

**COMPARATIVE STUDY BETWEEN SERUM CYSTATIN C AND CREATININE LEVELS IN CHRONIC
KIDNEY DISEASE PATIENTS AND BLOOD DONORS**

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A dissertation submitted to the University of Nairobi in part fulfillment of the requirements for the degree of Master of Medicine in Human Pathology.

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DECLARATION

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I, Mwaludindi Dixon Mchana, declare that this dissertation for Master of Medicine in Human Pathology is my original work and has not, to the best of my knowledge, been presented by any other individual at any other institution of higher learning.

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DEDICATION

This work is dedicated to my family and true friends

APPRECIATION

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LIST OF ABBREVIATIONS

ACE	Angiotensin-Converting Enzyme
BMI	Body Mass Index
BTU	Blood Transfusion Unit
CG	Cockcroft-Gault formula
CKD	Chronic Kidney Disease
CRF	Chronic Renal Failure
Cr-EDTA	Chromium-labeled EDTA
Cys C	Cystatin C
CV's	Coefficient of Variance
^{99m}Tc-DTPA	Technecium-labelled diethylene-triamine-pentacetate
eGFR	Estimated Glomerular Filtration Rate (eGFR)
EPO	Erythropoietin
ESRD	End-Stage Renal Disease
HAART	Highly-active antiretroviral therapy
HbA_{1c}	Hemoglobin A _{1c}
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HIVAN	HIV-associated nephropathy
IFCC	International Federation of Clinical Chemists
IQC	Internal Quality Control
KDIGO	Kidney Disease Improving Global Outcome
KNBTS	Kenya National Blood Transfusion Service

KNH	Kenyatta National Hospital
MDRD	Modification of Diet in Renal Disease
NBTC	National Blood Transfusion Centre
NBTS	National Blood Transfusion Services
SCr	Serum Creatinine
SCr LN	Serum creatinine natural logarithm
SLE	Systemic Lupus Erythematosus
TGs	Triglycerides
USA	United States of America
VDRL	Venereal Diseases Research Laboratories

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SUMMARY

Background

Chronic Kidney Disease (CKD) is a disease spectrum characterized by progressive loss of renal function over a period of time. Chronic kidney disease has reached epidemic proportion with current reported incidence of approximately 14.5% among the adult population in the United States of America. While no data exists on the Kenyan situation, risk factors for CKD such as post-streptococcal glomerulonephritis, hypertension, diabetes mellitus and lately HIV-associated nephropathy (HIVAN) are on the rise. Recent studies show that early diagnosis allows for institution of therapy to either arrest or reverse progression of the CKD. Glomerular filtration rate (GFR) is accepted as the best overall measure of renal function and is determined by the renal clearance of exogenous markers such as inulin, iohexol and chromium-labeled ethylene diamine tetrachloroacetic acid (^{51}Cr -EDTA). Currently, creatinine clearance (CrCl) is the widely used endogenous marker for estimating glomerular filtration rate.

While a number of equations based on serum creatinine (SCr) are in use to estimate GFR, case diagnosis and stratification of CKD is poor. Estimated GFRs based on serum cystatin C (cys C) levels have been shown to be more sensitive to early CKD compared to serum creatinine, hence a suitable alternative.

In this study, estimated GFRs for CKD patients at the Kenyatta National Hospital and blood donors at the National Blood Transfusion Centre (Nairobi) were derived from serum cystatin C and serum creatinine. Secondly, the reference ranges for both serum cystatin C and serum creatinine were compared to those from studies done in other parts of the world.

Main Objectives:

- To compare serum cystatin C and creatinine levels among CKD patients and blood donors.

Specific Objectives:

- To determine the serum cystatin C and creatinine levels in CKD patients and blood donors.
- To determine reference ranges for serum cystatin C and creatinine using blood donors as the reference population.
- To compare the estimated GFRs based on the two markers and stratify the CKD patients as per the National Kidney Foundation (NKF) guidelines.

Study Design

Comparative descriptive study between two assay markers.

Study Setting and Population/Subjects

1. Confirmed CKD patients on follow-up at the renal clinic in KNH.
2. Volunteer blood donors at the blood transfusion unit (BTU) - KNH and those at the various outreach tents organized by the NBTC, Nairobi.

Methods

A total of 124 blood donors, well above the minimum requirement of 40 by IFCC for establishment of reference range were recruited. One hundred and fifty (150) CKD patients were also recruited into the study according to the formula for prevalence study. Minimal demographic data was obtained by direct interviews using a study questionnaire.

Additional information on CKD patients was obtained by perusal of the treatment files on clinic day followed by a clinical evaluation.

Once informed consent was guaranteed, 5 ml of whole blood was obtained from each study participant for eventual determination of serum cystatin C and creatinine at the KNH main laboratory.

Data Management

The data obtained from the laboratory was entered into a computer database. Spreadsheets were generated and analyzed using Windows SPSS version 17.

Results

Out of the 124 blood donors recruited into the study, 78 (63%) were males while 46 (37%) were females. The age group pattern was: ≤ 20 years were 24%, 48% were in the 21 – 25 years; 26% in the 26 – 30 years and 2% were above 30 years. The serum cystatin C levels for blood donors had 0.6mg/L as the lowest and 1.6mg/L as the highest level while it was 65 μ mol/L and 120 μ mol/L for serum creatinine respectively. The reference ranges for cystatin c and creatinine (irrespective of age and gender) were 0.8- 1.4mg/L and 63 – 109 μ mol/L respectively.

Of the 150 CKD patients, 57% were males and 43% were females. The youngest CKD patient was 18 years and the oldest was 77 years. The younger CKD study participants had glomerulopathies

(17%) as the commonest underlying morbidity while hypertension with diabetes co-morbidity was seen in the older CKD patients.

Staging of the CKD study participants using derived estimated GFR as per serum cystatin C had 75% in early disease (stages 1-3) with only 2% in ESRD. However, eGRF based on serum creatinine had 64% of participants in early CKD (stages 1-3) and 15.4% had ESRD.

Correlation between cystatin C and creatinine levels among CKD study participants had an r value of 0.849 and a p value of 0.00. The correlation between cystatin C and its derived eGFR had an r value of -0.837 and p value of 0.00. The correlation between serum creatinine and eGFR-serum creatinine had an r value of -.678 and p value of 0.00.

Conclusions

1. The reference ranges for blood donors aged 18 – 34 years for both serum cystatin C and creatinine in this study were 0.8 - 1.4 mg/L and 62 – 120 mol/L respectively.
2. There is a difference in the staging for CKD participants as per National Kidney Foundation (NKF) guidelines using the estimated GFR derived from the two markers separately
3. There is a difference in the staging for CKD participants as per NKF guidelines using the estimated GFR derived from cystatin C and serum creatinine separately. Cross tabulation of the staging of the CKD participants yielded a kappa value of 0.359.

Recommendations

Laboratory input in the management and follow-up is central and at all times staging of known and at risk CKD patients as per the National Kidney Foundation should be done on every clinical request for renal function tests.

A follow-up study involving a larger sample size of blood donors be carried out to capture more variables such as age, gender and race.

A comparative study involving a 'gold standard' for measured GFR be carried out to assess the sensitivity and specificity of serum cystatin C and serum creatinine.

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Normal Renal Function and biochemical markers

In health, the body requires normal functioning kidneys which are involved in:

- Secretion of hormones: Erythropoietin (EPO) regulates the red cell population in erythropoietin marrow, Renin which is central in the renin-angiotensin –aldosterone system, Calcitriol and Prostaglandins.
- Maintenance of extracellular fluid volume and composition such as glucose, amino acids, sodium chloride, water, bicarbonate, protons, potassium, calcium, magnesium and phosphate. Maintenance of acid-base of acid-base balance is critical for optimal enzyme function.
- Excretion of metabolic waste: water, sodium, urea among other substances.

The kidney achieves the above via fundamental physiological mechanisms of filtration, reabsorption and secretion. When assayed, the above indices are maintained within the reference intervals.

1.2 Renal Dysfunction

This occurs when the kidney cannot effectively carry its body functions.

Kidney failure may be a complication of many disease entities and/or syndromes. Glomerular diseases such as post-streptococcal glomerulonephritis contribute a large proportion of early CKD. Chronic pyelonephritis and tuberculosis are notable infectious risk factors of which HIV-associated nephropathy is routinely encountered. Not to be left out are congenital anomalies e.g. polycystic kidney disease and obstructive processes such as calculi. Collagen disease e.g. SLE and vascular diseases such as renal nephrosclerosis may lead to CKD. Nephrotoxic agents e.g. aminoglycosides are occasionally implicated. Chronic kidney disease may be a progression from acute renal failure. These disease processes interfere with the 3 mechanisms of kidney function mentioned above.

Studies done in the USA show that diabetes and hypertension account for two thirds of cases with CKD with a significant other contribution by glomerulonephritis and cystic lesions of the kidney [1].

Chronic Kidney Disease (CKD) is a continuum in which there is progressive loss of renal function over variable time duration. While CKD has reached epidemic proportions(1) with rising prevalence and incidence worldwide, insensitivity of diagnostic markers has continuously hampered efforts in case identification and monitoring disease progression.

In 2002, the National Kidney Foundation (NKF) under the umbrella of Kidney Disease Outcomes Quality Initiative (K/DOQI) was formed to address CKD in the USA and to facilitate the development and implementation of clinical guidelines (2). Through a series of conferences, consensus was reached on i) definition of CKD and staging regardless of underlying cause, ii) laboratory measurement of kidney disease, iii) association of the level of kidney function with complications of CKD, and iv) stratification of the risk for loss of kidney function and development of cardiovascular disease.

Table1: Staging of CKD and prevalence in the USA between 1998 and 2004 (2)

Stage	Description	GFR mL/min/1.73m ²	Prevalence
-	Increased Risk	≥ 60	-
1	Kidney disease with normal or increased GFR	≥ 90	3.3
2	Mild reduction in GFR	60 – 89	3.0
3	Moderate reduction in GFR	30 – 59	4.3
4	Severe reduction in GFR	15 – 29	0.2
5	End-Stage renal Disease (ESRD)	< 15	0.1

From the table above, approximately 10.6% of the general USA population in the period between 1998 and 2004 had early CKD (stages 1 – 3) and are normally asymptomatic. Asymptomatic cases will miss out on early treatment with risk of progression to ESRD.

1.3 Markers of Renal Function

Urea, electrolytes and creatinine. Serum Na^+ , K^+ , Cl^- , HCO_3^- and creatinine levels are assayed during routine examination or in suspected renal impairment to assess electrolyte imbalance and acid and base disturbance. The body pH which is maintained at a narrow range of 7.35 – 7.45 gives additional information of renal involvement in various metabolic syndromes. Levels of acidosis rise depending on the degree of renal failure.

The Anion gap is a measure of $[\text{Na}^+] + [\text{K}^+] - (\text{Cl}^- + \text{HCO}_3^-)$. The gap is maintained at 8 – 12 mmol/L with interpretation of either elevation, normal or decrease useful in the eventual search for the cause of acid-base disturbance.

