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Validation of glomerular filtration estimation equations adjustable by race/colour in adults from Vitória, Espírito Santo, Brazil

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Abstract *The assessment of renal function is performed using the glomerular filtration rate (GFR) whose measurement by creatinine clearance (ClCr) and is dependent on a 24-hour urine sample, hindering it use in primary healthcare. The equations that estimate GFR from serum creatinine make the test more accessible, however, their adjustments by race/color have been questioned in mixed populations. To test the agreement between CrCl and GFR estimated by formulas (Modification of Diet in Renal Disease [MDRD-4] and Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI]), with or without adjusting for race/color, data were used from a sub-study of the National Health Survey (NHS) including 272 adults from Vitória/Espírito Santo who underwent a 24-hour urinary sampling. Analysis of variance (ANOVA) and the Bland-Altman method were adopted. There was adequate agreement between ClCr and equations, but the adjustment by race/color decreases the accuracy of both equations. In the race/color factor, there was similarity between groups for CrCl (p=0.21), suggesting that there is no difference in creatinine metabolism induced by skin color. It is concluded that MDRD and CKD-EPI equations perform satisfactorily in the evaluation of renal function, and the use of corrections for race/color is not recommended.*

Key words *Glomerular Filtration Rate, Kidney Function Tests, Chronic Renal Insufficiency*

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Introduction

Population aging has produced a significant increase in the prevalence of chronic non communicable diseases (NCDs), including the chronic kidney disease (CKD)[1]. According to the Nation Health Survey/2013, the prevalence of CKD in the Brazilian adults was 6.48%, estimating the existence of around 10 million Brazilians living with this condition[2].

The term CKD is used to indicate the presence of a wide range of heterogeneous diseases that affect the structure and function of the kidneys, being commonly asymptomatic in its initial stages[3]. Thus, early diagnosis requires tracking the disease in susceptible individuals, which should be done at the primary health care level[3].

One of the most important quantitative indicators to identify patients with CKD is the glomerular filtration rate (GFR), which approximately expresses the mass of functioning nephrons[4]. The maximum number of nephrons of an individual is established at the end of pregnancy, tending to decrease progressively[5]. GFR has a progressive decrease with advancing age, which is accelerated in the presence of risk factors for CKD such as arterial hypertension and diabetes mellitus, in addition to kidney diseases *per se*[5,6]. According to the current guidelines, an adult should be diagnosed with CKD in presence of GFR less than 60 mL/min.1.73 m^2 for more than three months[3,7].

The gold standard method to estimate the GFR is the measurement of depuration of fully filterable substances in the glomerulus, without reabsorption or secretion in the renal tubules, such as inulin and iothalamate. These markers, however, must be administered intravenously, being unfeasible for use in clinical practice[8]. Thus, there has been an intense search for endogenous markers that may account for this purpose. So far, serum creatinine (SCr) has been the most accessible endogenous marker for use in primary care, where CKD screening should be investigated[9].

However, the influence of liver and muscle metabolism on SCr makes the simple measurement of this marker insufficient to estimate the degree of renal functioning[10]. Therefore, renal depuration of creatinine or the endogenous creatinine clearance (CrCl) has been used as an easily accessible method for estimating GFR[11]. However, in order to calculate CrCl, in addition to determining plasma and urinary creatinine concentrations, it is necessary to measure the urinary flow, which requires measuring the urine volume produced in a predetermined time interval[11,12].

The standard time for urine collection is 24 hours (h) to mitigate the impact of fluctuations in SCr levels throughout the day[11]. The extension of this collection time represents a considerable inconvenience to most of patients. In addition, the incorrect measurement of the collection time is the main error in establishing the urinary flow[12]. Creatinine is fully filterable in the glomeruli. However, it also shows a little tubular secretion[13], what may compromise the measurement of GFR, especially in more advanced stages of CKD[14].

To overcome these problems, several formulas were developed to estimate the GFR using only the SCr associated with other data easily obtained at the primary care service, such as gender, age, weight and height[15]. In some equations, race/color and plasma albumin concentration were also included[15].

In this context, two equations have been most frequently used. One was developed in the study Modification of Diet in Renal Disease (MDRD), originally aimed to identify CKD in patients with some renal impairment. This equation was subjected to several variations and adaptations[16]. For this study, the version with 4 variables (MDRD-4) was used. The other equation was developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) group and is more accurate to be used in healthy individuals[17]. MDRD-4 and CKD-EPI estimate GFR using the same set of variables (SCr, sex, age and race/color) and are widely recommended for use in the Brazilian population, being very relevant in CKD outpatient screening[18,19].

However, to date there are no robust studies that test and compare the performance of these formulas with the GFR calculated by the gold standard (CrCl) with 24-hour urine collection. Additionally, the introduction of a correction factor for black individuals in these equations has been questioned[20], which is even more controversial in the case of Brazil, considering that most of the population show a highly mixed genetic background[21]. Regarding the detection of CKD via estimation of the GFR, the National Health Survey (NHS) indicated that adjustment by race/color is not recommended, reinforcing the understanding that there are no differences in the creatinine metabolism related to different skin color in Brazilians[22].

Thus, the objective of this study was to validate the CKD-EPI and MDRD-4 equations with

and without adjustment to race/color, analyzing the concordance of the GFR estimated by these equations with the CrCl measured in 24h urine.

Methods

Participant recruitment and orientation

In the NHS (2013-2015), blood and urine samples were collected at home aiming to estimate salt consumption by the Brazilian population using the Tanaka and Kawasaki equations based on the sodium/creatinine ratio in the urine casual[23]. To validate these equations, an additional parallel study was carried out in Vitória, Espírito Santo (ES) State where a 24-hour urine collection, as well as a casual urine collect, was performed in a random sample of adult population. Additional methodological details of this study were published elsewhere[22-25].

Therefore, this article used the data obtained in the parallel study that consisted of a cross-sectional study carried out between August and October 2015, in a stratified sample of adults aged 18 to 69 years living in Vitória[25]. The sampling process was carried out by selecting 20 households in 20 censitary sectors of the city which were visited by trained research assistants. In each domicile only one resident was invited to participate in the study, following a distribution by sex (50% male) and age group (20% in each decade). The sample size (N=400) was defined to obtain a final sample of at least 250 individuals uniformly distributed by sex and age group, being sufficient to obtain 95% accuracy in verifying the degree of agreement between two diagnostic methods by the Bland-Altman protocol[25]. A total of 396 individuals were selected in domiciles. From this sample, 30 did not attend to do the clinic exams and 36 did not undergo the 24-hour urine collection. Thus, 330 volunteers remained in the study, with distribution by sex and age group similar to the planned sample.

