COMPARISON OF ESTIMATED GLOMERULAR FILTRATION RATE (eGFR) USING SERUM CREATININE AND CYSTATIN C IN APPARENTLY HEALTHY INDIVIDUALS

1*Fashina AI and 2Abbiyesuku FM
1Department of Medical Services, University of Lagos, Lagos
2Department of Chemical Pathology, University of Ibadan.
Corresponding author: *abfash2@gmail.com*

ABSTRACT
SEVERAL STUDIES HAVE SHOWN THE LIMITATIONS OF CREATININE IN THE DETECTION OF EARLY CHANGES IN GLOMERULAR FILTRATION RATE (GFR). MEASUREMENT OF SERUM CYSTATIN C HAS BEEN PROPOSED IN ASSESSMENT OF RENAL STATUS IN ORDER TO IMPROVE MANAGEMENT. THE AIM OF THIS STUDY WAS TO COMPARE THE ESTIMATED GLOMERULAR RATE GFR (eGFR) CREATININE AND eGFR CYSTATIN C AMONG APPARENTLY HEALTHY INDIVIDUALS WITH A VIEW TO ESTABLISHING THE PARAMETER THAT WILL DETECT THE CLINICALLY SIGNIFICANT LEVEL eGFR<60ML/MM/1.73M^2 FIRST.

A TOTAL OF 100 MALE WITH A MEAN AGE 46.5 YEARS (±1.15) WERE RECRUITED FOR THIS STUDY. SERUM CYSTATIN C AND CREATININE WERE ASSAYED USING THE IMMUNOTURBIDOMETRIC AND KINETIC MODIFIED JAFFÉ'S METHOD (TRACEABLE TO IDMS) RESPECTIVELY. PEARSON CORRELATION AND BLAND-ALTMAN PLOT WERE USED FOR COMPARISON.

THE MEAN EGFR FOR SERUM CYSTATIN C WAS 96.5 ML/MM/1.73M3±18.18 AND MEAN EGFR CREATININE WAS 96.8 ML/MM/1.73M3 ±22.39. THE EGFR USING SERUM CYSTATIN C HAS SIGNIFICANT POSITIVE CORRELATION WITH EGFR USING SERUM CREATININE (R=0.671; P=0.000). THE EGFR USING SERUM CYSTATIN C REVEALED THAT 3% OF THE PARTICIPANTS SHOWED REDUCED RENAL FUNCTION BASED ON THE GROUPING OF KIDNEY DISEASE BY THE KIDNEY DISEASE: IMPROVING GLOBAL OUTCOMES (KDIGO) CLASSIFICATION BUT THIS WAS NOT DETECTED BY SERUM CREATININE METHOD. THERE WAS AGREEMENT BETWEEN THE EGFR CREATININE AND EGFR CYSTATIN C (97% OF THE PLOT FELL WITHIN ±2SD). CONCLUSION: EGFR CYSTATIN C SHOWED EARLY STAGE OF REDUCED RENAL FUNCTION NOT DETECTED BY EGFR CREATININE.

Key words: *Estimated glomerular filtration rate creatinine (eGFR), creatinine, Estimated glomerular filtration rate cystatin C (eGFR cystatin C), serum Creatinine and serum Cystatin C*

INTRODUCTION

Glomerular filtration rate (GFR) is considered one of the good indicators of renal function, and serum creatinine is the most commonly used biochemical parameter to estimate GFR in routine practice (Hay *et al.*, 2014). But it can be estimated