Traditionally GFR is considered the best overall index of renal function in health and disease. The 'gold standard' of measuring GFR is by the renal clearance of exogenous markers inulin, radio-labeled EDTA (^{51}Cr -EDTA) and technetium-labeled diethylene-triamine-pentacetate ($^{99\text{m}}\text{Tc}$ -DTPA) and iohexol. These tests are however time-consuming, labor-intensive, invasive, costly and require specialized equipment restricting their use in routine individual cases monitoring or in large epidemiological studies.

While an ideal endogenous marker should meet 3 criteria of complete filtration at the glomerulus, absent tubular secretion and no tubular reabsorption, serum creatinine is widely accepted as an endogenous marker for assessing renal function despite its well-documented limitations. Previously, the laborious and error-prone creatinine clearance (CrCl) involving 24-hour urine collection was in use. The formula for CrCl is

$$\text{Creatinine clearance} = \frac{U \times V}{P} \quad (3)$$

U = urinary creatinine concentration ($\mu\text{mol/L}$)

V = urine flow rate [mL/min or (L/24h)/1.44]

P = plasma creatinine concentration ($\mu\text{mol/L}$)

Currently, a spot serum creatinine level is favored with creatinine-based equations for estimating GFR being employed. They are the Cockcroft-Gault (CG), the 4-variable Modifications of Diet in Renal Disease (4v-MDRD) and the Mayo Clinic Quadratic formulae (see Appendix xii). These equations are used inter-changeably to suit various study populations with no consensus on which equation is more accurate ((4, 5)).

Limitations of serum creatinine

There is an inverse relationship between plasma creatinine and GFR. Glomerular filtration rate can decrease by 50% before plasma creatinine concentration rises beyond the upper limit of the reference range hence the poor sensitivity in early CKD case detection. Thereafter, serum creatinine doubles for each further 50% fall in GFR [6].

Levels of serum creatinine are influenced by creatinine generation, renal function, extra renal elimination and tubular handling. Confounders that alter the serum creatinine levels are age, gender, race, muscle mass, protein load in diet and drugs that interfere with colonic flora [7]. These variables are factored in the various equations for calculation of eGFR.

In their study, McClellan et al (8) showed that tubular secretion increases creatinine clearance by 10-20%, thus overestimating the glomerular filtration rate by nearly a similar proportion.

In the method of Jaffé which employs alkaline picrate, there are multiple interfering substances both exogenous and endogenous. These include proteins, glucose, and acetoacetate and drugs especially the cephalosporin family (9, 10). These non-creatinine chromogens are present in serum but absent in urine. The end-result is underestimation of creatinine clearance. However, recent modifications such as optimization of kinetic assays have improved method specificity and minimized susceptibility to interfering substances (10).

Creatinine clearance requires 24 hour urine collection which is laborious and prone to standardization and preservation errors especially when carried out in an out-patient setting.

Rigelleau et al (11) in their study on diabetic patients showed that CG equation had hyperglycemia and BMI-related bias with overestimation of the eventual eGFR.

Perkins et al (12) and separately Mclsaac et al (13) demonstrated that MDRD equation overestimates normal or raised GFR missing out early stages of CKD.

Sensitivity of serum creatinine is low with detection of renal dysfunction occurring when more than half the initial population of nephrons is non-functional. The diagnostic accuracy of Cockcroft & Gault (CG) and Modification of Diet in Renal Disease (MDRD) prediction equations in mild and moderate renal function is poor thus the acronym 'creatinine blind' range of 40-70mL/min/1.73m²(14).

Accordingly, only 70% of renal subjects (15) can be considered well stratified as per the NKF guidelines thus the need for a precise marker for GFR. Cystatin C is considered an 'ideal' marker and is being proposed as a suitable alternative endogenous marker of renal function.

Cystatin C

Biochemistry

It is a 122 amino acid protein with molecular weight of 13 KDa. It belongs to a family of 11 other potent, non-covalent, competitive inhibitors of mammalian lysosomal cysteine proteinases. Known functions of cystatins include control of proteolysis (intracellular, extracellular and intravascular), immune modulation, antimicrobial activities and modification of the body's response to injury (16).

Properties of cystatin C that make it a potentially more "ideal" endogenous marker of GFR compared to creatinine include:

- i). Constant rate of production in all nucleated cells
- ii). Reported lack of effect of age, gender or muscle mass on cystatin C generation
- iii). Free filtration at the glomerulus due to its small size and basic pH
- iv). Complete reabsorption and catabolism by proximal tubular cells; lack of renal tubular secretion and lack of reabsorption into the bloodstream and
- v). Absence of problems with analytic control.

Clinical Utility of Cystatin C

Cystatin C is freely filtered by the renal glomeruli, is not secreted by the tubules or eliminated via any extra-renal route but almost completely reabsorbed and metabolized by proximal tubular cells (17).

Clinical usefulness of cystatin C as a measure of GFR was first proposed in 1985 by Grabb *et al* (18) and separately by Simonsen *et al* (19) triggering widespread research interest.

Advances in assay of serum cystatin C have established its place as suitable alternative to serum creatinine in estimating GFR. In their meta-analysis of cystatin C and serum creatinine in predicting renal dysfunction, Ross *et al* (20) found that cystatin C had higher pooled sensitivity (81%) and similar specificity (88%) as compared to serum creatinine (69%;88% respectively).

Results mirrored those by Harmoinen *et al* (21) while comparing the diagnostic accuracy of cystatin C with serum creatinine and creatinine clearance (CG) and CrCl (MDRD): Cystatin C was superior to SCr when BMI was abnormal, or when GFR was normal or moderately impaired.

Absence of tubular secretion makes it extremely sensitive to early CKD as shown by Laterza *et al* (22). Maahs *et al* (23) showed that cystatin C predicts progression of sub-clinical atherosclerosis in type 1 DM. Like HbA1c, cystatin C is reflective of cumulative effect GFR over time, but also sensitive to recent changes in renal function (24). It allows for estimation of the slope of decline in GFR and possible progression to End-Stage Renal Disease (ESRD). Other studies concur with additional role of cystatin C levels in predicting cardiovascular events and death in CKD patients (25).

Cystatin C is unaffected in hepatic disease and in intestinal bacteria overgrowth (26). Studies done in the pediatric age groups and other patient categories such as the elderly, pregnancy and transplant recipients stress its widening application in monitoring disease progression and increased risk of mortality (27, 28). Levels of cystatin C in extracellular fluids such as in malignant effusions correlate closely with disease burden.

In an elaborate study in the pediatric population (from premature infants to 17 years), Finney *et al* (29) had 3 conclusions.

- ✓ Cystatin C is a better marker than creatinine of glomerular filtration rate in preterm infants .
- ✓ A single reference range for plasma cystatin C can be used, regardless of gender, from 1 year of age.
- ✓ Cystatin C offers a more specific and practical measure for monitoring GFR in the pediatric population than does creatinine.

Cystatin C levels have been found to remain stable from 1 year to 80 years of age effectively covering the spectrum for possible CKD. Cystatin C is currently in routine use for estimating GFR employing nephelometric, turbidimetric and Elisa techniques (30).

Immunoturbidimetric Assay: highly accurate, excellent precision with coefficient of variance of less than 5%. It is cost-effective with easy instrument adaptation. One may use either serum or plasma.

Immunonephelometric Assay: No blank correction required, is highly sensitive with lower detection limit but not widely available.

Elisa: Easy to perform, rapid, more cost-effective and allows quantification.

Cystatin C remains stable during storage for both plasma and serum samples for up to 1 week at 2-4°C. Levels are not affected by most drugs, acute phase reaction or diet (31).

Correlation between Marker and Usefulness

An increase in serum cystatin C corresponds to decreased GFR. Normal cystatin C values for adults are between 0.8 and 1.2 mg/L, but may rise 2 to 5 times the upper limit in cases of chronic kidney diseases.

The formula for estimating GFR in this study was:

$eGFR = 100/\text{cystatin C}$ expressed as ml/min (32). This is used for adults and referred to as the simple cystatin C equation.

Limitations of cystatin C.

1. Levels are not affected by standardized high-dose corticosteroid therapy but may increase in patients with renal dysfunction receiving corticosteroids (33).
2. Levels are sensitive to changes in thyroid function: Cystatin C levels rise in hyperthyroidism and fall in hypothyroidism. This is as opposed to the inverse relationship between serum creatinine and thyroid function (34).
3. Higher cost with limited availability of current assay techniques compared to serum creatinine. This may eventually change with emerging evidence in favor of cystatin C (35).

RATIONALE OF THE STUDY

While no data is available on the prevalence of CKD in Kenya, it remains a major global health problem (1). There is reported rise in the risk factors for CKD such as chronic glomerulonephritis, diabetes mellitus, hypertension and recently HIV-associated nephropathy (HIVAN) (36). Chronic kidney disease is characterized by high cost of management and poor outcomes chiefly being cardiovascular disease and death. Remuzzi *et al* (37) found that adverse outcomes in CKD can be prevented or delayed by early detection and treatment using ACE-inhibitors.

Despite the existence of guiding principles on approach to CKD, it remains greatly underdiagnosed and undertreated. Laboratory input is central in staging and risk stratification. Creatinine clearance is widely used despite its numerous limitations. Cystatin C is an emerging marker for detecting early renal dysfunction and a big step towards preventive renal medicine.

This study aims to assess whether serum cystatin C equals or is a better marker for renal dysfunction than serum creatinine. Levels from volunteer blood donors will be assessed and used to determine reference ranges for cystatin C and creatinine and their respective estimated GFRs.

NULL HYPOTHESIS

There is no difference in the staging of the Chronic kidney disease patients at Kenyatta National Hospital as per National Kidney Foundation guidelines using serum cystatin C and serum creatinine.

ALTERNATE HYPOTHESIS

The reference ranges for serum cystatin C and serum creatinine among blood donors at the NBTS, Nairobi are comparable to those in literature.

STUDY QUESTIONS

- What are the serum cystatin C and serum creatinine levels in blood donors and CKD patients at NBTS and Kenyatta National Hospital?
- What is the staging of CKD patients using estimated GFR as per the National Kidney Federation guidelines based on serum cystatin C and serum creatinine?

2.0 OBJECTIVES

2.1 Broad Objective:

To compare the serum cystatin C and creatinine levels in CKD patients and blood donors.

2.2 Specific Objectives:

- To determine serum cystatin C and creatinine levels in CKD patients and blood donors.
- To determine reference values of cystatin C using blood donors as reference population.
- To compare the eGFR values obtained using cystatin C and serum creatinine in blood donors and CKD patients.

3.0 METHODOLOGY

Study Design

This was a comparative descriptive study involving both CKD patients and blood donors

A) RENAL PATIENTS

Study Site

The renal clinic at the Kenyatta National Hospital which is conducted every Friday from 8 a.m. with an average attendance of 40 patients of all age groups.

Study Population

Adult males and females on follow-up at the renal clinic.

Definition of cases

- Referred patients with $\geq 3/12$ established renal disease with/without proteinuria, elevated serum creatinine levels or deranged eGFR.