The project was approved by the institutional Ethics Committee of the Health Sciences Center of the Federal University of Espírito Santo (UFES) under Protocol No. 201,110. All participants signed the Informed Consent Form during the home visit where demographic data (age, gender, self-reported skin color, monthly income and education) and life habits were obtained by research assistants.

Demographic data were collected at home, where the day for the 24-hour urine collection and the performance of clinical and laboratory tests at the University Hospital (HUCAM-UFES) was also scheduled. Participants were instructed to not interrupt the use of chronic medication and to maintain their usual eating habits. On the day before the exams, participants should fast after 8 pm, abstain from alcohol consumption and from vigorous physical activities.

Clinical and Laboratory Exams

On the day scheduled for the exams, each participant was instructed to void in the toilet upon waking up and to annotate the exact time of this void in a form, marking the beginning of the 24-hour urine collection. After this first void, the volunteer should go to the University Hospital. At arriving at the hospital, the exact time of void was checked and the fasting blood sample was collected. The first 24-hour urine sample was performed at the examination site, corresponding to the second urinary emptying of the day.

The anthropometric (weight and height) and blood pressure (oscillometric method, Onrom765CP, Japan) were then obtained. After the exams, participants returned to their home or workplace, carrying two bottles (2L) to collect all the urine produced until the next morning. Special recommendations were given to avoid losses and to keep the bottles in a refrigerator. The last collection was carried out the following morning at home and at the closest time of 24 hours after the start of collection the day before. The time of the last void in the bottle was also annotated in the form. The bottles with 24-hour urine were collected in domiciles along with the form with participant annotations (beginning and end of collection, urine loss, etc.). Urine volume was measured in a test tube with a precision of 10 mL and an aliquot was sent on the same day to a central laboratory for creatinine measurement using the Jaffé method[26]. The blood analysis collected in fasting were performed in the same laboratory using commercial kits.

In addition to SCr and CrCl, which are the objects of this study, other data related to clinical and laboratory tests were recorded in this article to characterize the sample and were used to characterize the clinical condition of the sample, and secondarily, to help in the evaluation of possible outliers. In order to characterize the participants in relation to the prevalence of the main risk factors for CKD, individuals with a body mass index ≥30 kg/m² were defined as obese, and those using antihypertensive drugs or with

blood pressure ≥140/90 mmHg were considered hypertensive. Presence of diabetes was indicated to those reporting using insulin or oral hypoglycemic agents or with fasting blood glucose >125 mg/dL[27-29].

Validation of the 24-hour urine collection

According to information provided by the participants in the urine collection form, samples with no report of loss, with a volume greater than 500 mL and with collection time between 23-25h were considered valid. The 24-hour urinary volume was calculated by interpolation[25].

Of the 330 participants with a 24-hour urine collection, we excluded those reporting urine loss (N=8), with collected volume <500 mL (N=4) or with a collection time outside the 23-25-hour interval (N=2). After the biochemical analyses, 44 participants who showed 24-hour creatinine excretion out of limits of 14.4 to 33.6 mg/kg for men and 10.8 to 25.2 mg/kg for women were also excluded[30]. Thus, the final study sample consisted of 272 participants. No loss bias was observed, given that the proportions of gender and age group remained similar to the initial sample. CrCl was calculated by multiplying urinary flow by the ratio between urinary and plasma creatinine concentrations (in mg/dL), adjusted for 1.73m² of body surface area calculated by the DuBois & DuBois formula[31].

Equations to estimate the Glomerular Filtration Rate

CKD-EPI and MDRD-4 are the most used formulas in Brazil to estimate GFR, being used in protocols and medical Apps, including in the website of the Brazilian Society of Nephrology[18,19]. In these equations, SCr is given in mg/dL and age in years.

• Modification of Diet in Renal Disease with 4 variables (MDRD-4)[16]:

GFR= 175 x $SCr^{-1;154}$ x $age^{0.203}$ x 1.212 (only for blacks) x 0.742 (only for females)

• Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)[17]:

Male

$GFR = C \times (SCr/0.9)^{\alpha} \times 0.993^{age}$

Where C=141, except for blacks where C=163; α=-0.411 for SCr <0.9 mg/dL or α=-1.209 for SC r≥0.9 mg/dL.

Women

$GFR = C \times (SCr/0.7)^{\alpha} \times 0.993^{age}$

Where C=144, except for blacks where C=166; α=-0.329 for SCr < 0.7mg/dL or α=-1.209 for SCr≥0.9mg/dL.

Statistical analysis

Data were expressed as mean, standard deviation (SD) or median for continuous variables or as proportions and percentages in counts. Adherence to the normal distribution was checked using the Kolmogorov-Smirnov test.

For comparisons between means of the GFR calculated by different methods, we defined the measured variable (ClCr) and estimated variables (GFR_{MDRD4} and $GFR_{CKD-EPI}$), using the formulas in three possibilities of adjustment by race/color: only for blacks, for blacks and brown or unadjusted. One-way analysis of variance (ANOVA) for repeated measures compared CrCl vs. GFR_{MDRD4} and CrCl vs. $GFR_{CKD-EPI}$ in the three adjustment possibilities (4 means in each test), associated with the Sidak post-hoc test to compare pairs of means. Student's t-test was used to assess gender-grouped GFR data.

Additionally, one-way ANOVA associated with post-hoc Bonferroni was used to analyze the CrCl, GFR_{MDRD-4} and $GFR_{CKD-EPI}$ data (with the three adjustment modes) in the subgroups by race/color (grouping factor) namely: whites, blacks, brown and others (indigenous, yellow or undeclared).

To elucidate the type of association between CrCl, GFR_{MDRD-4} and $GFR_{CKD-EPI}$ values, Pearson's correlation coefficients were determined, in the presence and absence of correction for skin color. The agreement analysis between CrCl and the CKD-EPI and MDRD-4 formulas was performed using the Bland-Altman method[32]. According to this method, concordance is assumed when 95% of the individuals are within ±1.96 SD of the total concordance point (reference "0" which corresponds to the mean of differences between methods). In the Bland-Altmann plot, the minimum and maximum points of the aforementioned interval are called limits of agreement and all data located outside this interval are considered outliers.

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS 20.0, Chicago, IL, USA) and statistical significance was set at $p < 0.05$.

Results

The general characteristics of the participants (47.4% male) are shown in Table 1. Age mean was 44±14 years, with no difference (p>0.05) between genders. There was balance in the number of participants in different age groups.

Regarding the risk factors for CKD in the whole sample (N=272) 23.5% were obese, 31.2% with hypertension and 7.0% with diabetes. The prevalence of hypertension was higher (P<0.01) in men and the prevalence of overweight, obesity and diabetes were similar (P>0.05) between genders.

