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Agreement between the CKD-EPI serum creatinine- and cystatin C- based eGFR equations in pregnant and non-pregnant women: A cross-sectional study in Ghana

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ABSTRACT

Introduction: The serum cystatin C (sCys)-based estimated glomerular filtration rate (eGFRcys) is usually preferred to that of serum creatinine (eGFRcrt). However, the former is relatively more costly and may not be suitable for low- and middle-income countries such as Ghana. The study sought to determine the agreement between the sCrt- and sCys-based eGFR in pregnant and non-pregnant.

Materials and Methods: This was a cross-sectional study conducted from May 2020 to April 2022 in the Upper East Regional and War Memorial Hospitals. The study involved 281 women, 50 of whom were non-pregnant (nonP) and 231 of whom were normotensive pregnant (NP). Serum Crt and Cys were measured using a routine biochemistry analyzer and ELISA and which were then used to calculate the eGFR based on the CKD-EPI equations.

Results: A percentage difference in eGFR (bias) <40% was considered a measure of high agreement between the two methods. The sCrt was significantly higher (P<0.001), while eGFRcrt was significantly lower (P=0.001) in the normotensive pregnant women. The eGFRcys were significantly higher than eGFRcrt and the combined (eGFRcrt+cys) in both non-pregnant and pregnant women (P<0.001). There was no high agreement between eGFRcrt and eGFRcys (bias: -61% to -76%). Reduced eGFR (<60 mL/min/1.73m^2) was detected only by eGFRcrt with a frequency of 4.7% and 2.0% in pregnant and non-pregnant women, respectively.

Conclusion: There is a marked difference in eGFR based on Crt and Cys in pregnant and non-pregnant women. The sCrt—and sCys-based CKD-EPI equations need to be validated in a given population before use.

Keywords: Humans, Female, Glomerular filtration rate, Creatinine, Renal insufficiency, Ghana.

INTRODUCTION

Pregnancy alters kidney function due to hyperfiltration and pregnancy-associated kidney injury (PAKI) (1, 2). These factors contribute to altered glomerular filtration rate (GFR) and could lead to chronic kidney disease (CKD). It is therefore recommended to monitor pregnant women for PAKI or CKD and other kidney-related complications by measuring the glomerular filtration rate (GFR) (3).

Several methods have been used in assessing renal function or injury, including histological, urine albumin to creatinine ratio, proteinuria, etc. (4). However, the most widely used method for the determination of renal function is the measurement of GFR, of which the gold standard is the direct measurement (mGFR) using the urine or plasma

clearance of exogenous markers such as iothalamate, inulin or iohexol (5, 6). However, these methods may be cumbersome, invasive, time-consuming, and costly (5, 6). These have led to the formulation of equations based on endogenous analytes such as serum creatinine (sCrt) to estimate the GFR (eGFR). Several model equations have been formulated for eGFR, which are mainly based on sCrt levels, including the Full Age Spectrum (FASage), Modification of Diet in Renal Disease (MDRD), the Chronic Kidney Disease-Epidemiology collaboration (CKD-EPI), and the Cockcroft-Gault (CG)(7). The use of sCrt for estimating GFR has been controversial as its reliability has been questioned (6, 8). A new biomarker, serum cystatin C (sCys), has been advocated as a better measure of eGFR than sCrt (6).

The suggestion of superior performance of sCys to Crt has not been conclusive as previous studies are inconsistent in their findings (6, 8). While some studies recommend using Cys over sCrt or their combination over their equations, some have found no marked difference (4, 6, 9). One previous study categorically recommends against using sCys in estimating GFR in pregnancy, citing its unreliability (10). Moreover, multicenter studies among Africans and Indigenous Australians favour using sCrt over sCys in estimating GFR (4, 7). The performance of sCrt and sCys-based eGFR equations may depend on patient characteristics (11).

The measurement of sCys is relatively more costly than sCrt and may not be suitable for low- and middle-income countries (LMIC) such as Ghana (7). The sCrt-based eGFR will be preferred to the sCys-based if there is a high agreement between them in a given population. While previous studies have used eGFR equations in predicting CKD in varied study populations in Ghana (12, 13), hitherto, the agreement between the sCrt- and sCys-based CKD-EPI eGFR equations in pregnancy has not been adequately assessed, hence the aim of the study.