Case Selection

Inclusion Criteria

- Age 18 to 80yrs.
- Gender: Male and Female.
- Renal disease for $\geq 3/12$.

- Mild to moderate chronic kidney disease as per clinical staging in the treatment file.
- Informed consent.

Exclusion Criteria

- Patients with advanced renal disease or End-stage renal failure requiring dialysis.
- Patients with known malignancies or thyroid illness through clinical history and physical examination.
- Patients on high-dose steroids
- Declined consent

Sample Size

The sample size for CKD patients was 150. This was arrived at using the formulae for cross sectional studies (38). The minimum sample size n was thus obtained by the formulae

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

Z value is the upper $\alpha/2$ point of the normal distribution with a value of 1.96.

P is the assumed prevalence of CKD in Kenyatta National Hospital, based on the prevalence of 11% among adult population in the USA.

d is the precision, 0.05 with which to determine the prevalence

$$n = \frac{1.96^2 * 0.11(1-0.11)}{0.05^2}$$

$$n = 150$$

Sampling Technique

In a study on renal patients at KNH carried out by Maritim (39), it was found that early CKD (Stage I-III) comprised 53% of cases 30 years old and above. In this study, every second adult patient on follow-up at the renal clinic was assessed for eligibility and recruitment until the desired number of 150 was achieved.

Recruitment

On the morning of a clinic day, files were perused to identify candidates for screening. Once the patients were done with the physician, they were ushered into a designate study room where the purpose of the study, the benefits and risks of participation were explained. Informed consent was sought. The screening proforma (Appendix I) was then administered by direct interview and responses entered into respective sections. Successful patients were assigned study numbers. Further information was then obtained followed by a clinical assessment, which were entered into the study proforma (Appendix II). Five mls of whole blood was then obtained and put into a plain vacutainer and thereafter into a cool box. The recruited patients were then advised to check the results of their renal function status in their next visit. Those who declined at the screening stage were allowed to continue with the physician's instructions. An average of 10 CKD patients was recruited on a single clinic day. A sticker was put on the file to avoid duplication in recruitment.

B) HEALTHY BLOOD DONORS

Study Site

The blood transfusion unit (BTU) at Kenyatta National Hospital and the National Blood Transfusion Services (NBTS) outreach tents within Nairobi province.

Study Population

Volunteer donors at the BTU in KNH and in outreach campaigns by the NBTS team.

Definition of suitable donors

Eligible study participants were male or female volunteer donors aged between 18 and 65 years, weighed 50 kgs and above and had a hemoglobin count of/or above 12.5g/dl (determined using copper sulphate method). They agreed to test for HIV, HBsAg, HCV and syphilis as required by NBTS guidelines.

Subject selection – donors

Inclusion Criteria

- Volunteers who qualified as blood donors as per NBTS guidelines (Appendix VI).
- Those who gave informed consent to voluntarily take part in the study.

Exclusion Criteria

- Volunteers who did not qualify as blood donors as per NBTS guidelines.
- All blood donors who did not give consent to participate in the study.

Sample Size

According to the International Federation for Clinical Chemists (IFCC) (40), a minimum of 40 cases are required for establishment of reference ranges though 120 is most desirable.

In this study, 128 blood donors were initially recruited to make up for any anticipated pre-analytical sources of error. Four specimens tested positive for HIV and were eliminated leaving 124 blood donors' specimens for eventual analysis.

Sampling Technique

Every third blood donor irrespective of age and gender was recruited until the desired sample size of 124 was achieved. A research assistant assisted in administering both the screening and study proformas for blood donors (Appendix VI).

Recruitment and Sample Collection

During a blood donation exercise, potential donors were briefed on the purpose of the study, the advantages and risks alongside the NBTS donor screening questionnaire (Appendix VI). Their consent was sought before obtaining of vital socio-demographic data by direct interviews, which was entered into the relevant section in the donor screening/study questionnaire (Appendix VI). Those who declined consent were excluded from the study but proceeded to donate blood normally.

Five ml of whole blood was then obtained from the venepuncture just before connecting the pilot tube, into well-labeled plain vacutainers. An average of 20 blood donors was recruited on a single day.

The vacutainers bearing samples from study participants were transported in a cooler box to NBTC- Nairobi offices.

Separation and Storage:

The vacutainers were left to settle and serum pipetted into labeled cryovials (in duplicates) for storage at -40°C at the NBTC. The precipitant was discarded safely. Once the desired sample size

was reached, all the specimens were removed from the freezer for batch analysis in one single day.

Specimen Analysis:

The alkaline picrate method (modified kinetic Jaffe reaction) using an *Olympus 400/640* autoanalyzer was employed in this study (Appendix III).

Cystatin C was measured using Latex-enhanced immuno-turbidimetric assay on *Olympus 400/640* autoanalyzer (Appendix VIII).

The samples, reagents, controls and calibrators were retrieved from the refrigerators and brought to room temperature. The tests were run as per manufacturer's instructions.

Quality Assurance

Stringent measures were followed to ensure accurate and precise data to within the recommended coefficient of variance (<5%) by the manufacturers. Both the calibrators and controls were run in duplicates.

ETHICAL CONSIDERATIONS

The study commenced upon approval by the Department of Human Pathology (UoN) and the KNH/UoN Scientific & Ethical Review Committee (Appendix XI).

There was also clearance from the office of the Director, National Blood Transfusion Services (Appendix XII).

Pre-consent counseling involved the following:

- i. Information and explanation on the research nature and overall goal
- ii. Detailed explanation of the procedures involved, outlining their safety or lack of.
- iii. Assurance that participation is voluntary and one can withdraw at any point without losing other benefits from KNH or blood donation services
- iv. Confidentiality and custody of patient information, specimen and results
- v. Assurance on free access to their results and their medical interpretations. Appropriate referrals for medical intervention shall be carried out.
- vi. The benefits and unforeseen harm of participating in the study shall be explained in unambiguous language as contained in the Study explanations for both renal patients and healthy blood donors (Appendices III and V respectively).

Thereafter, the study participants signed the consent form (Appendix IV and VI) before undergoing a physical examination.

DATA HANDLING AND ANALYSIS

Demographic data on study participants was obtained by direct interviews and entered into the study proforma. Laboratory results for serum cystatin C and creatinine levels were in the form of a computer print out. The eGFR using the two equations below:

1. Serum creatinine: the MDRD study equation (also available online at the NKF website) .
2. Serum cystatin C: $100 / [\text{cystatin C}]$ – the simple cystatin C equation.

All data was then entered into a computer database from which spreadsheets were generated and transferred to the Statistical Package for Social Sciences (SPSS) software version 17 for analysis. Summary of the statistics was determined during the analysis and presented as proportions and percentages in the form of tables and graphs.

In the determination of reference range, there was no partitioning of blood donors into age or gender because of the small sample size and the narrow age spread. Histograms were prepared using the SPSS; visual inspection was then done to ensure a Gaussian distribution. Outliers were identified and excluded in the final analysis. The data derived from cystatin C was linear and Gaussian distribution was obtained. For serum creatinine, the data was non-linear hence transformation into the natural logarithm to obtain a Gaussian distribution. The reference limits were set at $X \pm 1.96 \text{ SD}$ where X = the mean and SD = standard deviation.

Statistical methods employed included correlations, cross tabulations and T-tests for comparison. A p value of <0.05 was considered a statistically significant result.

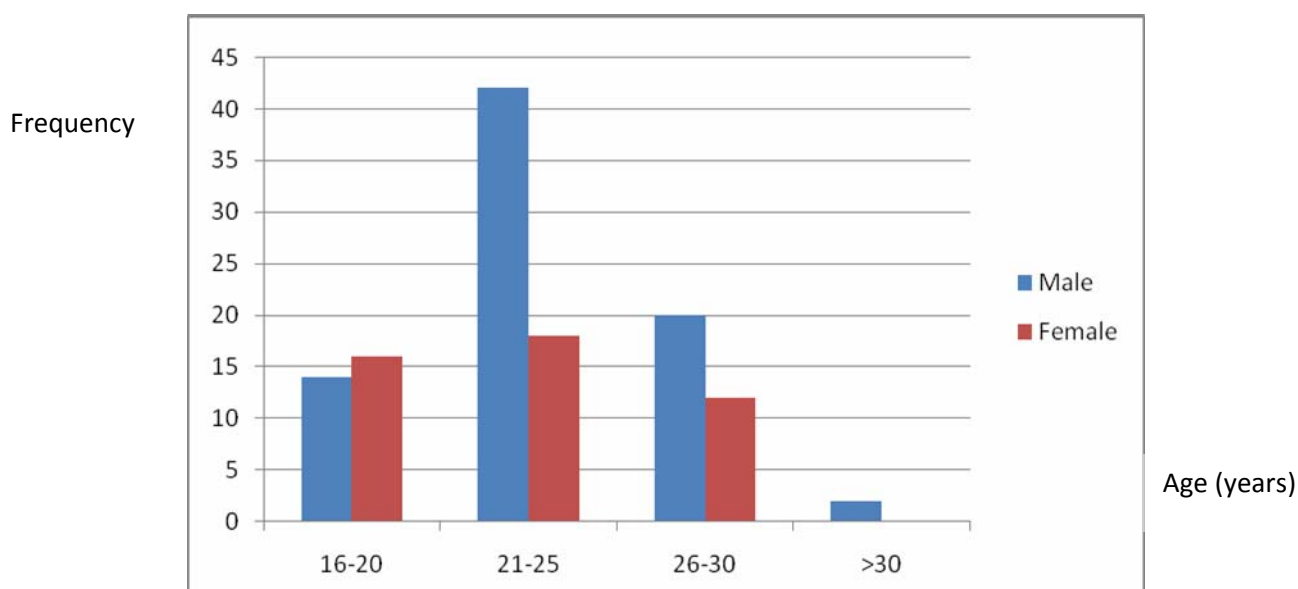
RESULTS

A. BLOOD DONORS

Socio-demographic characteristics of the study participants

A total of 124 blood donors were recruited into the study. Of the 124, 78 (63%) were males and 46 (37%) were female. Majority, 60 (48%) were aged between 21 – 25 years. The mean age was 23.3 years and the standard deviation was 3.587. The mode was 21 years and the median was 23 years. The youngest donor was 18 years and the oldest was 34 years. Most of the donors were single and naïve. The age-gender distribution is depicted in figure 1.