Mean values of CrCl (Table 2) were similar (P>0.05) in men and women (111±22 ml/min/1.73 m^2 vs. 109±25 ml/min/1.73 m^2), with normal distribution of this parameter. Normal distribution was also identified in both the $GFR_{CKD-EPI}$ and GRF_{MDRD-4} data, regardless of adjustment for skin color (P>0.05).

The one-way ANOVA of CrCl using the skin color factor showed that among the subgroups composed of whites (n=125), blacks (n=27), brown (n=117) and others (n=3) there was no statistical difference among means [F(3.268)=2.951; p=0.21]. On the other hand, analyzing the equations without correction for race/color, the one-way ANOVA showed that there is an effect of skin color on GFR_{MDRD-4} [F(3,268)=4,314; p=0.01], where the post hoc Bonferroni test showed that only brown and black with similar means (p=0.99). An identical situation was observed in the $GFR_{CKD-EPI}$ analysis, where the one-way ANOVA showed difference in the subgroups by race/color [F(3,268)=4,297; p=0.006], and the post-hoc Bonferroni test revealed similarity only between the means of blacks and brown (p=0.98). Testing the formulas after adjusting only for blacks, the result of the ANOVA in the groups by race/color showed a significant difference between means [F(3.268)=15.909; p<0.001 for MDRD-4 and for CKD-EPI [F(3.268)=13.532; p<0.001], and the post-hoc Bonferroni identified that all comparison between pairs differ from each other (p<0.001 in all cases). Repeating the ANOVA with the formulas adjusted for blacks and brown, it is also verified that there is a difference between means in the subgroups by skin color [F(3,268)=35,531; p<0.001] for MDRD and for CKD-EPI [F(3.268)=30.745; p<0.001], where the post-hoc Bonferroni showed that only blacks and brown have similar means (p=0.99 for MDRD and p=0.98 for CKD-EPI).

One-way ANOVA with repeated measures comparing the means of ClCr, GFR_{MDRD-4} and $GFR_{CKD-EPI}$ showed that the three variables differ among themselves both using the formulas without adjustments [F(1.474; 396.46)=162.710; p<0.0001); post hoc Sidak with p<0.001 in all pairs] and after adjustment in blacks [F(1.143; 307.54)=119.95; p<0.0001); post hoc Sidak with p<0.001 in all pairs] and also after adjustment in blacks and brown [F(1.166; 313.64)=33.187; p<0.001); post hoc Sidak with p<0.001 in all pairs].

Comparing ClCr, GFR_{MDRD-4} and $GFR_{CKD-EPI}$ it was observed that the latter showed a positive and moderate correlation (Pearson's r) with CrCl, regardless of adjustment for skin color (GFR_{MDRD-4}: r=0.453 without adjustment and r=0.446 after adjustment; $GFR_{CKD-EPI}$: r=0.461 without adjustment and r=0.451 after adjustment). In the analysis by sex, the correlation between adjusted formulas and CrCl was slightly better in women (GFR_{MDRD-4}: r=0.483 and $GFR_{CKD-EPI}$: r=0.490) as compared to men (GFR_{MDRD-4}: r=0.408 and $GFR_{CKD-EPI}$: r=0.419). In the subgroup composed only of self-declared blacks (n=27) the test suggested a negligible positive correlation with ClCr (r=0.101 for MDRD=4 and r=0.113 for CKD-EPI). An even weaker correlation was observed when adjustment for skin color was applied to the brown subgroup (n=117; r=0.078 for the MDRD and r=0.076 for the CKD-EPI).

Figure 1 shows the analysis of correlation (A, B, E, F) and agreement (C, D, G, H) between CrCl with the GFR calculated by MDRD-4 and CLD-EPI equations with and without adjustments for skin color. Graphs C, D, G and H show that there is agreement between ClCr and both formulas, since more than 95% of the points, related to data on differences between methods, are within the agreement limits (±1.96 x SD).

Additionally, there was a tendency of the equations to concentrate the differences below the mean of the differences (^{2}C, ^{2}D, ^{2}G, ^{2}H), indicating a proportion bias due to underestimation of the real value. It was also noticeable that the differences between the results estimated by the equations and those measured by CrCl was higher in the highest GFR values. However, at lower GFR values (less than 90 mL/min x 1.73 m^2), the performance of the two formulas was much better, given the absence of values outside of the agreement limits.

After stratification by gender (Figure 2), the performance of the MDRD-4 and CKD-EPI

Table 1. Demographic and clinic characteristics of the adult sample, by sex. Vitória-ES, Brazil, 2015.

	Men	Women	All
N (%)	128 (47.2%)	143 (52.8%)	272 (100.0%)
Age (years)	43±14	45±14	44±14
Height (cm)'	173±7	159±7*	166±10
Weight (kg)	79.9±14.4	68.1±13.6*	73.7±15.1
Race/Color			
White	57 (44.4%)	68 (47.6%)	125 (45.9%)
Black	16 (12.5%)	11 (7.7%)	27 (9.9%)
Brown (*Pardo*)	54 (42.2%)	61 (41.6%)	115 (42.3%)
Other or not declared	1 (0.8%)	3 (2.1%)	4 (1.5%)
Years in school			
<4	0 (0.0%)	4 (2.8%)	4 (1.5%)
4-8	20 (15.6%)	28 (19.6%)	48 (2.8%)
9-11	76 (59.4%)	73 (51.0%)	149 (54.8%)
≥12	31 (24.2%)	37 (25.9%)	68 (25.0%)
Not informed	1 (0.8%)	1 (0.7%)	2 (0.7%)
Smoking			
Current	13 (10.1%)	13 (9.1%)	26 (9.5%)
Past	40 (31.3%)	32 (22.4%)	72 (26.5%)
Never	74 (57.8%)	100 (69.9%)	174 (64.0%)
Clinical parameters			
BMI (kg/m²)	26.67±4.55	27.01±5.41	26.74±5.21
SBP (mmHg)	124±14.7	118±16.5	122±16.0
DBPPAD (mmHg)	76±9.9	74±9.2	76±9.5
Glucose (mg/dL)	93.81±19.18	95.16±34.05	94.52±27.99
Cholesterol (mg/dL)	184±38.1	185±39.5	185±38.7
Triglycerides (mg/dL)	191±114.6	159±77.5	174±98.0
Serum creatinine (mg/dL)	0.96±0.16	0.75±0.15	0.85±0.19
Clinical groups (N, %)			
Normal weight (<25kg/m²)	49 (18.1)	58 (21.4)	108 (39.5)
Overweight (25.0-29.9 kg/m²)	56 (20.7)	46 (17.0)	102 (37.7)
Obesity (≥30 kg/m²)	23 (8.5)	39 (14.4)	62 (22.9)
Normotensive (23)	55 (20.3)	91 (33.6)	147 (53.9)
Hypertensive (23)	73 (26.9)	52 (19.2)	125 (46.1)
Diabetic (23)	28 (10.3)	20 (7.4)	48 (17.7)

Notes: Data are given as mean±standard deviation or as number (N) and percentage (%). BMC: Body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. *p<0.05 men vs. women (Student-t test for independent samples).