MATERIALS AND METHODS

Study design and settings

The cross-sectional study was conducted from May 1, 2020, to April 30, 2022, at the Upper East Regional Hospital (UERH), Bolgatanga, and the War Memorial Hospital (WMH), Navrongo. The UERH is the secondary-level healthcare facility that serves as a referral hospital in Ghana's Upper East Region (UER). The hospital also renders healthcare services to patients referred from healthcare facilities in neighbouring Burkina Faso and Togo. The WMH is a district hospital that renders health-related services to the residents of Navrongo and neighbouring communities.

Study population

The study participants were part of a larger study involving 281 women, 50 of whom were non-pregnant and 231 were pregnant. The pregnant women included first-trimester (n=51), second-trimester (n=73), and third-trimester (n=107) women. All participants had no known history of preeclampsia, CKD, thyroid dysfunction, melanoma, HIV, or other chronic disease that could markedly influence the outcome of the study.

Sample size

A previous study found the mean ± standard deviation of the eGFR based on serum creatinine and cystatin C in pregnant women to be 80 ± 26 and 73 ± 31 in mL/min/1.73 m^2, respectively (14). Using power analysis at 0.8 power, 5% alpha, and a 95% confidence interval, the minimum sample size for the non-equal two-sided test was 264.

Variables

The study variables included dependent and independent variables. The dependent variables were (i) serum creatinine, (ii) serum cystatin C, (iii) serum NGAL, (iv) the eGFR, and (v) urea. The independent variables included pregnancy status and the trimester of pregnancy. Possible confounding variables included the age and BMI of women at the time of sampling. The calculation of the eGFR, however, considers the participant's age and body surface area.

Data collection and measurements

Socio-demographic and anthropometric data

Socio-demographic data were collected using an interviewer-administered semi-structured questionnaire. Clinical data were also obtained from the women's clinical records held at the health facility. Body weight and standing height were measured to the nearest 0.1 Kg and 0.1 cm using a body scale and a stadiometer, respectively. The body mass index was calculated in Kg/m^2 following recommended guidelines (15).

Laboratory analysis

A single venous blood sample was collected and dispensed into a gel-separator vacutainer tube**.** The

blood was allowed to clot before centrifugation to obtain serum. The serum samples were aliquoted in duplicates and stored at -20°C before analysis. The serum levels of urea and creatinine were determined from a routine automated biochemistry analyzer using manufacturer-recommended calibrators, controls, and reagents. The enzyme-linked immunosorbent assay (ELISA) technique was used to measure the serum cystatin C.

Calculating the glomerular filtration rate

The GFR was estimated using the online calculators based on the formulae below from the website: https://www.kidney.org/professionals/KDOQI/gfr_calculator

A. eGFRcrt = 142 x min(Scr/κ, 1)α x max(Scr/κ, 1)-1.200 x 0.9938Age x 1.012

where:

Scrt = standardized serum creatinine in mg/dL or µmol/L

κ = 0.7

α = -0.241

min(Scr/κ, 1) is the minimum of Scr/κ or 1.0

max(Scr/κ, 1) is the maximum of Scr/κ or 1.0

Age (years)

B. eGFRcys =133 x min(Scys/0.8, 1)-0.499 x max (Scys/0.8, 1)-1.328 x 0.996Age x 0.932

Scys (standardized serum cystatin C) = mg/l

min = indicates the minimum of Scys/0.8 or 1

max = indicates the maximum of Scys/0.8 or 1

age = years

C. eGFRcrt+cys = 135 x min(Scr/κ, 1)α x max(Scr/κ, 1)-0.544 x min(Scys/0.8, 1)-0.323 x max(Scys/0.8, 1)-0.778 x 0.9961Age x 0.963 [if female]

where:

Scr = standardized serum creatinine in mg/dL or µmol/L

κ = 0.7

α = -0.219

min(Scr/κ, 1) is the minimum of Scr/κ or 1.0

max(Scr/κ, 1) is the maximum of Scr/κ or 1.0

Scys = standardized serum cystatin C in mg/L

Age (years)