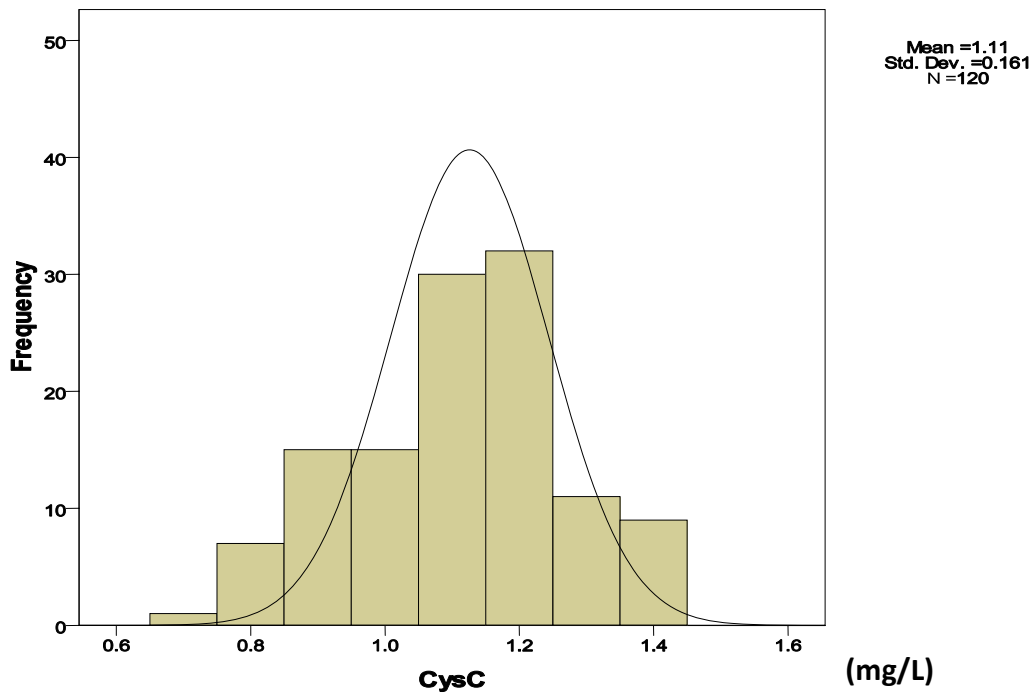
Figure 1: Age-Gender distribution of blood donors (n = 124)



Majority (48%) of the blood donors were in the 21 -25years age bracket, 24% were 20 and below years, 26% were 26 – 30 years and only 2% were 30 years and above (Figure 1).

The serum cystatin C levels (expressed in mg/L) obtained from the donors yielded continuous data with a histogram plot resulting in a Gaussian distribution as shown in figure 2. Four outliers were excluded in the analysis to obtain the reference range for serum cystatin C.

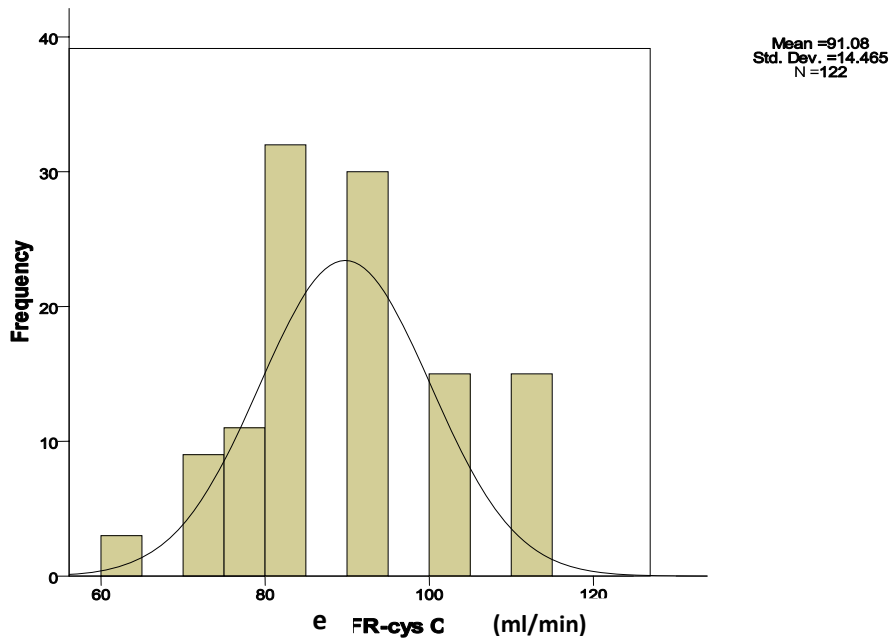
Figure 2: Serum cystatin C levels in blood donors ($n = 120$)



The cystatin C levels ranged from 0.7 to 1.4 mg/L on exclusion of four outliers. The mode was 1.2 mg/L while the mean was 1.11 mg/L. The standard deviation was 0.161 mg/L (Figure 2).

The estimated GFR derived from cystatin C ($eGFR_{cysC}$) was also continuous. Histograms were prepared and visual inspection of the frequency plot yielded a Gaussian distribution as shown in figure 3. Two outliers were excluded during analysis.

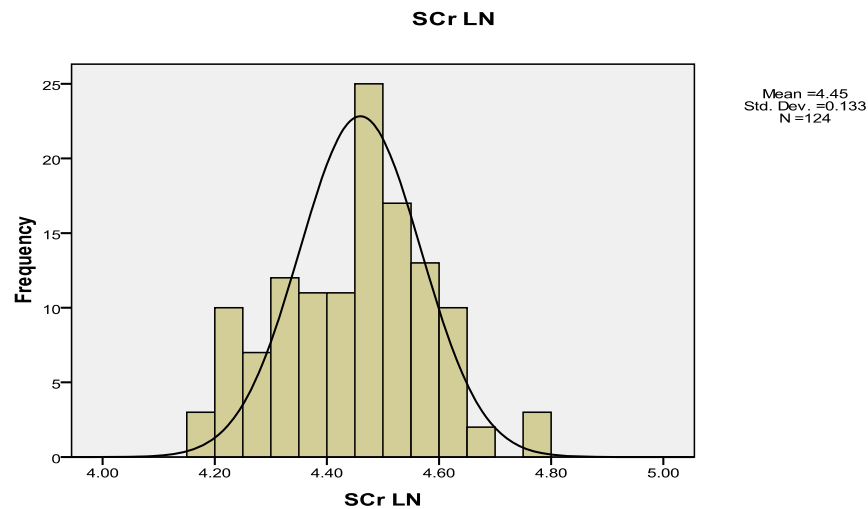
Figure 3: eGFR-cystatin C in blood donors (n = 122)



The derived eGFR-cystatin C of study participants ranged from 63 to 125 ml/min after removal of two outliers. The mean was 91.08 ml/min and the standard deviation 14.465 ml/min. The reference range obtained was 62 – 120 ml/min (figure 3).

The histograms for the data for serum creatinine levels among blood donors were non-linear. Transformation into natural logarithm was necessary in order to achieve gaussian distribution as shown in figure 4. No outliers were found in the inspection of the plot.

Figure 4: Distribution of the Natural Log SCr (n=124)



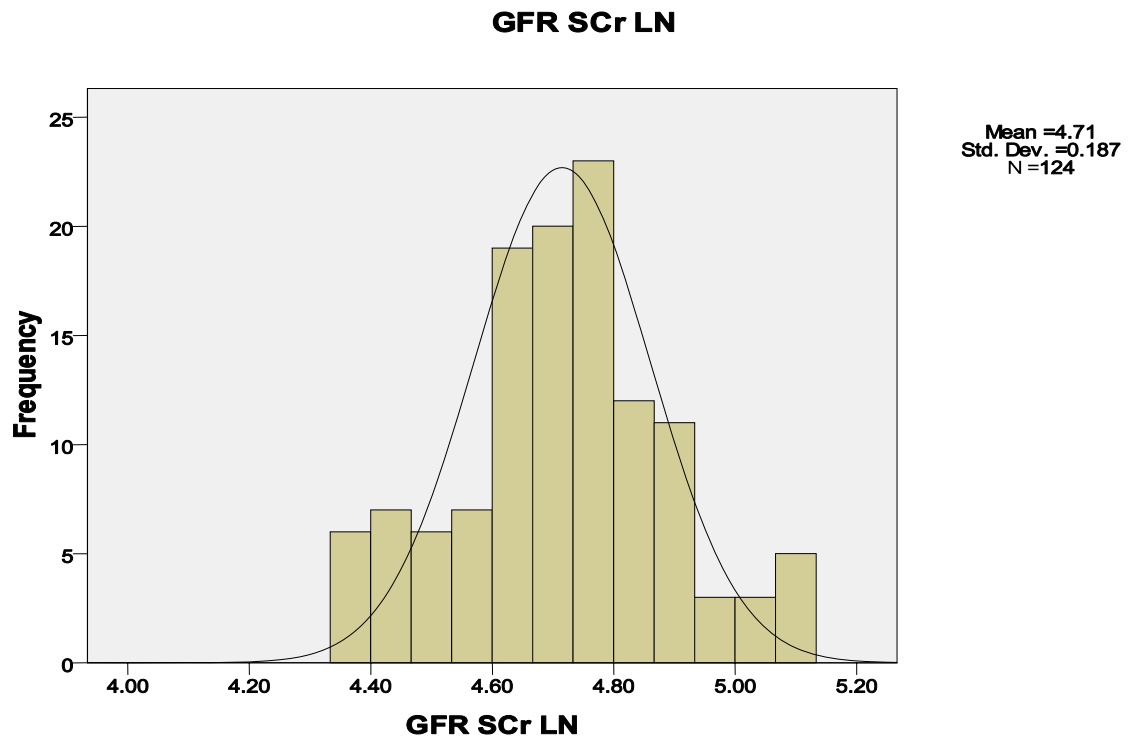
SCrLN: the natural logarithm of the serum creatinine levels. The range was from 4.15 to 4.75. The mean was 4.45 with a standard deviation of 0.133 (figure 4).

The retransformed data into corresponding serum creatinine levels was as per table 2 below:

The lowest and highest values for serum creatinine were 65 and 120 respectively. The mean was 86.32 μ mol/L and the standard deviation 11.46. The reference range was 63 - 109 μ mol/L (table 2).

Similarly, the estimated GFR derived from serum creatinine yielded non-linear data and required transformation into natural logarithm. The plot for the histogram for the natural logarithm had a Gaussian distribution shown in figure 5 below. No outliers were noted.

Figure 5: Distribution of the Natural Log for eGFR-SCr for blood donors (n=124)



GFR SCr LN: the natural logarithm for eGFR derived from serum creatinine levels. The range was from 4.35 to 5.10. The mean was 4.71 and the standard deviation was 0.187 (figure 5). This information was retransformed into usable values as summarized below.