Source: Authors.

equations was slightly better in females, showing a stronger positive correlation. In both sexes, the CKD-EPI equation showed slightly greater strength and less data dispersion, and both equations tend to underestimate GFR when compared to the reference.

In comparison with females, the agreement analysis in the Bland-Altman diagram (C, D, G and H) shows closer limits in males, signaling a smaller difference between means (in the relationship between each equation and CrCl). On the other hand, the scatter plot in females shows less dispersion, suggesting a stronger agreement between the formulas and CrCl. In both sexes, there is greater agreement between the methods in individuals with GFR less than 90 mL/min x 1.73m².

An additional analysis was performed to verify the accuracy of the two equations using the adjustment for race/color only in self-declared black volunteers (n=27). In this group,

Table 2. Creatinine clearance (ClCr) and Glomerular Filtration Rate (GFR) calculated according to the MDRD-4 and CKD-EPI formulas in adults, by sex. Vitória-ES, Brazil, 2015.

	Men	Women	All
ClCr	111 (110)±22.0	109 (107)±25.2	110 (108)±23.7
[a]GFR^{MDRD4}	92 (89)±20.9	91 (88)±22.6	91 (89)±21.8
[a]$GFR^{CKD\text{-}EPI}$	98 (98)±18.5	99 (100)±21.1	98 (98)±18.7
[b]GFR^{MDRD4}	89 (88)±17.9	89 (87)±21.7	89 (88)±20.0
[b]$GFR^{CKD\text{-}EPI}$	96 (96)±16.2	97 (99)±20.6	97 (98)±18.7
One way ANOVA (factor: skin color) - Student t-test (factor: sex)			
Method	**F**	**P**	**t**	**P**
ClCr	2.951	0.21	0.31	0.578
[a]GFR^{MDRD4}	15.909	<0.001	0.11	0.742
[a]$GFR^{CKD\text{-}EPI}$	13.532	<0.001	0.08	0.812
[b]GFR^{MDRD4}	4.314	0.01	0.10	0.920
[b]$GFR^{CKD\text{-}EPI}$	4.297	0.006	0.44	0.506
[c]GFR^{MDRD4}	35.531	<0.001	0.27	0.871
[c]$GFR^{CKD\text{-}EPI}$	30.745	<0.001	0.08	0.772

Notes: Data are given as mean (median) and ± standard deviation. [a] Adjusted only in blacks; [b] No adjust; [c] Adjusted in black and brown subjects.

Source: Authors.

the Bland-Altman plots suggest that adjusting for skin color results in a small increase in data dispersion (A, B, C and D of Figure 3), and the CKD-EPI formula seems to perform slightly better, given the greater arrangement of differences between methods near the midline (^{2}C and ^{2}D).

Discussion

The present study confirmed that MDRD-4 and CKD-EPI equations show good agreement with the CrCl based on a 24-hour urine collect and signaled that the correction factor of the formulas by race/color is unnecessary and even worsens their performance. The study was carried out in a stratified sample of the adult population of Vitória-ES, including both healthy people and patients with the most common NCDs in this public, such as diabetes, hypertension and dyslipidemia, with prevalence similar to those verified in the Brazilian population[33]. In the specific case of renal complications, the proportion of individuals with reduced CrCl was compatible with the prevalence of intermediate stages of CKD in Brazil[2].

Analyzing the behavior of the equations for estimating GFR, it was found that both exhibit a moderate and positive correlation with CrCl, which is slightly higher in females. The data suggest that the MDRD-4 and CKD-EPI equations tend to underestimate the GFR, with CKD-EPI showing greater proximity and slight predictive superiority. This fact corroborates the literature, since the MDRD-4 was initially validated in subjects with compromised renal function[16], while the CKD-EPI was validated in homogeneous groups and with a predominance of healthy individuals[17], as in the present study.

The Bland-Altman methods represents the best tool to compare the performance of different diagnostic tests given as a continuous variable, as is the case of GFR. Basically, it aims to identify the proportion of individuals outside the ±1.96 SD interval, called outliers. More importantly, this method allows checking whether a method, such as a formula, has valid performance for the entire spectrum covered by the reference method, that is, whether there is bias[34-36]. In this study, it was possible to verify that almost all of the outliers refer to individuals with very low body mass index and/or with very low SCr values. In all cases, among the equations, the MDRD-4 intensified the discrepancies calculated in the outliers by inducing greater dispersion in the data.

The concordance analysis also demonstrated the better performance of these formulas to individuals with CrCl lower than 90 mL/min x 1.73 m² (data contained within the concordance intervals), with residues of absolute value lower than 30 mL/min x 1.73 m² for the two formulas. This is particularly important when considering the diagnosis of CKD and its classification in stages, according to the GFR values[3]. In the lit-

