Evaluation of serum cystatin C as an early marker of renal disease in Chronic Kidney Disease patients in Kano

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Abstract

Chronic Kidney Disease (CKD) is a public health problem with rising incidence worldwide. Nigeria appears to be badly hit by this epidemic; therefore, there is a need to assess a more reliable marker devoid of limitations. This study evaluated serum cystatin C as an early biochemical marker of renal disease in chronic kidney disease patients in the Kano metropolis. A cross-sectional study was conducted at Aminu Kano Teaching Hospital and Muhammad Wase Specialist Hospital (MWSH) in Kano, Nigeria. A total of 150 subjects comprised 100 chronic kidney disease patients, and 50 apparently healthy subjects as controls. The serum creatinine was measured by the Jaffe Method, cystatin C by immunoturbidometric method, and glomerular filtration rates were estimated using CKD-EPI and modified diet in renal disease formulae. One-way Analysis of Variance was used to compare the Estimated Glomerular Filtration Rate (eGFR) of the chronic kidney disease patients with the control groups. In this study, the multiple comparisons of the estimated glomerular filtration rate showed that cystatin C-based glomerular filtration rate gave a direct and accurate measurement of independent of age, and muscle mass with the estimated glomerular filtration rate of ≤ 60 mL/min/1.73m^2 in chronic kidney disease patients substantially lower as compared to the control group and newly diagnosed chronic kidney disease patients. Serum cystatin C-based glomerular filtration rate gave a direct and accurate measurement of independent of age, and muscle mass and thus suggestive of a better marker of early detection of chronic kidney disease.

Creatinine-based glomerular filtration rate has been relatively inexpensive and widely used for the diagnosis of renal function. However, to address its limitations caused by the influence of some factors, cystatin C-based glomerular filtration rate gave a direct and accurate measurement independent of age, sex, and muscle mass.

Introduction

Chronic Kidney Disease (CKD) is a progressive renal impairment with early structural injury occurring in absolute clinical silence and classified into stages according to the level of glomerular filtration rate.[1] It has a global incidence of five to eight percent per year.[2-4] Egbi *et al.* also reported a 7.8% prevalence of CKD in a community-based study in Bayelsa, Nigeria.[5]

In Nigeria, CKD treatment, including hemodialysis, costs about $8000/patient/year.[2,6-8] At the University College Hospital, Ibadan, 70% of patients could not afford more than three sessions of dialysis.[9] The resultant effect is high rates of mortality amongst the End-Stage Renal Disease (ESRD) population in various countries of the sub-region.

The risk factors of kidney disease span the life course and environmental, infection, and lifestyle etiologies.[10] If risk factors are identified early, Acute Kidney Injury (AKI) and CKD can be prevented, and thus worsening of kidney function can be retarded or averted by inexpensive interventions.[10-12] Serum creatinine has been the mainstay by which renal function has been estimated for

decades, but it is crude and can sometimes be misleading due to the influence of some factors such as age, sex, weight, and race.[13] These limitations led to the search for additional renal disease biomarkers and the ultimate identification of markers like cystatin C. The National Institute for Health and Care Excellence (NICE) recommended cystatin C-based equations of eGFR (estimated Glomerular Filtration Rate).[14] Cystatin C, a non-glycosylated protein, has a low molecular weight that makes it freely filterable by the glomerulus and unlike creatinine it is not affected by age, sex or spectral interferences.[15] More so, cystatin C is almost completely reabsorbed by the tubules and has no known route of extra-renal route of elimination.[16] Thus, this study evaluated the diagnostic accuracy of cystatin C in the determination of eGFR.

Materials and Methods

Study Design

A cross-sectional study design was adopted for this research. The participants were divided into 3 groups composed of 2 test groups and a control group as follows: Group A, control group, comprising 50 apparently healthy subjects, including apparently healthy blood donors, volunteers, and students; Group B, test group 1, comprising 50 newly diagnosed CKD patients with GFR≥60 mL/min/1.73m^2; Group C, test group 2, comprising 50 patients diagnosed with CKD with GFR≤60 mL/min/1.73m^2.

The study was carried out in 2020 at the Nephrology clinic of Aminu Kano Teaching Hospital (AKTH), Kano State, Nigeria, and Muhammad Wase Specialist Hospital (MWSH), Kano, Nigeria.