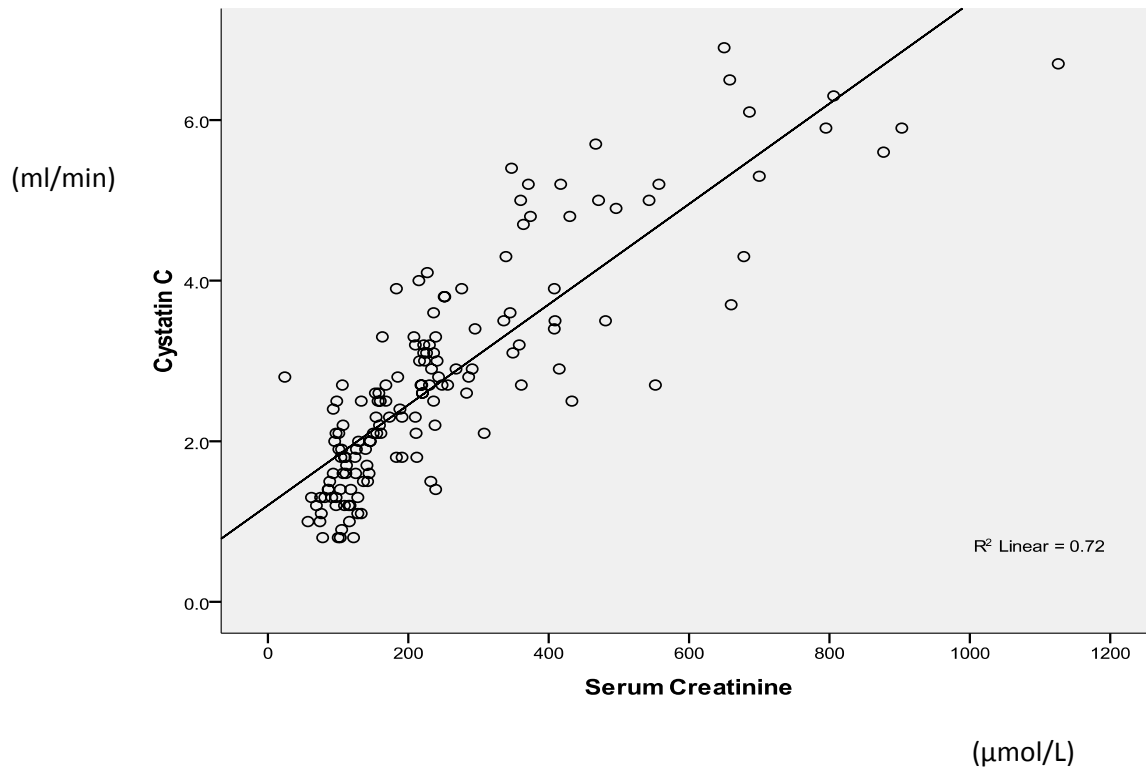
Summary of eGFR-serum creatinine for blood donors

The lowest and highest derived values for blood donors were 63 and 167 $\mu\text{mol}/\text{min}/1.73\text{m}^2$ respectively. The mean was 112.74 $\mu\text{mol}/\text{min}/1.73\text{m}^2$, the standard deviation was 20.86 and the median was 113 $\mu\text{mol}/\text{min}/1.73\text{m}^2$. The reference range was 71 - 155 $\mu\text{mol}/\text{min}/1.73\text{m}^2$.

Correlative Statistics

There was a linear relation between serum cystatin C and serum creatinine for blood donors as shown in figure 6.

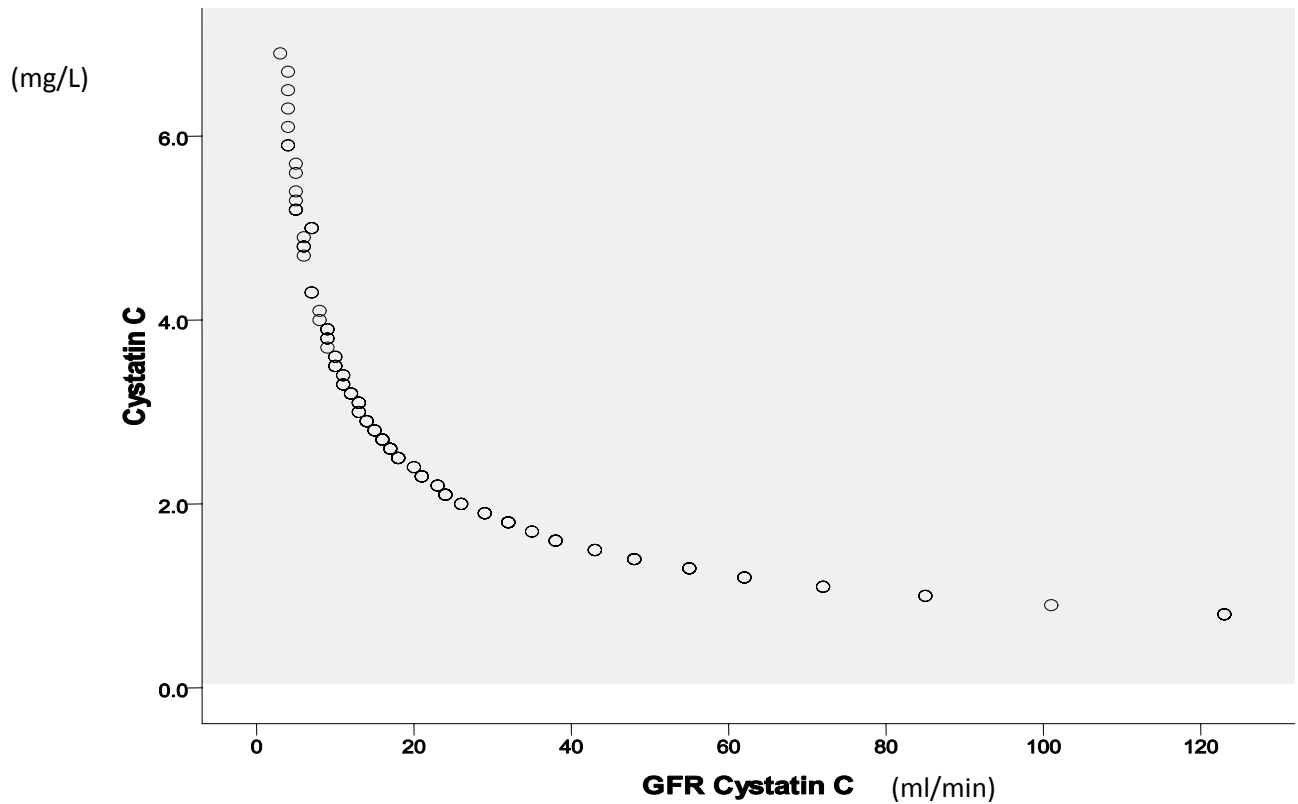
Figure 6: Correlation between Serum Cystatin C and creatinine for blood donors (n = 124)



The linearity of the correlation is represented in the equation: **Cystatin C = 1.203 + 0.006(SCr)**. The R2 value was 0.72 and the p value 0.00 which is statistically significant (figure 6).

There was an inverse relationship between serum cystatin c and eGFR-cystatin C meaning that as the renal function deteriorated with impaired removal of cystatin C from the circulation, there was a corresponding rise in the levels of serum cystatin C. This relationship is shown in figure 7.

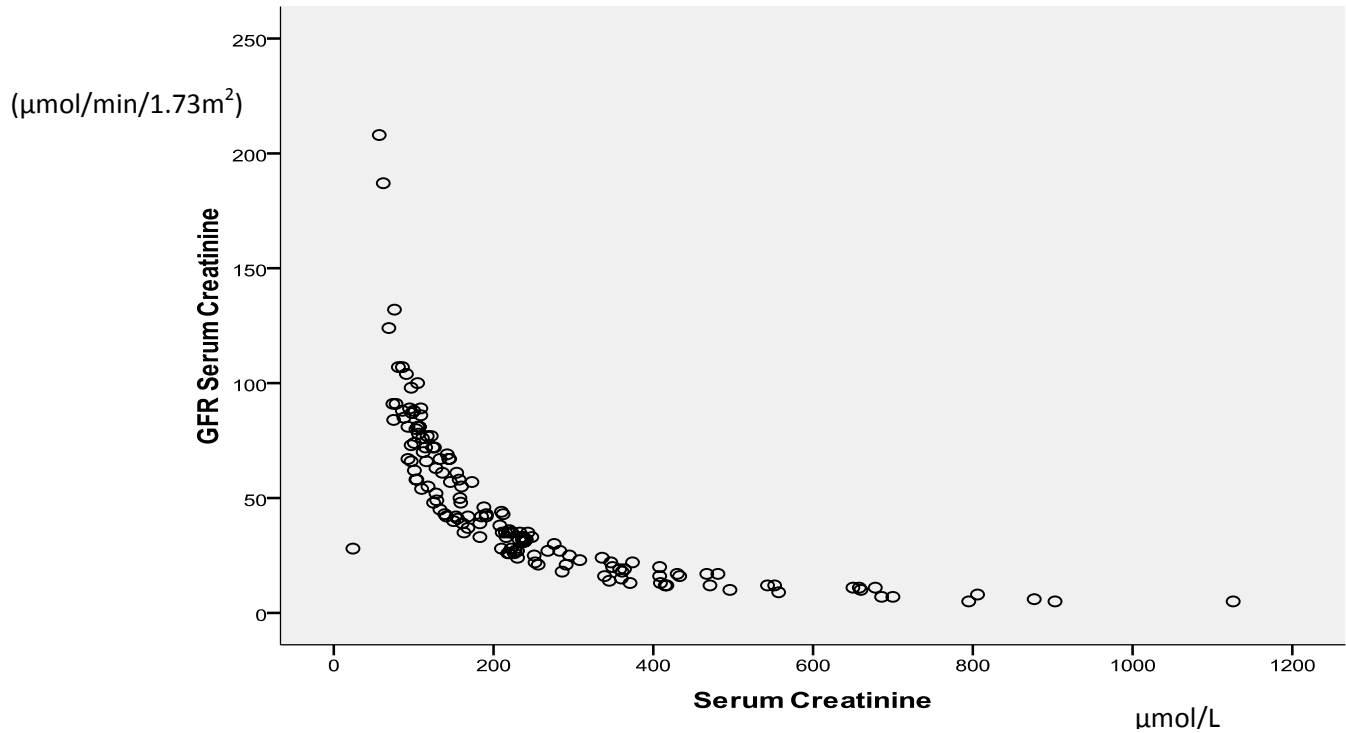
Figure 7: Correlation between cystatin C and eGFRcystatin C for blood donors (n=124)



The correlation was curvilinear represented by the quadratic equation: $\text{Cystatin C} = 3.871 - 0.41(\text{eGFRcystatin C})$. As cystatin C levels rose, there was a corresponding decrease in eGFR-cystatin C (figure 7).

Similarly, there was an inverse relationship between serum creatinine and eGFR-serum creatinine as shown in figure 8 below. As renal function decreased hence fall in eGFR, there was a corresponding rise in the serum creatinine levels and vice versa.