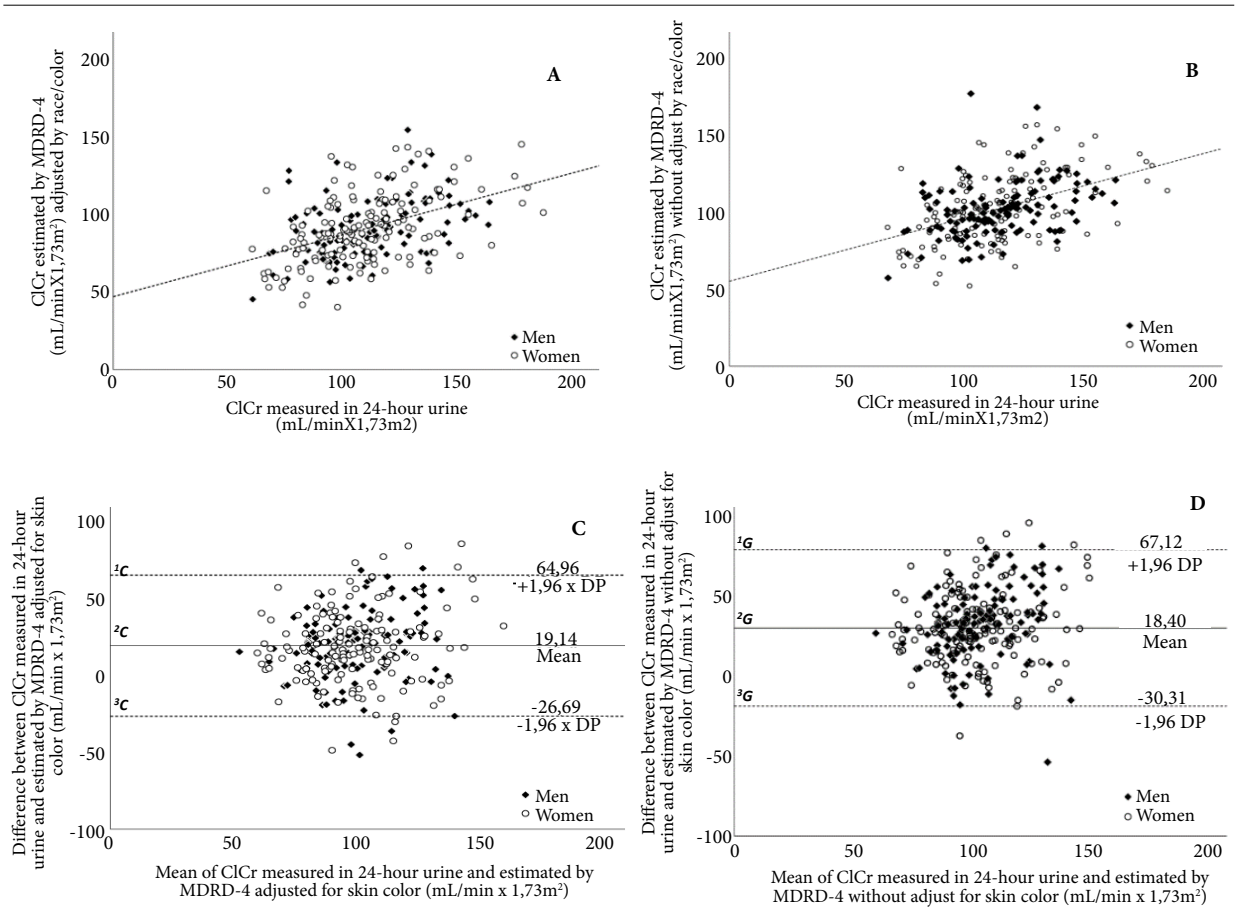

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Figure 1. Correlation (A and B; E and F) and agreement (C and D; G and H) analysis among the Creatinine Clearance (ClCr) and GFRMDRD4 and GFRCKDEPI formulas (Adults, 18-69 years). PNS - Vitória-ES, Brazil, 2015.

erature, it is stated that the difference between the result of these equations and the actual GFR value can alter the classification of a possible stage of CKD and, consequently, alter conducts and treatments to be adopted in patients[37,38]. The fact that the agreement was excellent in the range of GFR less than 90 mL/min x 1.73 m², regardless of race/color correction, indicates that these formulas can be used reliably in the screening of CKD in the general population. Classification divergences between the more advanced stages of CKD, analyzing the difference between $GFR_{M-DRD-4}$ vs. ClCr and between $GFR_{CKD-EPI}$ vs. CrCl, were not presented given the reduced number of individuals with GFR <60 mL/min x 1.73 m². However, it was noticed that the introduction of correction for skin color tends to increase the frequency of this divergence, that is, to increase the rate of diagnostic errors, since the adjustment increased the dispersion of data, mainly in the case of MDRD-4. The increase in dispersion promotes an increase in the proportion of outliers that are nothing more than individuals in which the formula does not adequately predict GFR.

In a similar work, it was reported that the equations tend to underestimate the GFR in the elderly and also in those with lower GFR, and tend to overestimate the result in younger individuals and individuals with higher GFR[38]. In this study, older participants had slightly higher residuals in the analyses, with the CKD-EPI having slightly higher accuracy than the MDRD-4 in all age subgroups. In general terms, the results showed that the CKD-EPI equation worked as a