Statistical analysis

The data were collected onto an Excel Spreadsheet and then analyzed using SPSS (v26), and GraphPad Prism (v8) statistical packages. The Shapiro-Wilk test was used to test for the normality of the data and check for outliers. Categorical variables were summarized as frequencies (percent), while continuous non-parametric variables were summarized as median (IQR-interquartile range). The differences between variables of two unpaired and multiple unpaired groups were determined using the Mann-Whitney U and Kruskal-Wallis tests, respectively. The comparison of multiple paired samples was performed using the Friedman test. The agreement between methods for estimating GFR was measured using the Bland-Altmann test, adopting the %difference/average method. A %difference/average <40% was considered a high agreement between the methods (5). All statistical analyses were 2-tailed at a significance level of $P<0.050$. All findings were reported following The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

RESULTS

Socio-demographic variables

Table 1 shows the socio-demographic characteristics of the study participants. The majority of the participants were from the Mole-Dagomba cultural group (92.5%). Married women and self-employed women constituted 97.9% and 48.8% of the study participants, respectively. More women had tertiary-level education.

Comparing variables of kidney function between pregnant and non-pregnant women

From Table 2, serum creatinine ($P<0.001$) and NGAL ($P<0.001$) were significantly elevated in pregnant than non-pregnant women, while the effect was reduced considerably in pregnant than non-pregnant women. From Table 3, only serum NGAL was significantly higher in the second and third trimester of pregnancy.

Within group comparison of eGFR

The eGFR based on creatinine, cystatin C, and, when combined, were compared in non-pregnant and pregnant women stratified by the trimester of pregnancy (Table 4). The eGFR based on sCys was significantly higher than sCrt-based eGFR and the combined equation in non-pregnant and pregnant women regardless of the trimester of pregnancy ($P<0.001$).

Agreement between creatinine and Cystatin C-based eGFR

Considering a percentage difference of <40% as a measure of high agreement between the two methods, there was no high agreement between the sCrt- and sCys-based CKD-EPI equations in estimating the GFR (bias: -61% to -76%) as shown in Figure 1. Moreover, the eGFR based on sCys were consistently higher than those based on sCrt (negative bias). Similarly, there was no high agreement between the sCrt-based eGFR and the combined sCrt +sCys eGFR (bias: -41% to -53%). However, from Figure 2, there was high agreement between the Cys-based equation and the combined sCrt+Cys eGFR equation (bias: 24% to 27%).

Frequency of reduced GFR (<60 mL/min/1.73m2)

An eGFR<60 mL/min/1.73m^2 was regarded as reduced and a risk factor for CKD based on the guidelines of the National Kidney Foundation (16). Only sCrt-based equations produced eGFR<60 mL/min/1.73m^2. From Figure 3, the frequency of reduced eGFR was 2.0% in non-pregnant and 4.7% in pregnant women. Second-trimester pregnancy recorded the highest number of women with reduced eGFR CKD (13.7%), while third-trimester pregnancy recorded the least (1.9%)

Table 1. The socio-demographic characteristics of the study population

Variable	Frequency	Per cent
Cultural group		
Mole-Dagomba	260	92.5
Akan	12	4.3
Other	9	3.2
Married		
Yes	275	97.9
No	6	2.1
Employment status		
Unemployed	72	25.6
Salary worker	72	25.6
Self-employed	137	48.8
Educational level		
None	16	5.7
Basic	78	27.8
Secondary	81	28.8
Tertiary	106	37.7
Trimester of pregnancy		
Non-Pregnant	50	17.8
1st Trimester	51	18.1
2nd Trimester	73	26
3rd Trimester	107	38.1

The results are summarized as frequency (per cent). nonP=none pregnant, 1T=first trimester, 2T=second trimester, 3T=third trimester, NP=normotensive pregnancy.