using different equations. Kidney Disease Improving Global Outcomes (2012) has stated that estimated GFR should be included when creatinine and cystatin C are requested in order to enhance diagnostic accuracy and proper patient management. Estimated glomerular filtration rate (eGFR) is more precise if derived from both cystatin C and creatinine levels in serum (Inker *et al.,* 2012). The recently proposed CKD-EPI equation is known to be more accurate (KDIGO, 2012). Hence, it is recommended in assessment of renal function, especially with cystatin C, for the detection of early renal dysfunction not easily detectable by routine creatinine assay. Estimated glomerular filtration rate (eGFR) is an important index of renal function which can be calculated from creatinine or cystatin C concentration in serum (Mysliwiec *et al*. 2013). Creatinine and cystatin C are important biomarkers of kidney function. However, several studies have shown the limitations of creatinine in the detection of early changes in glomerular filtration rate (GFR). Thus Cystatin C has recently emerged as more reliable, early marker of renal dysfunction, compared with creatinine (Artunc et al., 2005). Cystatin C is a better indicator of small changes in glomerular filtration rate (GFR); at which stage creatinine values are still within the reference range (Artunc et al., 2005). Cystatin C is not affected by age, diet gender or muscle mass. There are many proposed formulae based on cystatin C concentrations for estimating GFR but the recently proposed chronic kidney disease-epidemiology collaboration information (CKD-EPI) equation is more accurate (KDIGO, 2012). Therefore, measurement of serum cystatin C has been proposed in assessing renal status in order to improve clinical outcome (Artunc *et al.,* 2015). There is insufficient literature available, to the best of our knowledge, on the relationship between creatinine and cystatin C and the performance of the CKD-EPI equation for GFR in apparently healthy population in Ibadan, Nigeria. This study compared the eGFR creatinine and eGFR cystatin C among apparently healthy individuals with a view to establishing the parameter which will detect first the clinically significant level eGFR <60ml/mm/1.73m^2. 5ml of venous blood samples were collected aseptically from a total of hundred (100) participants and analysed for cystatin C level and creatinine. Immunoturbidometric and modified kinetic Jaffé methods (traceable to IDMS) were employed in the estimation of cystatin C and creatinine respectively. Pearson correlation and Bland-Altman (BA) plot were used for comparison.

MATERIALS AND METHODS

The study was approved by the Health Research Ethics Committee of the University of Ibadan/University College Hospital, Ibadan (UI/UCH Registration number: NHREC/05/01/2008a) and informed consent was obtained from the participants prior to specimen collection. This is a cross-sectional study on the

relationship between GFR serum creatinine and GFR cystatin C using CKD-EPI equation.

Recruitment of Subjects

A total of 100 male participants with mean age 45.5 years were recruited for the study voluntarily. They were randomly recruited from areas in Ibadan which included: Bodija, Aleshinloye, and Mokola areas of Ibadan. The procedure was explained to the participants both in English and Yoruba before sample collection and written informed consent was obtained.

Sample Size Determination

The prevalence of kidney disease in Nigeria has been shown by various studies to have an approximate average of 7.0% (Odubajo *et al.*, 2011). The sample size for this study is therefore given by the formula:

$$n = \frac{(1.96)^2 P(1-P)}{d^2}$$

n=Sample size,

P=Estimate of expected proportion= 7.0%, 1.96= Standard normal deviation of α, d= Confidence limit, desired level of precision is 95% (α=0.05),

Estimated design is 1

(Mangani,1997).

$$n = \frac{(1.96)^2 (0.07)(1-0.07)}{(0.05)^2} = 100$$

Therefore, n=100 ie One hundred (100) participants were studied.

Inclusion and Exclusion Criteria

There were no amputees or any with muscle wasting disease. They were between the ages of 18 and 75 years. The following people were excluded: pregnant and breastfeeding mothers, persons who refused consent and those with chronic and debilitating illnesses.

Method

Anthropometric and clinical data were collected. Weight was taken with bathroom scale placed on a flat surface and the subjects stood on it as the reading was recorded in kilogram. Height (in metres) was measured against a flat, vertical surface with subjects standing upright on a firm level ground without raising the heels from the ground. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Blood pressure (BP) was measured three

times with a standard mercury Accosson's sphygmomanometer on the left arm after about 10 minutes rest and average taken.