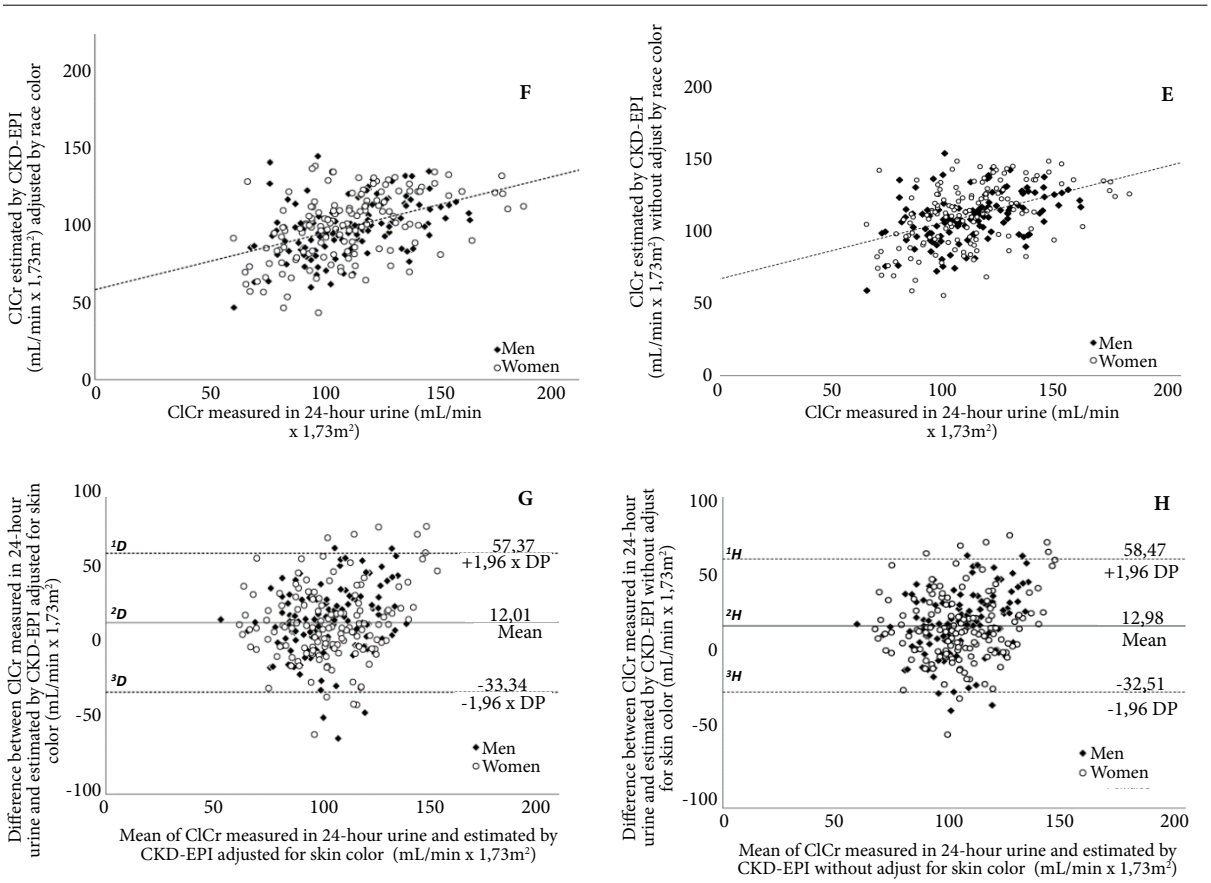


Figure 1. Correlation (A and B; E and F) and agreement (C and D; G and H) analysis among the Creatinine Clearance (ClCr) and GFRMDRD4 and GFRCKDEPI formulas (Adults, 18-69 years). PNS - Vitória-ES, Brazil, 2015.

Notes: [1]C and [3]C - limits of agreement; [2]C - Average difference (24h-urine vs. MDRD-4 without race adjustment). [1]D and [3]D - limits of agreement; [2]D - Average difference (24h-urine vs CKD-EPI without race adjustment). [1]G and [3]G - limits of agreement; [2]G - Average difference (24h-urine vs. MDRD-4 adjusted by race/color). [1]H and [3]H - limits of agreement; [2]H - Average difference (24h-urine vs. CKD-EPI adjusted by race/color).

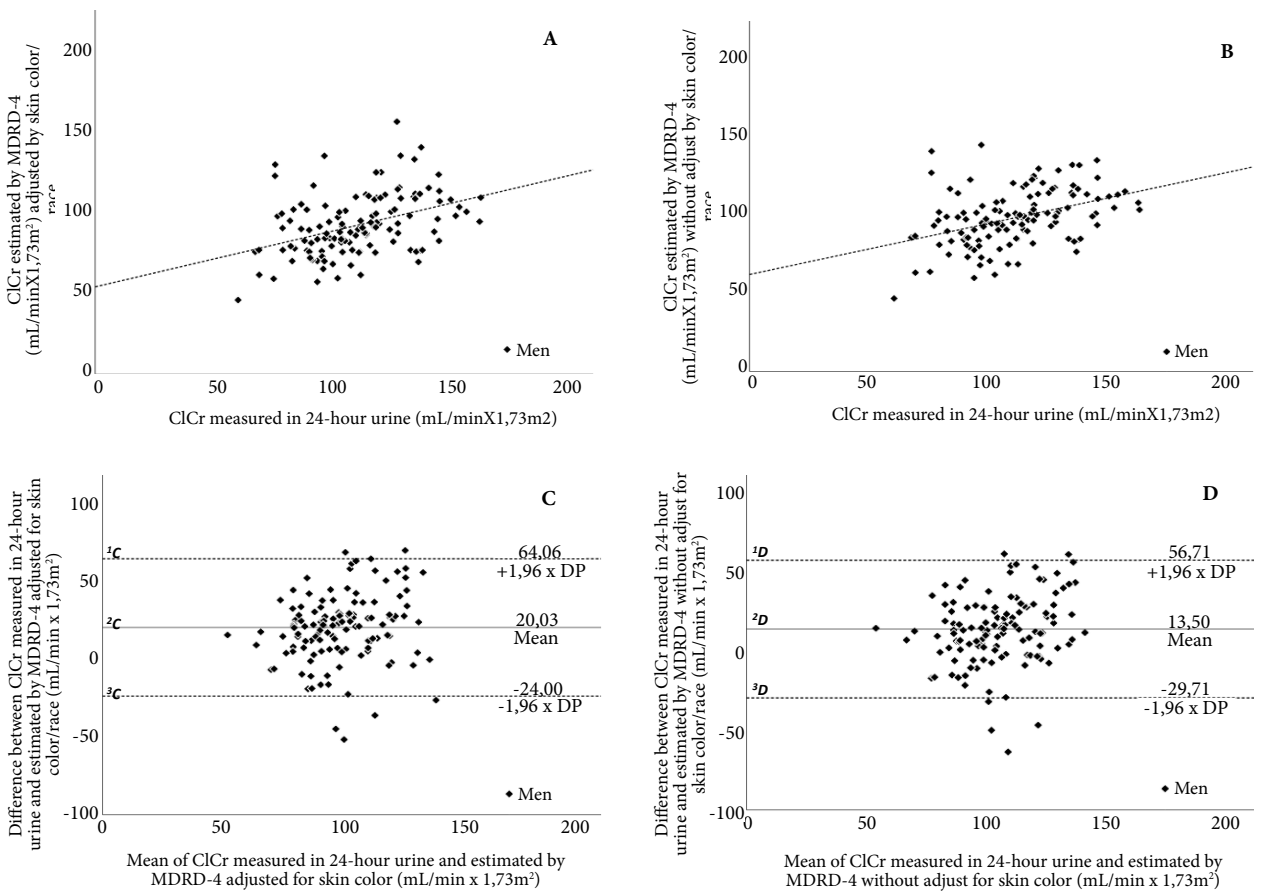
Source: Authors.

slightly more relevant predictor for GFR since, in the studied population, the performance of the equation was superior with greater proximity and less dispersion of data in the graphical and numerical comparison with ClCr, suggesting that preference should be given to its use in preliminary screening for CKD, which is usually done in primary care. However, the distinction of accuracy between the two formulas was not the objective of this study demanding more detailed analysis in larger population groups.

It should be noted that both formulas were developed and validated in populations in the northern hemisphere and that, despite being widely used in other countries, had not been tested in the Brazilian context, where ancestry, dietary patterns and other characteristics related to lifestyle are quite different. In Brazil, the issue of adjustment by race/skin color is fundamental, since the majority of the population is predominantly mixed and with a high degree of African ancestry[21,39]. The correction for individuals with black skin was introduced with the argument that creatinine production would be higher in blacks, however, this correction seems to be irrelevant and even harmful to the diagnosis of the status of renal function in very mixed populations[38.39], as was the case of this sample that represents, approximately, the diversity of race/color in the Brazilian population.