Table 2. Comparing anthropometric variables and markers of kidney function of the study population

Variable	nonP	NP	P-value
Age (years)	30(27-34)	28(26-32)	0.031
BMI (Kg/m^2)	30.3(24.6-32.8)	27.7(23.8-30.5)	0.033
Urea (mmol/L)	4.3(3.4-5.3)	4.5(2.9-6.6)	0.364
Serum creatinine (µmol/L)	69.4(59.0-83.6)	83.1(67.3-97.2)	<0.001
Serum cystatin C (mg/L)	0.21(0.13-0.65)	0.24(0.13-0.78)	0.898
NGAL (µg/L)	21.6(18.7-24.5)	29.2(21.4-38.2)	<0.001
eGFRcrt (mL/min/1.73m^2)	105(82-120)	85(69-108)	0.001
eGFRcys (mL/min/1.73m^2)	212(121-266)	203(113-270)	0.945
eGFRcrt+cys (mL/min/1.73m^2)	148(114-193)	142(102-175)	0.109

The results are summarized as median (IQR). The between-group median comparison was performed using the Mann-Whitney U Test. nonP=non-pregnant, NP=normotensive pregnant, NGAL=neutrophil gelatinase-associated lipocalin, eGFR=estimated glomerular filtration rate.

Table 3. Comparison of markers of kidney function by trimester of pregnancy

Variable	1TNP	2TNP	3TNP	P-value
Urea (mmol/L)	5.9(3.9-8.3)	6.0(3.8-7.7)	4.3(3.1-5.1)	0.809
Serum creatinine (µmol/L)	80.6(60.0-93.2)	95.3(83.3-102.9)	74.2(60.0-91.1)	0.425
Serum Cystatin C (mg/L)	0.17(0.12-0.55)	0.18(0.13-0.78)	0.47(0.15-0.84)	0.381
NGAL (µg/L)	29.0(20.6-38.8)	35.6(25.2-47.4)	22.0(17.8-26.0) ^	0.004
eGFRcrt (mL/min/1.73m^2)	89(75-117)	71(66-84)	97(75-119)	0.453
eGFRcys (mL/min/1.73m^2)	237(134-279)	229(114-275)	144(104-263)	0.354
eGFRcrt+cys (mL/min/1.73m^2)	164(111-193)	147(97-170)	128(100-180)	0.179

The results are summarized as median (IQR). The between-group comparison of median values was achieved using the Krusskal-Wallis test. nonP=non-pregnant, T=Trimester, NP=normotensive pregnancy, NGAL=neutrophil gelatinase-associated lipocalin, eGFR=estimated glomerular filtration rate, ^P<0.010 compared to 2NTP

Table 4. The within-group comparison of eGFR based on serum creatinine and cystatin C

Sample	eGFRcrt	eGFRcys	eGFRcrt+cys	P-value
nonP	103(85-119)	159(124-240) *	145(115-180) #^	<0.001
1TNP	89(75-117)	237(134-279) *	164(111-193) #^	<0.001
2TNP	71(66-84)	229(114-275) *	147(97-170) #^	<0.001
3TNP	97(75-119)	144(104-263) *	128(100-180) #^	<0.001

The results are summarized as median (IQR) and were compared using the Friedman test for multiple paired samples (non-parametric). nonP=non-pregnant, T=Trimester, NP=normotensive pregnancy.

*P<0.001 compared to eGFRcrt, #P<0.001 compared to eGFRcrt, ^P<0.001 compared to eGFRcys