Sample Collection and Storage

Five (5) ml of venous blood was aseptically obtained from the antecubital fossa vein with minimal stasis using pyrogen-free disposable needles and syringes. 5ml of blood specimen was carefully dispensed into plain vacutainer tubes. The blood serum was obtained after blood was allowed to clot and retract (30 minutes) and by centrifuging at 4000rpm for at least 5 minutes and stored at -20^{o}C until analysis within a month of collection. Temperature was monitored with Thermo scientific thermometer.

Laboratory Analysis

Serum levels of Cystatin C and creatinine were determined with immunoturbidometric method and kinetic Jaffe method (traceable to IDMS) respectively (Jaffe, 1886). Serum Cystatin C was measured by a particle-enhanced turbidimetric immunoassay (PETIA) method using the COBAS C311, Roche diagnostics, Germany automated systems. Creatinine was determined using the Roche diagnostics, Germany reagents and COBAS C311 automated systems with kinetic Jaffe slot method as described by Jaffe'(Jaffe, 1886).

Statistical Analysis

A Statistical Package for the Social Sciences (SPSS) software version-16.00 was used for the statistical analysis of the data. All the values were expressed as mean plus/minus standard deviation (SD) in the study group and performed within 95% confidence interval or 5% level of significance ($p<0.05$). Non parametric method was used for data analysis because the data were normally distributed when tested by Kolmogorov method. Pearson's correlation, Bland-Altman (BA) plot were used for comparison of the data.

RESULTS

A total of 100 male with age 46.5 (±1.15) years completed this study. The summary of the anthropometric indicators, serum creatinine, serum cystatin C, eGFR creatinine and eGFR cystatin C are shown in Table 1. The mean eGFR cystatin C and eGFR creatinine lies within the reference range for apparently healthy persons.

Table 1: Anthropometric factors, serum creatinine, cystatin C concentration and eGFR

	Mean (±SD)
Age (years)	35.0 (±6.15)
Weight (kg)	67.9 (±12.78)
BMI (kg/m^2)	23.3 (±4.13)
Creatinine (mg/dl)	1.11 (±0.46)
Cystatin C (mg/dl)	0.90 (±0.19)
eGFR cystatin C (ml/mm/1.73m^2)	96.4 (±18.1)
eGFR creatinine (ml/mm/1.73m^2)	96.8 (±22.3)

Legend

eGFR creatinine (ml/mm/1.73m^2) – Estimated glomerular filtration rate using creatinine

eGFR cystatin C (ml/mm/1.73m^2) – Estimated glomerular filtration rate using cystatin C

Creatinine (mg/dl) - Serum concentration of creatinine

Cystatin C (mg/l) – Serum concentration of cystatin C

BMI - Body Mass Index

Table 2: Comparison between the percentage of eGFR creatinine and eGFR cystatin C. The eGFR cystatin C detected 3% of the participants with clinically significant reduced GFR (eGRF<60ml/mm/1.73m^2) which was undetected by the eGFR creatinine

Stages	**GFR ml/mm/1.73m^2**	**Percentage (%) eGFR creatinine**	**Percentage (%) eGFR cystatin C**
G1	≥ 90	66	67
G2	60-89	34	30
G3a	45-59	0	3
G3b	30-44	0	0
G4	15-29	0	0
G5	<15	0	0

Legend

G1-Stage 1 of the KDIGO classification of Chronic Kidney Disease

G2-Stage 2 of the KDIGO classification of Chronic Kidney Disease

G3-Stage 3 of the KDIGO classification of Chronic Kidney Disease

G4-Stage 4 of the KDIGO classification of Chronic Kidney Disease

G5-Stage 5 of the KDIGO classification of Chronic Kidney Disease

Percentage (%) eGFR creatinine – Percentage of automechanics with the estimated glomerular filtration rate using creatinine