The result of one-way ANOVA of CrCl indicated that, in the studied population, there are no variations mediated by race/color aspect that significantly affect creatinine excretion. Even acknowledging the limitations of the photocolorimetric detection of creatinine[26], the similarity of the methods allows the same reasoning to be applied to SCr, corroborating with the criticism made to the adjustment by skin color in the formulas that is currently used[40]. Additionally, when GFR_{MDRD-4} and $GFR_{CKD-EPI}$ were analyzed in the subgroups by skin color, became evident that the adjustment applied only to blacks or to blacks and brown increased the difference between the means, moving away from the ClCr measured according to the 24h urine collect. The one-way ANOVA with repeated measures, comparing GFR_{MDRD-4}, $GFR_{CKD-EPI}$ and ClCr, also showed differences between the means when the race/color correction factor was used both in blacks and in blacks and brown subjects.

Analyzing the data in its entirety, the use of the correction factor for skin color graphically increased the dispersion in both agreement and correlation and, mathematically, decreased correlation coefficients between measured and estimated GFR values. These results indicate that the differential adjustment of the equations only for blacks or brown decreases accuracy of the formulas, contraindicate their use in clinical settings. However, it is also not possible to state that the ideal method would be to apply the correction



it continues

Figure 2. Correlation (A and B; E and F) and agreement (C and D; G and H) analysis among the Creatinine Clearance (ClCr) and GFRMDRD4 and GFRCKDEPI formulas in participants stratified by male (A, B, C and D) and female (E, F, G and H) gender (Adults, 18-69 years). PNS - Vitória-ES, Brazil, 2015.

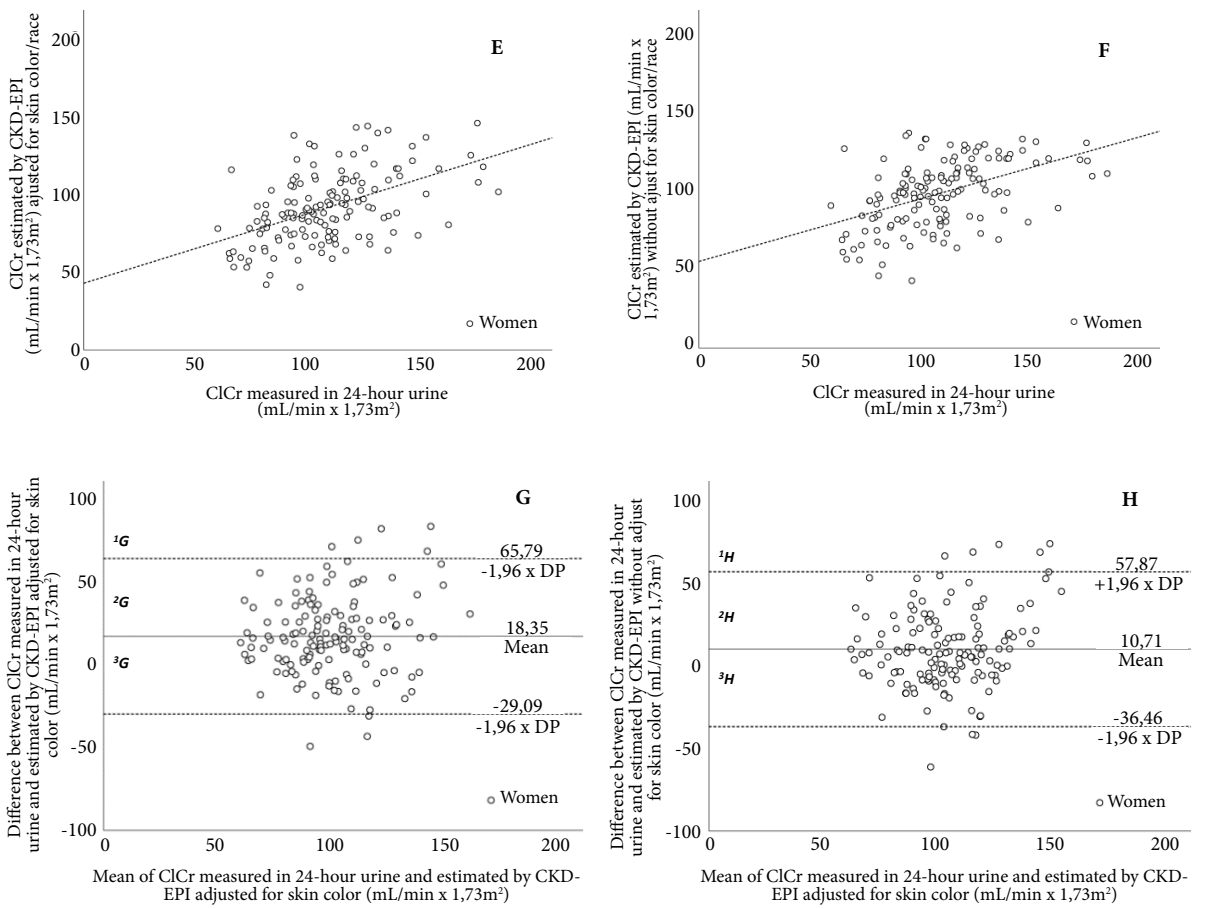


Figure 2. Correlation (A and B; E and F) and agreement (C and D; G and H) analysis among the Creatinine Clearance (ClCr) and GFRMDRD4 and GFRCKDEPI formulas in participants stratified by male (A, B, C and D) and female (E, F, G and H) gender (Adults, 18-69 years). PNS - Vitória-ES, Brazil, 2015.

Notes: [1]C and [3]C - limits of agreement; [2]C - Average difference (24h-urine vs. MDRD-4 without race adjustment). [1]D and [3]D - limits of agreement; [2]D - Average difference (24h-urine vs CKD-EPI without race adjustment). [1]G and [3]G - limits of agreement; [2]G - Average difference (24h-urine vs. MDRD-4 adjusted by race/color). [1]H and [3]H - limits of agreement; [2]H - Average difference (24h-urine vs. CKD-EPI adjusted by race/color).

Source: Authors.

to all individuals regardless of race/color, since tubular secretion of creatinine causes its urinary detection to overestimate glomerular filtration by between 10 and 30%[13].

Observing only the data for self-declared black participants (n=27), the estimates using the formulas only showed some positive (low) correlation with the reference ClCr after the mathematical suppression of the correction factor for race/color. This may be due to the fact that many individuals with strong African ancestry do not declare themselves as 'black', with the opposite also occurring in individuals with low African ancestry[40]. However, this aspect lacks evaluation in other studies.