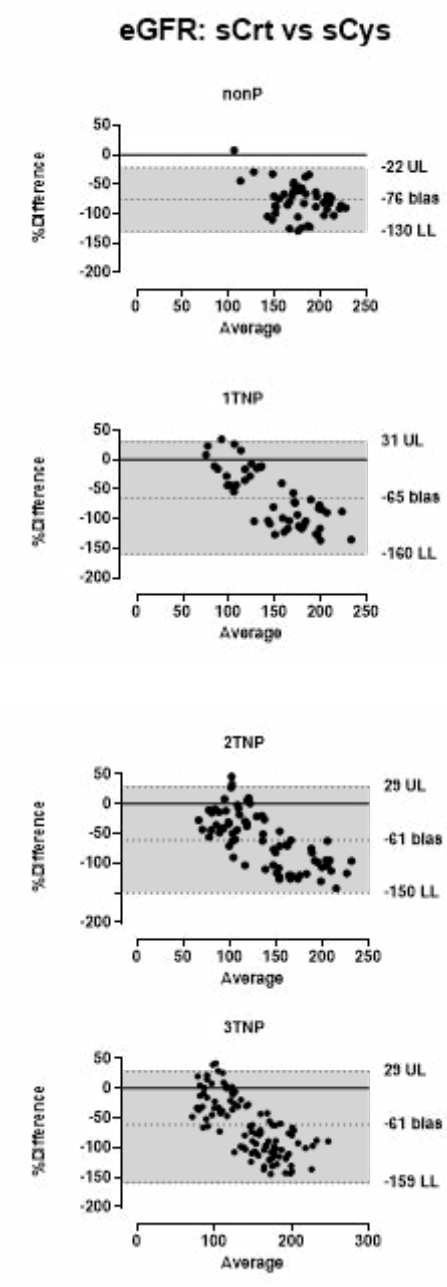

Figure 1. Bland-Altmann plots showing the percentage difference (bias) of the estimated glomerular filtration rates (eGFR) between serum creatinine- (sCrt) and cystatin C- (sCys) based equations. nonP=non-pregnant, T=trimester, NP=normotensive pregnancy, LL=lower limit, UL=upper limit