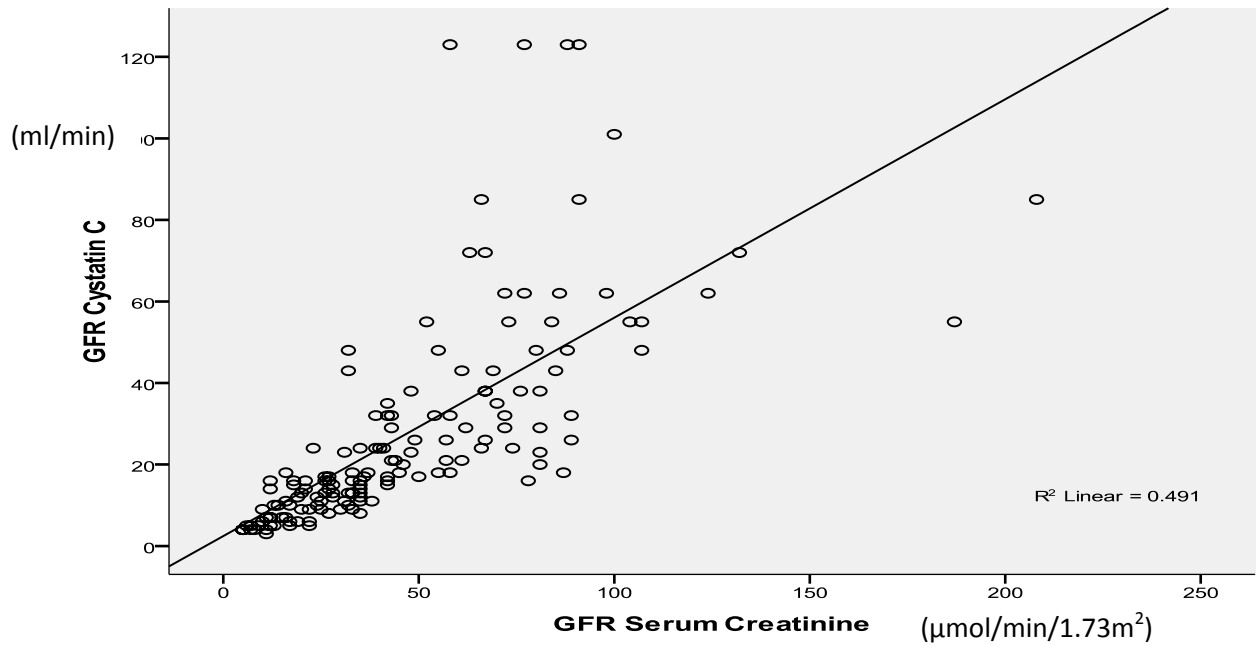
Figure 8: Correlation between Serum creatinine and eGFR_{SCr} in blood donors (n = 124)



The correlation was curvilinear represented by the equation: $eGFR_{SCr} = 75.663 - 0.118 \times SCr$. The R value was 0.678 and the p value 0.00 which is statistically significant (figure 8).

Lastly, there was a linear relationship between $eGFR_{\text{cystatin C}}$ and $eGFR_{\text{Scr}}$ as shown in figure 9 below.

Figure 9: Correlation between eGFR-cystatin C and eGFR-scr for blood donor (n=124)



The correlation yielded an r value of 0.701 and a p value of 0.00 which is statistically significant (figure 9) indicating that one could use GFR-cystatin C and GFR-serum creatinine to assess the renal function status.

Below is a summary of the correlations between primary and secondary results for blood donors.

1. Correlation between cystatin C and serum creatinine : r value = 0.849, p value = 0.01
2. Correlation between cystatin C and eGFR-cystatin C : Pearson's correlation (r value) = -0.837, p value = 0.01
3. Correlation between serum creatinine and eGFR-serum creatinine : r value = -0.678, p value = 0.01

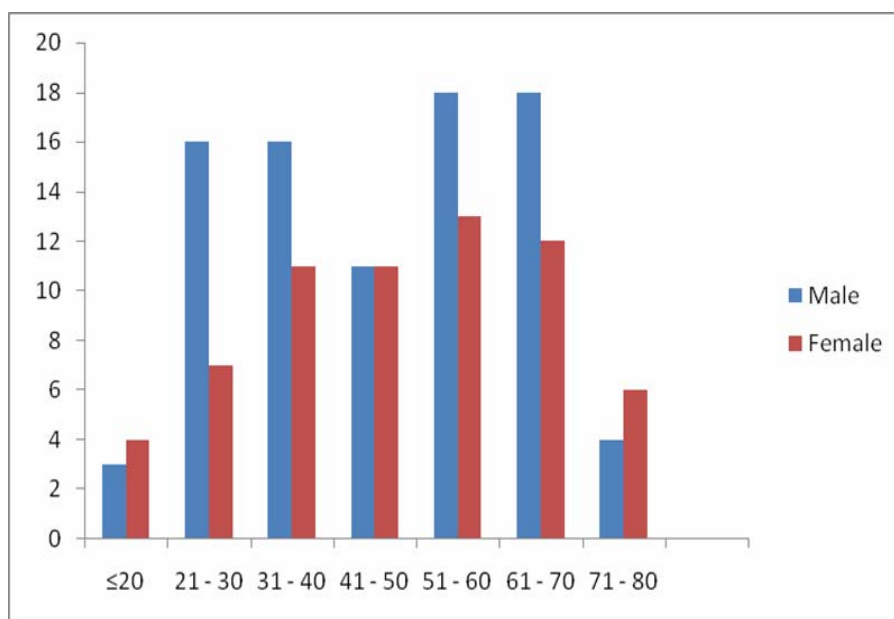
There is a positive correlation between cystatin C and serum creatinine (r value = 0.849) with p value of 0.01 which is statistically significant. Additionally, cystatin C is inversely correlated with eGFR with r value of -0.837, p = 0.01. Similarly, serum creatinine and its derived eGFR are inversely correlated, r value = -0.678, p = 0.01. However, cystatin C has stronger correlation with eGFR compared to serum creatinine (r value -0.837 vs -0.678).

B. RENAL PATIENTS

Socio-demographic characteristics of CKD patients (n = 150)

Of the 150 patients, 86 (57%) were males and 64 (43%) were females. The youngest patient was 18 years and the oldest was 77 years. The mean age was 47.7 years with a standard deviation of 16.7 years. The median age was 48.5 years and the mode was 60 years.

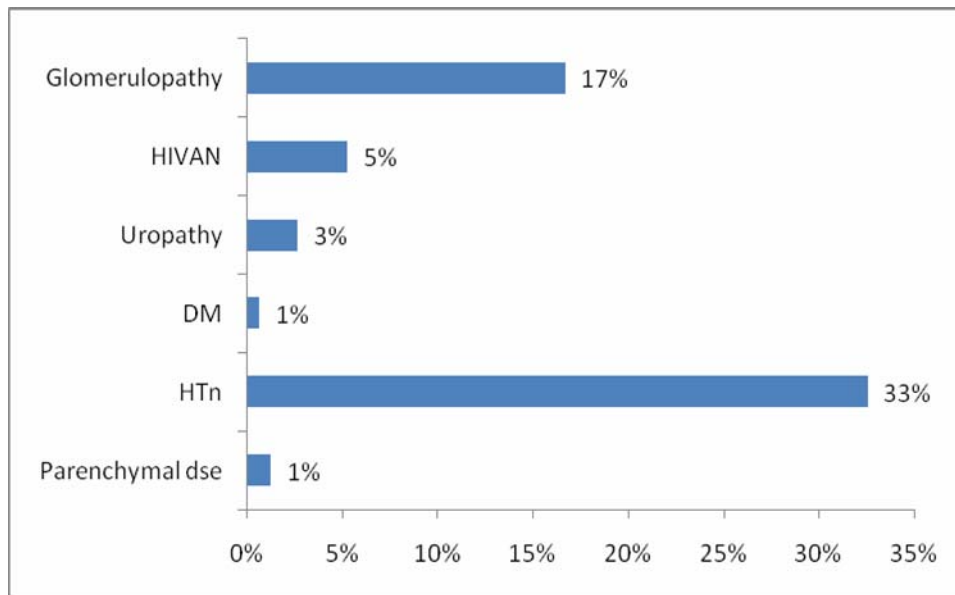
Figure 10: Graph showing the age-gender distribution of CKD patients



Majority (21%) of the CKD participants were in the 51 – 60 years, 5% were ≤20 years, 15% were 21 – 30 years, 18% were 31 – 40 years, 15% were 41 – 50 years, 20% were 61 – 70 years and 6% were in the age 71 – 80 years (figure 10).

In the clinical diagnosis, majority of the patients had hypertension. In the age distribution, the younger CKD patients had glomerulopathies either as a monodiagnosis or with secondary hypertension. Majority of the older patients had hypertension as a monodiagnosis or combined with diabetes mellitus.

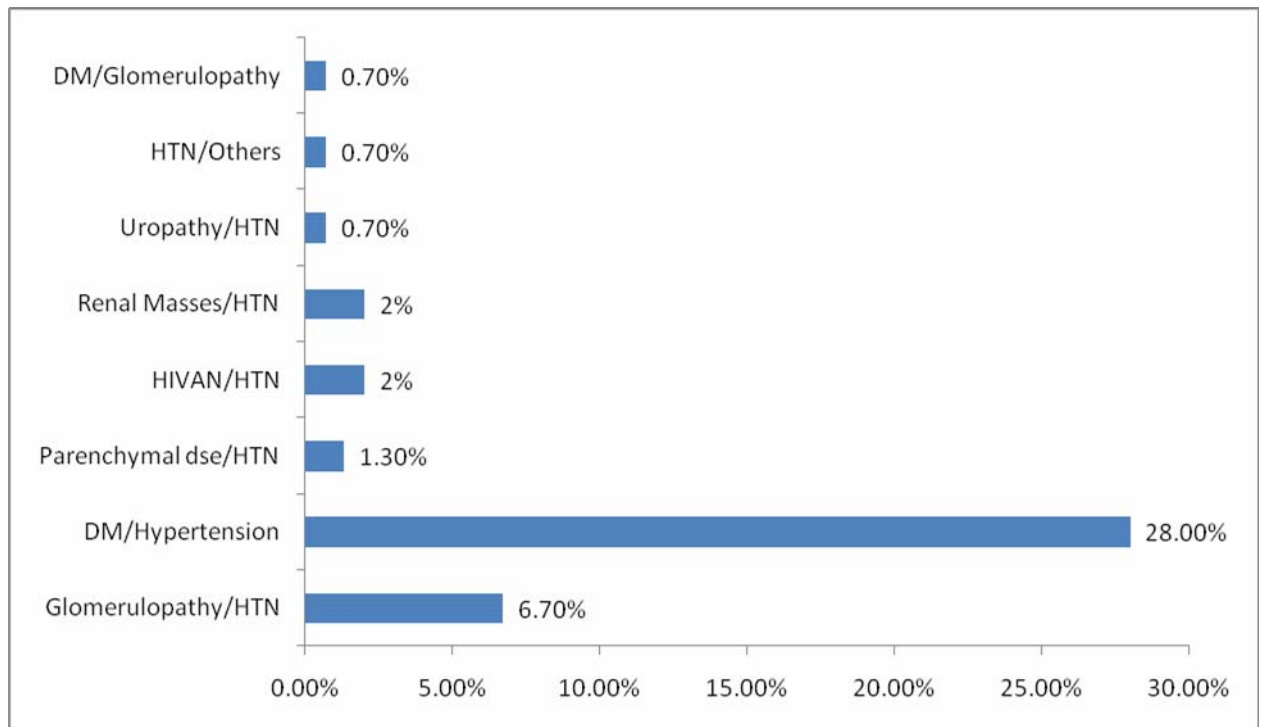
Figure 11: Graph showing monodiagnoses (n=150)



Monodiagnoses: Of the CKD study participants, 33% had hypertension, 17% had glomerulopathies while 5% had HIV nephropathy. Diabetes mellitus accounted for only 1% of the CKD participants (figure 11).