Study population

The study groups comprised of 100 CKD patients and 50 apparently healthy subjects.

Sample size determination

The targeted population is >10,000. The sample size was calculated using the formula described by Bolarinwa *et al.*[17] A prevalence of 7.8% from a previous study by Egbi *et al.* was used for the study.[5] Consequently, the minimum sample size was calculated to be 111 and was increased to 150 subjects to increase the power of the study.

Sampling technique

From the above considerations, 50 consenting newly diagnosed CKD patients and 50 known CKD patients attending the nephrology clinic were recruited as test groups I and II, while 50 consenting apparently healthy volunteers were recruited as controls. All consented participants were recruited into this study through a convenient random sampling technique.

Inclusion criteria

Male and female patients aged 18–75 years with chronic kidney disease and/or associated with any of the following were included: i) medical history/clinical evidence of kidney disease for more than three months accompanied with symptoms such as generalized body swelling/bipedal swelling, difficulty in breathing, weakness, persistent headache, hiccup, and oliguria; ii) renal ultrasound scanning revealing abnormality in the kidney structure; iii) urinalysis/microscopy showing evidence of persistent proteinuria or abnormal urinary deposits; iv) high-risk kidney disease patients (family history of diabetes/hypertension), and patients taking anti-inflammatory drugs.

Exclusion criteria

i) CKD patients on maintenance hemodialysis, those having any chronic systemic disease, those with thyroid dysfunction, those suffering from acute renal failure; ii) patients on medications known to affect kidney function (antidiuretics, corticosteroids) and those likely to interfere with the Jaffe's Method of creatinine analysis (cephalosporin antibiotics) or immunosuppressant including asthmatics and patients with malignancy.

Anthropometric measurement

Height**:** this was measured by a vertical board with an attached metric rule and a horizontal headboard. The subjects were asked to stand barefooted, arms by their sides, and to look straight, backing the vertical board. Then the height was recorded in meters.

Weight: this was measured while the subjects were wearing light clothing with the subject standing in the center of a weighing scale's platform. The weight was recorded in kilograms.

Body Mass Index (BMI): This was calculated as body weight divided by the square of height (kg/m^2), with body weight expressed in kg and height in meters.

Body Surface Area determination

The Body Surface Area (BSA) was calculated according to the formula described by Du Bois and Du Bois as:

$$BSA = (body\ weight^{0.425}\ [in\ kg]\ x\ height^{0.725}\ [in\ cm])\ x\ 0.007184.[17]$$

Specimen collection, processing and storage

Venous blood samples for cystatin C and creatinine assay were collected from the antecubital vein of participants following proper counseling. A tourniquet was applied to the upper arm above the cubital fossa, and the skin was cleaned with an alcohol-soaked swab which was allowed to air dry. Using a 5 mL syringe and needle, 3 mL of blood samples were collected from the most prominent antecubital vein into a clot gel activator (each tube well labeled with the participant's details). All samples collected were centrifuged at 10,000 rpm for 2-3 minutes, and transferred into clean plain specimen bottles. They were covered and stored at -20°C before analysis.

Serum creatinine determination

Creatinine was measured colorimetrically by Jaffe's Method.[18] Briefly, creatinine in an alkaline solution will react with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration**.**

Serum cystatin c estimation

Serum cystatin C was measured using the immuno-turbidimetric method.[19] Cystatin C reacts to the specific antibody-forming insoluble immune complexes. The turbidity caused by these immune complexes is proportional to the cystatin C concentration in the sample and may be spectrophotometrically measured.

Estimated Glomerular Filtration Rate

Modification of Diet in Renal Disease (MDRD) Method: The MDRD eGFR was calculated using the formula described by Levey *et al.*[20]

$$eGFR = 175\ x\ plasma\ creatinine^{-1.154}\ x\ age^{-0.203}\ (x\ 0.742\ if\ female;\ x\ 1.21\ if\ black).$$

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The estimated renal functions using the MDRD equation were expressed as GFR in ml/min per 1.73 m^2.

eGFR calculated according to simple cystatin C formula:

$$\frac{100}{\text{serum Cystatin C (mg/L)}}$$

Chronic Kidney Disease Epidemiology (CKD-EPI) creatinine and cystatin C formula:

177.6 x (serum creatinine (mg/dl) – 0.65 x (serum cystatin C (mg/L) – 0.57 x age – 0.2

The correction factor of 0.82 was used for women.[1]

Statistical analysis

The data generated from the study were subjected to statistical analysis using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were presented as mean ± standard deviations. The means of the three studied groups were compared by using One-Way Analysis of Variance (ANOVA), and the data was represented graphically by using bar charts. The level of significance was set at p≤0.05, and the confidence interval at CI=95%.