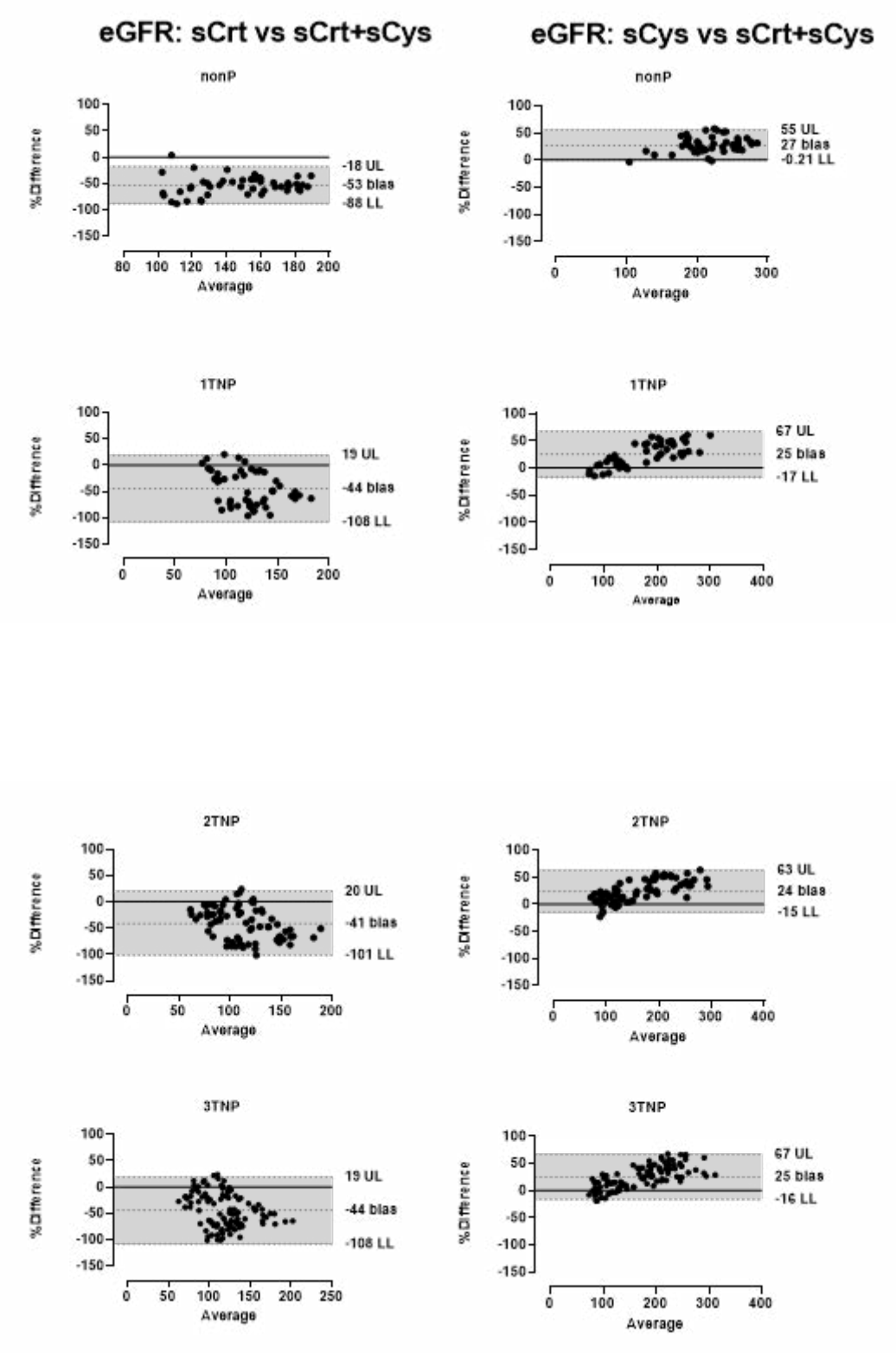

Figure 2. Bland-Altmann plots show the percentage difference (bias) in the estimated glomerular filtration rates (eGFR) between serum creatinine (sCrt) and cystatin C (sCys) based equation alone and when they are combined. nonP=non-pregnant, T=trimester, NP=normotensive pregnancy, LL=lower limit, UL=upper limit

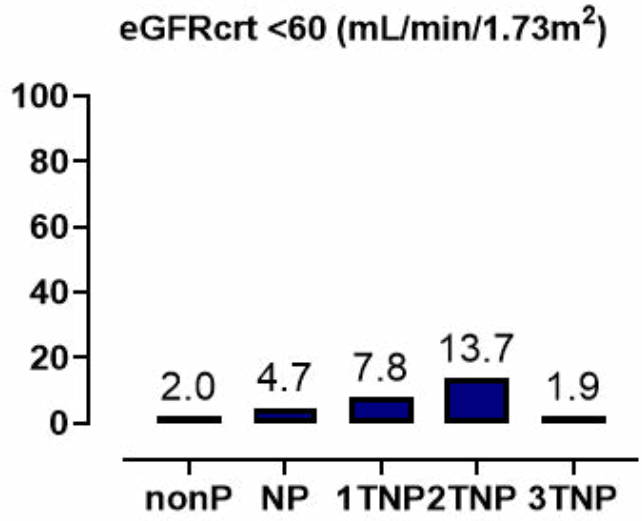

Figure 3. The frequency of reduced GFR (<60 mL/min/1.73m^2) in the study population stratified by trimester of pregnancy. nonP=non-pregnant, T=Trimester, NP=normotensive pregnancy.

DISCUSSION

The study sought to determine the agreement between eGFRs based on serum creatinine and Cystatin C in pregnant and non-pregnant women. The serum creatinine and NGAL were significantly higher, while the serum creatinine-based eGFR was significantly lower in pregnancy. There was no high agreement between serum creatinine- and cystatin C-based eGFR equations. The eGFR was markedly higher in the sCys- than the sCrt-based equation. While Crt- and sCrt + sCys-based equations did not show high agreement, there was high agreement between sCys- and sCrt + sCys-based eGFR equations. Only the sCrt-based equation yielded reduced eGFR with a frequency of 4.7% in pregnancy and 2.0% in non-pregnant women.

There was no high agreement between serum creatinine- and cystatin C-based eGFR equations. The eGFR was significantly higher in the latter than in the former equation. While sCrt- and sCrt + sCys-based equations did not show high agreement, there was high agreement between sCys- and sCrt + sCys-based eGFR equations. Previous studies have demonstrated that serum cystatin C-based equations show high agreement or less bias when compared to either the mGFR or the combined sCrt + sCys-based estimated GFR (5, 17, 18). It has been argued that creatinine excretion is affected by muscle mass and renal tubular effects. In contrast, the concentration of cystatin C, unlike creatinine, is unaffected by molecular mass or any tubular secretion effect (7). Recent studies have, however, identified some factors that may impact cystatin C levels. Previous studies have shown that persons with thyroid dysfunction, melanoma, HIV, and patients on glucocorticoid therapy may have elevated levels of cystatin C (6). Also, the extrarenal excretion of cystatin C tends to increase with decreasing GFR (6). Moreover, a person's height, weight, smoking status, and serum C-reactive protein levels tend to impact cystatin C concentration (6).