Percentage (%) eGFR cystatin C – Percentage of automechanics with the estimated glomerular filtration rate using cystatin C

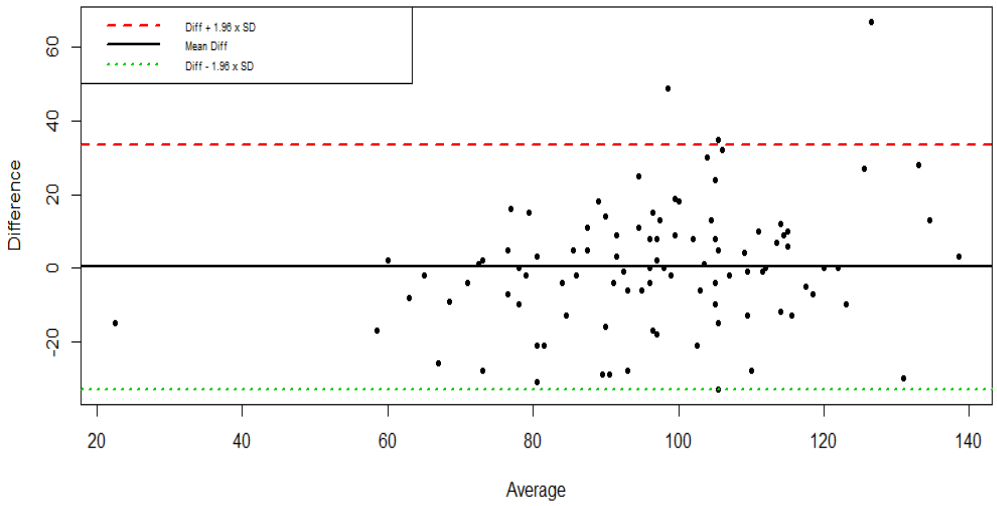


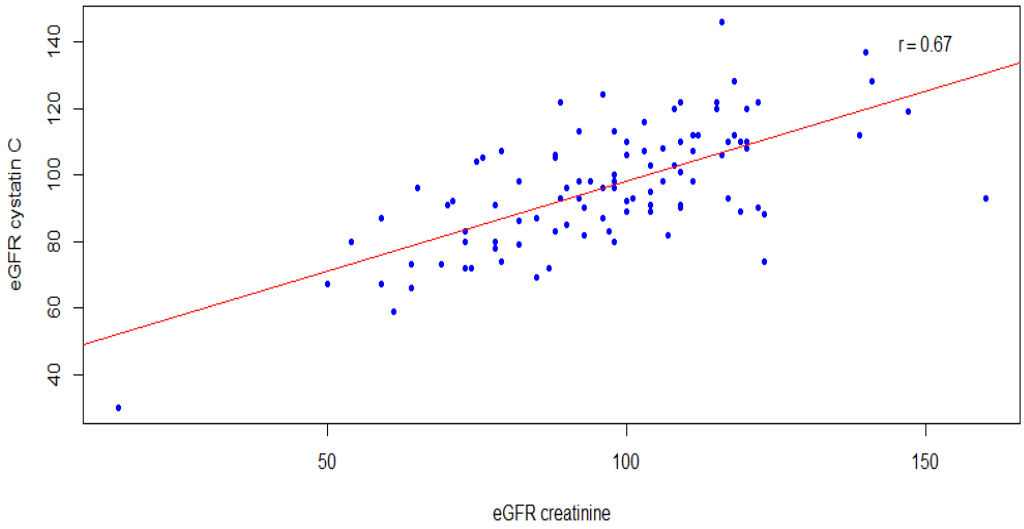
Figure 1: Bland-Altman plot of eGFR creatinine against eGFR cystatin C based on the KDIGO Stages 1-5 classification. There was agreement between eGFR creatinine and eGFR cystatin C using the KDIGO CKD stages based on Bland-Altman plot. It showed that there is significant agreement between the methods because 97% of the plot fell within ±2SD.

