other hand, the GFRcr showed in Table 3, Figure 4 and Figure 5 showed that AUC values higher than that for GFRcysC between the stage3 and 4 and between stage 4 and 5, this might reflect to some extent the poor ability of the GFRcysC in discriminating end stages of CKD this was in line with, Torre, *et al*, (2016) who reported that the accuracies of eGFRcysC and eGFRcr-cysC are

higher than eGFRcr in early stages, but is limited at end-stage of eGFRs.

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