The frequency of reduced eGFRcrt (<60 mL/min/1.73m^2) was higher in pregnancy (4.7%) compared to non-pregnant women (2.0%). Normal pregnancy changes kidney anatomy, function, and hemodynamics as the kidney in pregnancy tends to enlarge with a significant increase in renal blood flow (1, 10). Kidney enlargement in pregnancy is primarily physiological as an adaptation to increased fluid volumes and dilatation of the collecting system. However, further kidney enlargement may arise secondarily due to ureteric mechanical obstruction and subsequent hydronephrosis (19). Hormonal changes in pregnancy, such as progesterone, also play a pivotal role in kidney function by reducing systemic vascular resistance and mean arterial pressure, leading to heightened cardiac output, increased renal plasma perfusion, and subsequently hyperfiltration from increased GFR (1, 20). Studies have shown that there is an increased secretion

of renin from extra-renal sites such as the decidua and ovaries, estrogen-induced angiotensin production from the liver, and elevated levels of aldosterone and Relaxin from the corpus luteum (19, 21). The increased activity of the rennin–angiotensin–aldosterone system (RAAS) may lead to volume expansion and sodium retention (19). There is also haemodilution due to hypovolaemia, which reduces plasma protein concentration and plasma oncotic pressure (1). There is also a change in renal tubular function even in normal pregnancy, resulting in changes in the handling of nutrients and waste. In pregnancy, there may be a reduction in proximal renal tubular reabsorption and/or distal nephron reabsorption of waste and nutrients, coupled with increased GFR, which may result in glucosuria and proteinuria (1, 22). The higher frequency of reduced GFR in pregnancy is supported by the significantly higher serum NGAL in pregnant than non-pregnant women. There is a direct relationship between NGAL and proteinuria, indicating a direct excretion of NGAL into damaged renal tubular cells with the intent of repair since NGAL can induce re-epithelialization (23).

Only eGFRcrt was reduced (<60 mL/min/1.73m2) in the study population. A previous study showed that serum creatinine is better than cystatin C in assessing the risk factors of CKD (24). A multicenter study in Sub-Saharan Africa (SSA) and South Asia found no improvement in prediction accuracy by adding sCys to eGFR equations (7, 25). Similarly, an Indigenous Australian study found sCrt to be less biased compared to mGFR than sCys (4). It has been suggested that the tubular secretion of creatinine may differ between Africans and other populations, such as Western Europeans (7). Some authors have even advised against using sCys as a marker of GFR in pregnancy, where sCys levels could be influenced by differential renal handling (10). A study among pregnant women also reported the lack of correlation between sCys eGFR and inulin clearance. The authors thought this outcome could be due to placental sources of cystatin C (26). Cysteine protease production is essential to aid trophoblast invasion and angiogenesis into the decidua during the development of the placenta in pregnancy. Cystatin C allows decidua to limit trophoblast invasion by inhibiting cysteine protease. This protease-inhibitor balance is essential in the regulation of normal placenta development (26)

The current study has some strengths: it is among the limited studies to evaluate the agreement between the CKD-EPI eGFR equations based on serum creatinine and cystatin C in pregnancy among a Ghanaian population; the study also included non-pregnant women for purposes of determining the impact of pregnancy on the concordance between the equations. The study is, however, limited in that the GFR was not directly measured to determine whether the CKD-EPI equations were accurate in estimating the GFR. Also, the assumption that the combined sCrt + sCys eGFR equation is better than the individual equations may not be accurate without mGFR.

CONCLUSION

The findings of this study require that the CKD-EPI equations be validated among pregnant and non-pregnant women in Ghana. We recommend that future studies include a directly measured GFR for a fair comparison of the GFR estimating equations.

Data availability statement

The data supporting these findings can be obtained from the corresponding author upon a reasonable request.

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Conflict of interest statement

The authors have no conflict of interest to declare

Ethical statement

The study followed the guidelines for human subject studies as contained in the Declaration of Helsinki (1964) or its later amendments. The study was approved on 29 May 2020 by the institutional review board of Navrongo Health Research Center (NHRC) with the ethical approval number NHRCIRB378. The ethical approval was extended for one year on 26 May 2021. Written informed consent was obtained from all the women before they were included in the study. Participation in the study was voluntary.

Authors' contributions

IANA and NA: conceptualization, methodology, and writing–review. IANA and MAA: experimentation, data collection, and writing – review. MB and CN: statistical analysis, results interpretation, writing – original draft and writing – review: All the authors have reviewed the final draft and approved its contents.

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