Data presented here agree with other surveys on the prevalence of CKD in Brazil, which were carried out disregarding the correction factor for skin color[22] and strongly suggesting that its use be suppressed in national clinical practice. More robust works such as the Longitudinal Study of Adult Health (ELSA Brasil) already suggested the inadequate use of race/color adjustments because they did not find any difference induced by this proce-

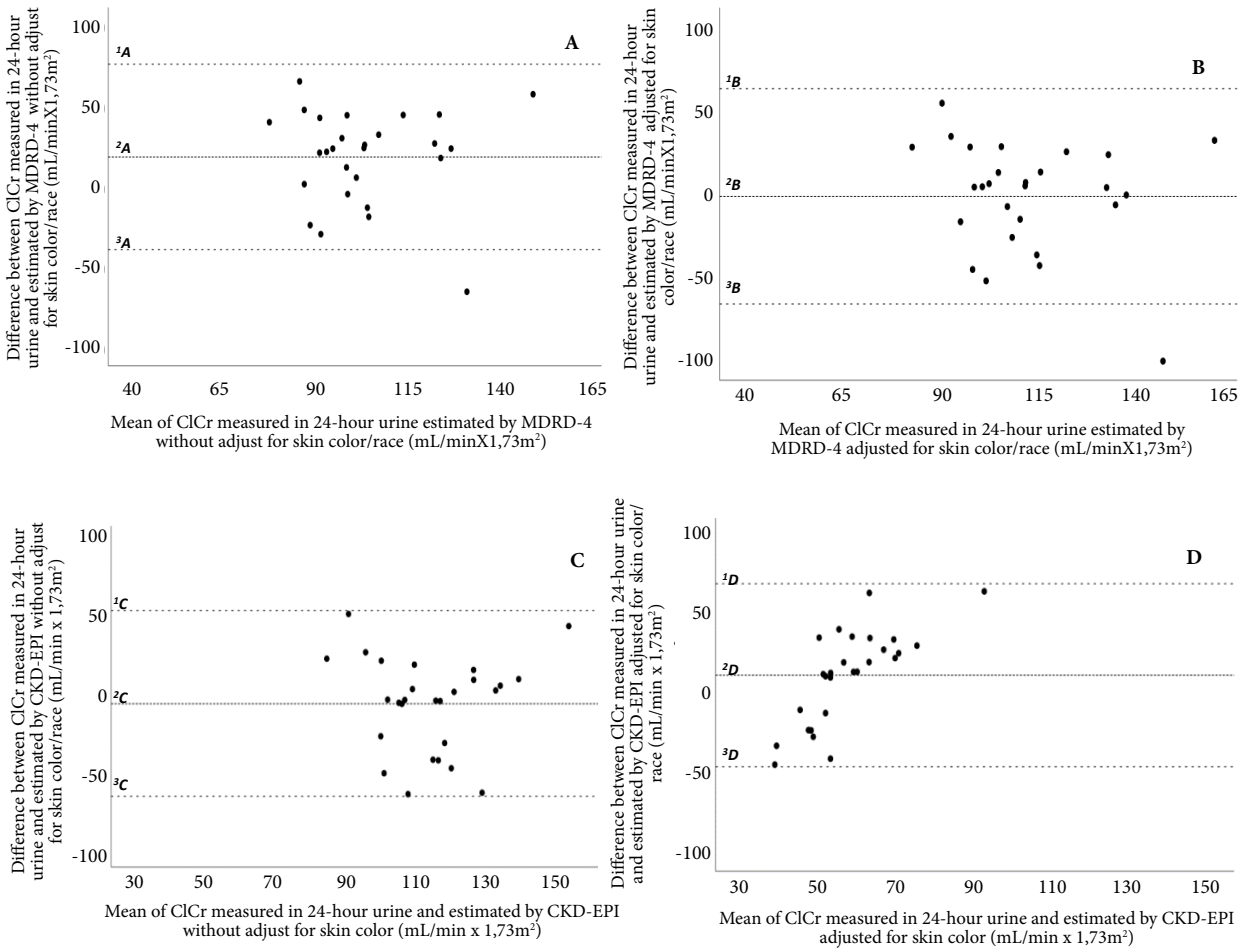


Figure 3. Agreement analysis among the Creatinine Clearance (ClCr), TFGMDRD4 and TFGCKDEPI in participants stratified by skin color (A, B, C, D) (Adults 18-69 years). PNS - Vitória-ES, Brazil, 2015.

Notes: [1]A and [3]A; [1]B and [3]B - limits of agreement; [2]A, [2]B - Average difference (24h-urine vs. MDRD-4). [1]C and [3]C; [1]D and [3]D - limits of agreement; [2]C, [2]D - Average difference (24h-urine vs CKD-EPI).

Source: Authors.

dure in the prevalence of CKD or even in SCr levels[39]. A position regarding the applicability of this correction in the Brazilian population is essential, considering that Brazil is a very mixed nation with a high prevalence of blacks in many states. In addition, the subjective nature of the attribution or self-declaration of race/color in clinical practice should be highlighted[40]. Observing the survey carried out in this study and the premise that possible errors in the diagnosis of CKD can have severe impacts on the management of the disease in black individuals, it is clear that the application of this correction factor of about 20% in the equations for these population groups is a practice that, at the very least, deserves to be reviewed.

This work has some limitations. The first is that it was not done on a random sample of the population. To cover a broader spectrum of age groups, we chose to select participants by sex and age quotas. However, the final result obtained was adequate, as both healthy individuals and those with the most prevalent morbidities in the general population are represented in the sample[33]. In addition, as expected in studies of this nature, problems in the 24-hour urine collect led to a loss of participants. But these losses did not

compromise the representativeness of the sample. Among the volunteers with apparently adequate urine collection, other removals were made due to the daily rate of creatinine excretion being outside the pre-established intervals in the literature[30]. These exclusions were necessary because they may be due to possible collection errors, resulting in a smaller sample, but with 24-hour urine data with a higher degree of internal validity, essential in validation studies[34]. Even with these removals, the final sample was robust and composed of a wide spectrum of ages, and can be considered representative of the adult urban population of Vitória[35]. Additionally, the sample size was insufficient for more consistent analyzes in subgroups, such as in patients with diabetes and already established CKD (CrCl<60 ml/min x 1.73 m^2). For these analyses, more robust samples or specific samples constituted for this purpose are required. However, it should be noted that the use of ClCr with 24-hour urine collection to validate the MDRD-4 and CKD-EPI equations is unprecedented in the Brazilian context, and may support specific guidelines on the subject.

In view of the results and considering the study limitations, it can be concluded that the MDRD-4 and CKD-EPI formulas performed adequately to estimate the status of renal function in adults, not recommending the use of corrections for race/color in the Brazilians because it reduces the accuracy of the GFR estimate, making the early detection of CKD more difficult.

Collaborations

JG Mill acted directly in the guidance and execution of the field research that originated the database. Additionally, he guided the writing of the article. WLC Almeida led the data processing, calculations and statistical analysis. Additionally, he created graphs and tables and wrote the article.

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