Results

Demographic and anthropometric characteristics

The mean age of control group participants (Group A) was 49.58±27.02, and the mean BMI was 28.50±3.10 kg/m^2. For Group B participants, the mean age was 46.40±15.47, and the mean BMI was 28.80±4.90 kg/m^2, whereas Group C participants had a mean age of 37.82±14.75 and a mean BMI of 26.40±2.33 kg/m^2. No significant difference in mean for age and BMI across groups was detected using One-Way Analysis of Variance (Table 1).

Serum creatinine and cystatine C measurements

In the control group (Group A), the mean creatinine value was 0.75±0.20, while the average values for serum cystatin C was 0.7±0.40. In Group B samples, the mean creatinine measurement was 2.42±1.03, while mean cystatin C was 3.28±1.11. For Group C, mean creatinine values was 0.98±0.14, while the mean value for cystatin C was1.12±0.13. These results showed that there was significant mean difference for creatinine, and cystatin C across the three studied groups (Table 2).

Glomerular Filtration Rate estimation

In the control group samples, the mean eGFR by MDRD was 94.31±20.42, the mean eGFR by CKD EPI creatinine was 104.16±18.74, the mean eGFR by CKD EPI cystatin was 83.65±11.80 and the mean eGFR by CKD EPI Cr-cys was 92.37±14.51.

These results showed that there was statistically significant mean difference across all eGFR across studied groups (Figure 1).

Discussion

Glomerular Filtration Rate (GFR) is the ideal (Gold standard) biochemical marker for kidney function. Unfortunately, the most accurate and precise methods of measuring GFR (like inulin clearance) are laborious and time-consuming in a clinical environment. Therefore, GFR is usually estimated from equations that consider endogenous filtration markers like creatinine and cystatin C.[1] GFR is presently being monitored by serum creatinine concentrations and calculated creatinine clearance using CKD-EPI method and MDRD formulae, among others.[19-21]

Creatinine production changes significantly according to muscle mass of the body and dietary factors. Creatinine is filtered by the glomeruli, and secreted by the renal tubules. This tubular secretion contributes approximately 20% of the total creatinine excretion by the kidney and it can increase with decrease in GFR.[22] However, serum creatinine does not increase until the GFR has moderately decreased (about 40mL/min/1.73m^2). This insensitivity for small to moderate decreases in GFR gives a false sense of

Table 1. Baseline characteristics of studied samples (n=150).

Parameter	Groups						p
	Group A (n=50)		Group B (n=50)		Group C (n=50)		
	Mean	SD	Mean	SD	Mean	SD	
AGE (Years)	49.58	27.02	46.40	15.47	37.82	14.75	0.23
BMI (Kg/m^2)	28.5	3.1	28.8	4.9	26.40	2.33	0.073

*p<0.05 was considered significant using One-way ANOVA.

Table 2. Mean comparison of creatinine and cystatin C across studied groups.

Parameter	Groups						p
	Group A (n=50)		Group B (n=50)		Group C (n=50)		
	Mean	SD	Mean	SD	Mean	SD	
Cystatin C (mg/dl)	0.7	0.40	3.28	1.11	1.12	0.13	<0.01*
Creatinine (mg/dl)	0.75	0.20	2.42	1.03	0.98	0.14	<0.01*

*p<0.05 was considered significant using One-way ANOVA.

security and leads to late detection of kidney damage. All these factors explain why creatinine concentration may not be a good parameter for accurate determination of GFR, especially at lower rates, and makes it less reliable for therapeutic decision making in CKD patients such as changing of nephrotoxic drugs or measures to increase renal perfusion.[13]

Cystatin C is a non-glycosylated low molecular weight (13.3 KDa) protein belonging to the cysteine protease inhibitors family, produced by all nucleated cells.[15] Its production in the body is constant, not influenced by renal conditions, increased protein catabolism, or dietary factors.[16] Also, it does not change with age or muscle mass, as creatinine does. Its biochemical properties allow it to be freely filtered in the renal glomerulus, and subsequent metabolism and reabsorption by the proximal tubules. The low molecular mass, and the basic nature of cystatin C in combination with its stable production rate, suggest that the GFR is the major determinant of cystatin C concentration in the peripheral circulation recommended by the NICE.[14]

In this study, comparisons of eGFR among study groups showed that the mean eGFR of control group samples by MDRD was significantly higher than Group B and Group C participants, whereas Group C participants had significantly higher eGFR by MDRD as compared to Group B participants.