In co-morbidities, hypertension and diabetes mellitus accounted for 28% of the CKD study participants. Cumulatively, a total of 41.6% of the study CKD participants had secondary hypertension (see figure 12 below).

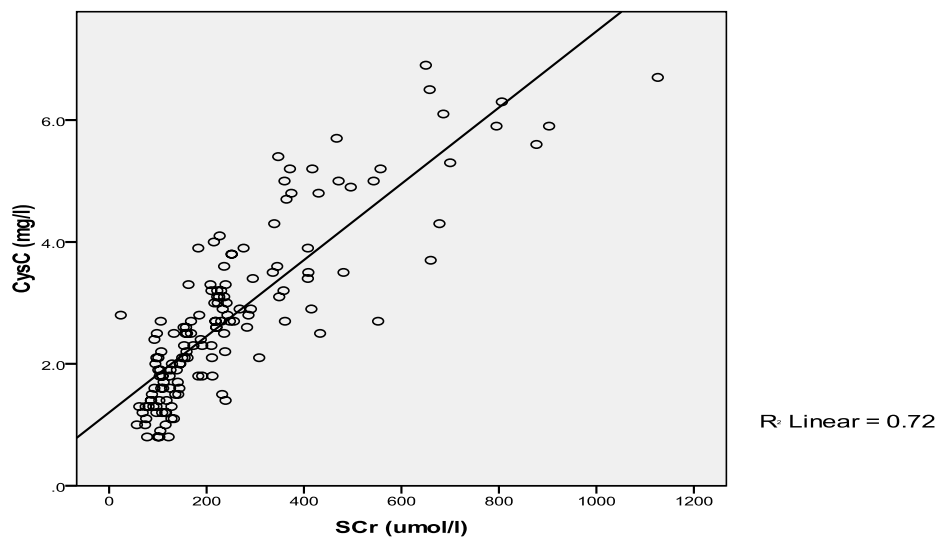
Figure 12: Graph showing co-morbidities among CKD patients (n=150)



Correlative Statistics

Correlations were done between serum cystatin C and serum creatinine and each with its derived eGFR. The results were as shown in the graphs below.

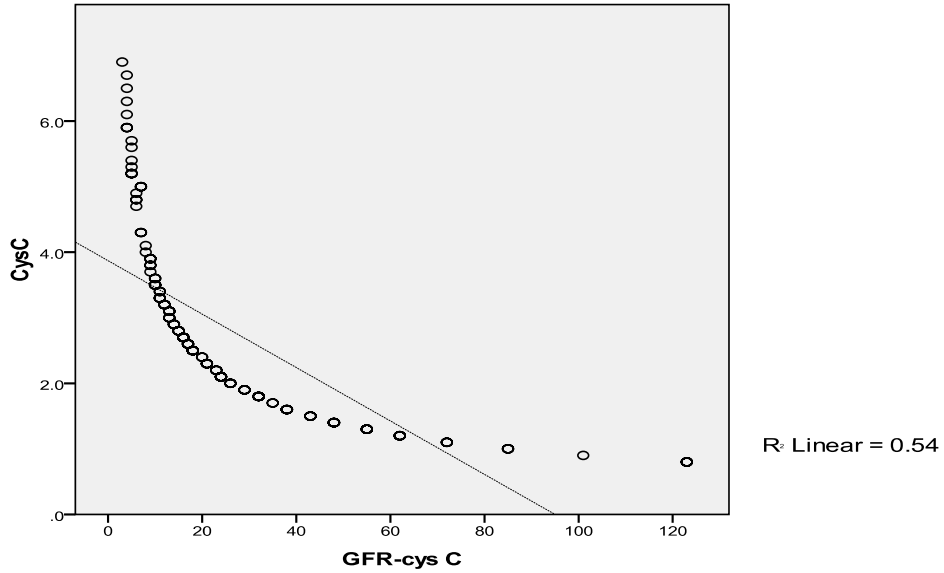
Figure 13: Correlation between Serum Cystatin C and creatinine for CKD patients (n = 150)



There is a positive correlation between serum cystatin C and serum creatinine with $r = 0.849$ and $p = 0.01$ which is statistically significant (figure 13).

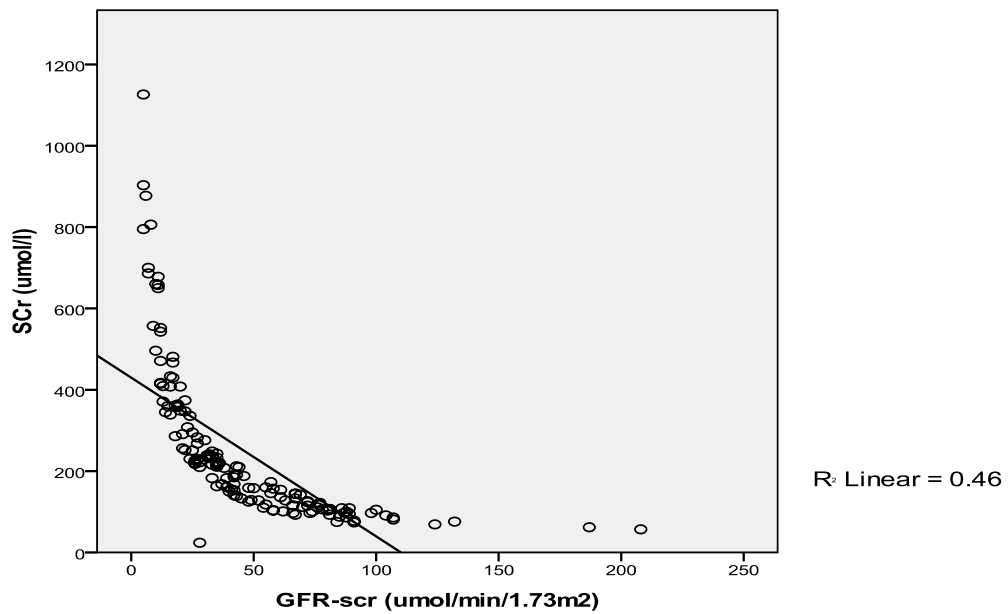
There is an inverse relationship between cystatin C and its derived eGFR with $r = -0.735$ and $p = 0.01$ which is statistically significant (see figure 14 below).

Figure 14: Correlation between cystatin C and eGFRcystatin C for CKD patients (n=150)



There was an inverse relationship between serum creatinine and its derived eGFR with $r = -0.678$ and $p = 0.01$ which is statistically significant (figure 15).

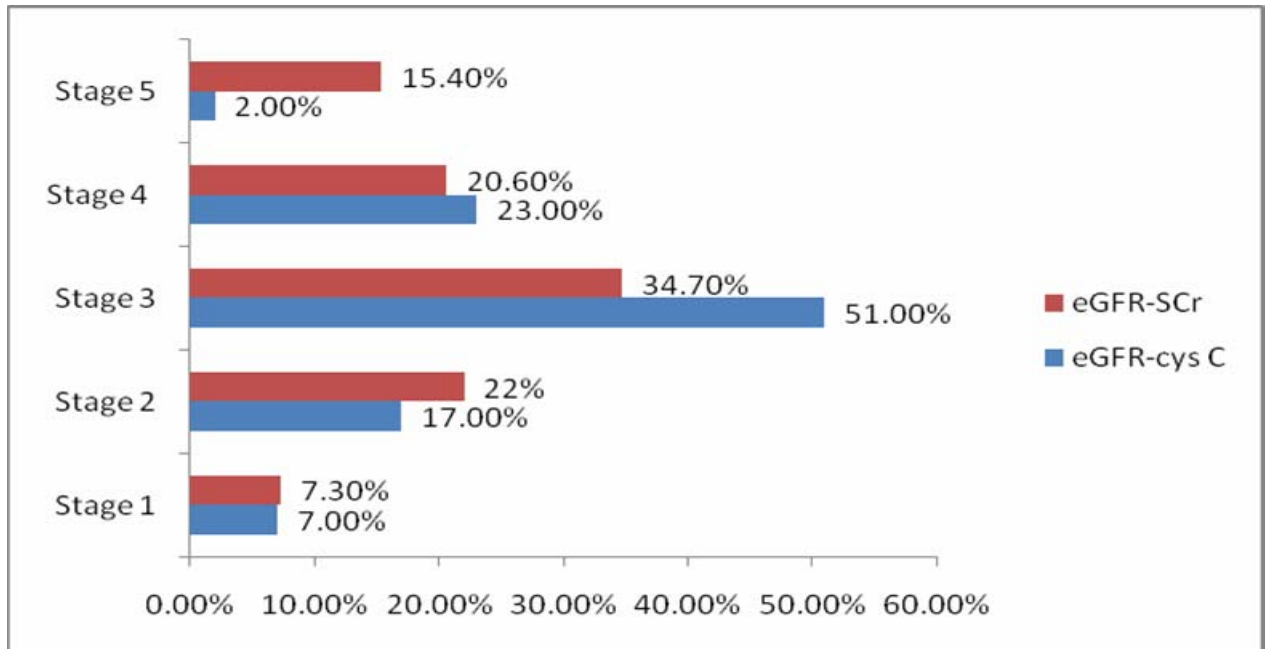
Figure 15: Correlation between eGFR-cystatin C and eGFR-scr for CKD patients (n=150)



Looking at the correlations of primary and secondary results CKD study participants, the findings were comparable to those obtained after analysis of blood donors.

Further to that, the entire CKD population was staged using the two markers separately. There was a bias for cystatin C derived eGFR towards advanced CKD while serum creatinine tended towards early disease.

Figure 16: Graph showing staging of CKD patients using the two markers (n=150)



Staging of CKD participants: 7%, 17%, 51%, 23% and 2% were in Stages 1, 2, 3, 4 and 5 respectively as per eGFR-cystatin C. However, according to eGFR-serum creatinine, 7.3%, 22%, 34.7%, 20.6% and 15.4% respectively. The proportion in early CKD was 75% while 25% had advanced renal disease using eGFR-cystatin C. Using eGFR-serum creatinine, 64% had early disease while 36% had advanced CKD (figure 13).

Lastly, cross tabulation was done for the staging of the CKD study participants according to both eGFR-cystatin C and eGFR-serum creatinine as depicted in table 2 below.

Table 2: Cross tabulation between GFR-cystatin C and GFR-SCr (n=150)

		SCr Stage					Total
		5	4	3	2	1	
Stage cystatin C	5	5	5	1	0	0	11
	4	5	14	4	0	0	23
	3	1	15	43	16	3	78
	2	0	0	4	15	16	35
	1	0	0	0	0	3	3
Total		11	34	52	31	22	150

Cross tabulation: The highlighted values indicate the CKD participants where both eGFR-cystatin C and eGFR-serum creatinine place them in the same CKD stage. The other values show the differential staging as per the two markers of renal function: cystatin C and serum creatinine. Five patients were in stage 5, 14 in stage 4, 43 in stage 3, 15 in stage 2 and 3 in stage 1 as per both eGFRcystatin C and eGFRscr (table 2).