Legend

Difference (Vertical axis) - The difference between eGFR cystatin C and eGFR creatinine

Average (**Horizontal axis**) – The average of eGFR creatinine and eGFR cystatin C

Figure 2: Scatter plot of eGFR cystatin C against eGFR creatinine. There was a



significant positive correlation between eGFR creatinine and eGFR cystatin C based on the scatter plot (r=0.67, p=0.000) as stated in Figure 2.

DISCUSSION

Many research findings revealed that early stages of reduced kidney function usually go undetected by the routine creatinine methods. Therefore, Cystatin C has been proposed because it detects early changes in the GFR (Artunc *et al.*, 2015). It is a medium size molecule (13kDaltons) synthesized by all nucleated cells with constant production rate, freely filterable through the glomerulus due to its small size, and metabolized by proximal tubular cells (Donadio *et. al.*, 2012). Glomerular filtration rate represents the plasma volume presented to the nephrons per unit time during urine formation and usually measured in milliliters per minute. Constant production of cystatin C makes it an ideal marker of GFR, especially in patients with reduced muscle mass or conditions that produce rapid change in muscle mass (Milić *et al.*, 2011). Creatinine is a chemical end product of creatine metabolism that is cleared from blood plasma by glomeruli and is excreted in the urine. While creatinine has been widely used to date to assess renal function, it is subject to variation due to a number of factors including age, gender, race, chronic illness, diet, and muscle mass (Stevens *et al.*, 2008). Estimated glomerular filtration rate (eGFR) is also an important index of renal function which can be calculated from creatinine or cystatin C concentration in serum (Mysliwiec *et al.*, 2013). This study compared the eGFR creatinine and eGFR cystatin C among apparently healthy individuals with a view to establishing

the parameter which will detect first the clinically significant level of eGFR <60ml/mm/1.73m^2. Bland-Altman plot was used to compare the methods as stated by Ramanathan and Padmanaban, (2011).

The Bland-Altman plot showed an agreement between the two methods. There was agreement between eGFR creatinine and eGFR cystatin C using the KDIGO CKD stages based on Bland-Altman plot. It showed that there is significant agreement between the methods because 97% of the plot fell within ±2SD.
Therefore, eGFR cystatin can be used in lieu of eGFR creatinine or together in assessment of GFR. This view agrees with the observation of Inker *et al.* (2012) who stated that estimated glomerular filtration rate (eGFR) is more precise if derived from both cystatin C and creatinine levels in serum (Inker *et al.,* 2012). It concurs with KDIGO (2012) which states that estimated GFR should be included when creatinine and cystatin C are requested in order to enhance diagnostic accuracy and proper patient management.

The eGFR cystatin C detected 3% of the participants with clinically significant reduced GFR (eGRF<60ml/mm/1.73m2) which was undetected by the eGFR creatinine. This view agrees with the observation of Zahran *et al.* (2007) and Dharnidharka *et al.* (2002) which states that serum levels of cystatin C rises earlier than serum creatinine levels in assessment of kidney function. Furthermore, it agrees with the observation of Artunc *et al.* (2015) which revealed that serum cystatin C levels showed small changes in glomerular filtration rate (GFR) when creatinine values are still within the reference range.

There is positive correlation between serum creatinine and cystatin C levels. This is in agreement with the view of Sjöström *et al.* (2005) which states that changes in glomerular filtration rate are reflected in serum creatinine and cystatin C levels.

CONCLUSION

In conclusion, eGFR cystatin C showed that 3% of participants had early stage of reduced renal function not detected by eGFR creatinine. Therefore, eGFR cystatin C should be combined with eGFR creatinine when assessing renal function in order to enhance diagnostic accuracy and proper patient management

RECOMMENDATION

It is recommended that eGFR cystatin C could be used in lieu of eGFR creatinine or both could be used together in assessing of renal status.

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