GFR estimated by CKD-EPI-Cr of Group B was significantly low as compared to Group A and Group C, whereas control group participants had significantly higher eGFR CKD-EPI-Cr as compared to Group C participants. Esitmated GFR by CKD-EPI-Cys of group B participants was substantially lower as compared to the control group and Group C participants whereas Group C has a significantly low mean as compared to control group samples. The pattern demonstrated that patients that are newly diagnosed (Group B) had lower eGFR than control group compatible with expected results of decline in GFR with renal disease.[23] Also, eGFR of newly diagnosed tend to be lower than known CKD patients possibly because of they are on treatment and having regular renal replacement therapy. Additionally, other confounding variables like lower serum albumin (seen in CKD), higher CRP, and white cell count can affect cystatin C levels and could explain this observation.[24] The eGFR by CKD-EPI-Cr-Cys of Group B participants was significantly low as compared to the control group and Group C samples. The mean of control group participants was also considerably higher when compared to Group C participants, which

correlates with the study of Shlipak *et al.* and Bibi *et al.*, who reported that the diagnostic accuracy of cystatin C in the detection of reduced kidney function is more reliable for the estimation of GFR compared to serum creatinine when cut-off values are set to 60 ml/min/1.73m^2. [25,26]

Our study showed that cystatin C-based calculation of eGFR is lower as compared to eGFR calculated based on serum creatinine when cut-off values are set to 60 ml/min/1.73m^2. This tends to support previous study findings, which suggest that cystatin C rises earlier than serum creatinine, making it a more sensitive marker for detecting CKD than serum creatinine.[27,28] A possible advantage of cystatin C over creatinine is that being a larger molecule, its blood levels might rise sooner than that of creatinine. As compared to conventional serum creatinine assays, serum cystatin C levels detection can also be done in addition to screening patients with serum creatinine, especially in individuals with a longer disease duration or uncontrolled diabetes mellitus or hypertension.[29,30]

In critically ill CKD patients, sudden changes in GFR are not instantly followed by parallel changes in serum creatinine.[31] Serum creatinine has been the mainstay, by which renal function has been estimated for decades, but it is crude and can sometimes be misleading due to the influence of some factors such as age, sex, weight, and race *etc.* on its production. Although the measurement of serum creatinine is relatively inexpensive and widely available, they do have significant limitations. Estimating GFR based on serum cystatin C shows to be more promising for evaluating the renal function of CKD patients. This study will help nephrologists in early detection of deranged kidney function and thus improve the patients' management.

Conclusions

Serum cystatin C is a better marker of renal function than serum creatinine in the early detection, diagnosis, and management of chronic kidney disease patients. Cystatin C-based GFR gives a direct and accurate measurement of GFR independent of age and muscle mass. Also, risk factors for CKD are common in our environment; therefore, there is a need for preventive strategies such as public enlightenment to avert the rising burden of CKD.

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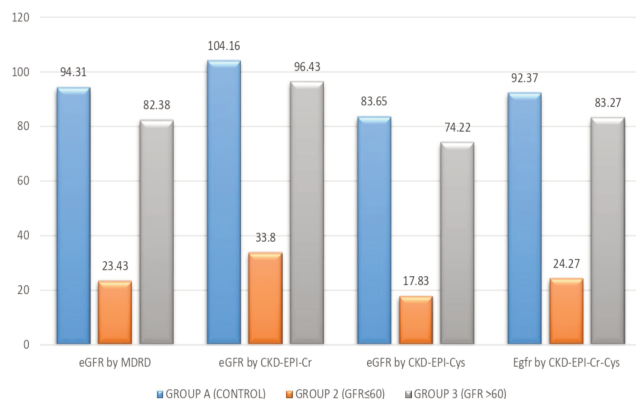6. United States Annual Data System (USRDS). Annual Data

Figure 1. Mean estimated Glomerular Filtration Rate (eGFR) across the studied groups.

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