The degree of agreement in stratification of CKD study participants using serum cystatin C- and serum creatinine- derived eGFRs' was expressed as kappa value which was 0.359. The p value was 0.00 which is statistically significant, indicating their utility in assessing renal function.

DISCUSSION

There were more male donors recruited into the study compared to female donors reflecting the true situation at the National Blood Transfusion Services (NBTS). This is despite the sampling technique favouring both genders equally. According to the Kenya demographic and health survey (KDHS)-2003 (41), women make up a larger proportion of the Kenyan population (57%). Women volunteers are likely to fail to qualify as donors because of low haemoglobin levels. Lower haemoglobin levels may be secondary to menstrual losses or increased iron requirements in pregnancy and lactation.

The 21 – 25 years age bracket had the highest proportion (48%) of donors. This is due to the fact that most of the blood donation exercises targeted the youth in the post-secondary colleges and universities. The youngest blood donor was 18 years and the oldest was 34 years. The volunteers were mostly single and naive donors. A good proportion were repeat donors responding to radio broadcast or faith-based appeal for urgent need for blood. This study was limited in the spread for blood donors where a wider age distribution of upto 65 years would have been more representative of the adult population. In her study on the seroprevalence of CMV antibodies in donated blood at the NBTC - Nairobi, Njeru (42) noted a similar trend where only 5% of study participants were above 30 years. Given that the sampling of donors was for every third volunteer irrespective of age and gender, there was no bias.

In health, cystatin C levels are maintained at a fairly narrow range of between 0.8 – 1.2 mg/mL from the age of one to eighty years with minimal gender variations. In this study, the 95% reference intervals for 124 blood donors for serum cystatin C and eGFR-cystatin C were 0.8 – 1.4 mg/L and 62 – 120 ml/min respectively while those for serum creatinine and eGFR-serum creatinine were 63 - 109 μ mol/L and 71 – 155 ml/min/1.73 m². These are comparable to findings from a study to determine adult reference ranges in the UK, where Hazel *et al* [43] found that the 95% reference intervals for creatinine, predicted creatinine clearance and cystatin C for 309 blood donors, regardless of gender were 68-118 μ mol/L, 58-120 ml/min/1.73 m² and 0.51-0.98 mg/L, respectively. As shown above, there is a slight difference the values for all the three parameters. This could have arisen due the difference in sample sizes between the two studies.

In the study mentioned above to determine the adult reference ranges, the recruited 309 healthy blood donors ranged from 16 years to 65 years. This enabled partitioning of the participants according to gender and age and the investigators were able to assess the effects of these two variables on the serum creatinine, creatinine clearance and serum cystatin C with no effect on the power of the study. There was minimal effect of gender on serum cystatin C levels

to warrant use of separate reference ranges for males and females. However, there was separation of reference range in those under 50 years of age and those above. In this study, no gender or age variations were calculated given the size and distribution of the sampled blood donors. While the effect of race on the serum creatinine is well-documented and factored in the MDRD equation, cystatin C levels are considered not to be affected by race. This fact can thus be cemented in the local setting by an appropriate follow-up study.

In impaired renal function serum levels of cystatin C rise proportionately due to impaired glomerular clearance. This gives an inverse relationship with estimated GFR. With a half-life of twenty minutes, cystatin C is a useful marker in the monitoring of subtle changes in renal function among CKD patients and staging necessary in the management and prognostication. Serum creatinine remains the most widely used routine marker for assessing renal function. Levels of serum creatinine rise with worsening kidney failure thus an inverse relationship with estimated GFR. Serum creatinine has an estimated half-life of 3.85 hours in a healthy male individual of between 20 and 39 years is 3.85 hours. In established chronic kidney disease irrespective of age and gender, the half-life of serum creatinine is prolonged up to 77 hours making it a lesser sensitive marker in monitoring (44). However, cystatin C sensitivity remains unchanged in established CKD.

Results for the CKD patients in this study showed that the younger patients had mainly the glomerulopathies (17%) with additional 7.4% having hypertension or diabetes mellitus co-morbidity. The recently recognised HIV-associated nephropathy (HIVAN) accounted for 7% of the CKD study participants. HIVAN had patients spanning the entire age range. The older age groups had mainly hypertension (46%) and hypertension-diabetes co-morbidities (27.3%).

Globally, the prevalence of impaired kidney function is estimated to range between 10% and 20% of the adult population (45) with rising incidence attributable to the epidemic of type II diabetes and the ageing population in developed countries. In a recent study, the prevalence of CKD in the general population was reported as 11% by Kissmayer et al (46) in the United Kingdom. In a 2007 weekly publication by CDC (47), the prevalence of CKD in the USA is estimated at 14.5% of the adult population. The leading risk factors are diabetes, hypertension, glomerulonephritis and cystic kidney in that order. Diabetes and hypertension account for two thirds of CKD cases.

Regionally, a prevalence study in a family practice population in Nigeria by Afolabi *et al* (48) is the closest that CKD in Sub-Saharan Africa has been investigated exhaustively. Accordingly, 12.4% of consecutive patients aged between 20 years and 74 years had CKD with demonstrable

association with modifiable risk factors namely hypertension, diabetes and abnormal waist-hip ratio. Available data from the South African society of nephrology (49) recognizes the high prevalence of HIV (up to 25%) in some centres but low incidence of HIV-related renal dysfunction (including HIVAN) due to early mortality before CKD can manifest.

In a study to determine the prevalence of and risk factors for renal diseases in a cohort of HAART-naïve adults attending an HIV clinic in western Kenya, Wools-Kaloustian *et al* (50) recognized the existence of renal insufficiency in non-diabetic, non-hypertensive HIV patients and recommended screening to allow for dose adjustments. There is no available data on the prevalence of CKD in the general Kenyan population. Borrowing from a recent Ugandan study among HIV-infected adults in the Home-Based AIDS Care clinical trial by Philip *et al* (51), renal dysfunction in advanced HIV disease improved by 16% after 2 years-follow-up while on HAART. This change accompanied other variables such as weight gain and rise in the CD4 counts

Staging of the CKD participants was done as per NKF guidelines, using eGFRs derived from both cystatin C and serum creatinine. As per eGFR-cystatin C, 76% had stages 3 – 5 chronic kidney disease while eGFR-serum creatinine showed 70% in stages 3 – 5. This indeed answers one of the study objectives: that there is differential staging of the CKD participants when the two markers are used separately. There is a bias for serum cystatin C towards more severe renal disease compared to serum creatinine. According to the KDIGO meeting of 2006 (52), the prevalence of CKD (stages 3 – 5) in the USA, UK, Netherlands, Australia and China was 4.7%, 4.9, 5.3%, 11.2% and 2.53% respectively. In a study at the St Georges Hospital in London in 2006 [51], 17.7% of all acute medical emergencies were due to stages 3 – 5 CKD, underscoring the need for clinical suspicion.

Correlative statistics in CKD patients in this study between serum cystatin C and serum creatinine had an *r value* of 0.849 and a p value of 0.01 which is statistically significant. This compares to recent comparative study between cystatin C and serum creatinine for detecting renal dysfunction among South Indian type II diabetes mellitus by Viswanathan *et al* (53) showed an *r value* of 0.5 and p value of <0.0001.

Correlation between cystatin C and eGFR-cystatin C yielded an *r value* of -0.837 due to the inverse relationship. Comparison between serum creatinine and GFR-serum creatinine yielded an *r value* -0.678. In their study, Hoek *et al* (54) found that the correlation between cystatin C and its derived eGFR ($r = 0.873$) while serum creatinine and eGFR-SCr yielded an *r value* of 0.876. They concluded that cystatin C had a better correlation with eGFR compared to serum creatinine.

CONCLUSION AND RECOMMENDATIONS

Conclusions

- 1.The reference ranges for blood donors aged 18 – 34 years for both serum cystatin C and creatinine in this study were 0.8 - 1.4 mg/L and 62 – 120 mol/L respectively and are comparable in literature.
- 2.There is a difference in the staging of CKD patients using eGFR derived from serum cystatin C and serum creatinine. Serum cystatin C had 76% of CKD patients with advanced disease (stages III – V) compared to serum creatinine which had 70.7%. Therefore, eGFR derived from cystatin C has a bias towards advanced disease.
- 3.The degree of agreement (kappa value 0 .359) of the two markers is statistically significant (p value – 0.001), meaning that both markers remain useful means of assessing renal function. Given that no ‘gold standard’ was used, the sensitivity and specificity of each marker could not be obtained.

Recommendations

- 1.A minimum report from the laboratory for all requests for renal function tests to include eGFR as a useful prognostic indicator in line with NKF guidelines. This will enable physicians to manage CKD patients better.
- 2.A study involving a larger sample size of blood donors is recommended. Further stratification with sufficient numbers in each stratum would remove the bias of comparing a relatively young reference group with a much older patient population.
- 3.A follow-up comparative study involving a gold standard (e.g. inulin) for measured GFR to be carried out to depict sensitivity and specificity serum cystatin C and serum creatinine. This will enable wider usage of either of them for routine follow-up of at-risk and confirmed CKD cases.

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Appendix 1: Screening Proforma Renal clinic

COMPARATIVE STUDY BETWEEN SERUM CYSTATIN C AND CREATININE LEVELS IN RENAL PATIENTS AT KENYATTA NATIONAL HOSIPTAL

<u>Diagnosis:</u>	YES	NO
a)Duration of illness		
b) Diabetic Nephropathy		
c)Hypertensive Nephropathy		
d) Post-Streptococcal Glomerulonephritis		
e)Polycystic Kidney Disease		
f) Other Renal Diagnoses		
g)Unknown Causes		
h) NO Renal Tumour		
i) NO Known Thyroid illness		
<u>Current Medication</u>		
NOT on High-dose Steroids		
<u>Eligibility</u>		
1. Are you willing to participate in this study?		

If answers to ALL questions are YES, Recruit and issue Study No. If NO, do NOT Recruit.

FOR OFFICAL USE:

RECRUITED (encircle) YES NO

STUDY NUMBER:

--	--	--

Once recruited, proceed to Study Questionnaire (Appendix II).

Appendix II: Study Questionnaire for Renal Patients

COMPARATIVE STUDY BETWEEN SERUM CYSTATIN C AND CREATININE LEVELS IN RENAL PATIENTS AT KENYATTA -NATIONAL HOSPITAL

--	--	--	--	--	--

Date

dd / mm / yy

A. Socio-demographic data

Name.....

--	--

Age (Years):

Study

--	--	--

No.

Hospital

--	--	--	--	--	--	--	--	--

No.

B. Medical History

Diagnosis:

1. Diabetes Mellitus
2. Hypertension
3. Post-Streptococcal Glomerulonephritis
4. Polycystic Kidney Disease
5. Others

Duration of illness

--	--	--

(months)

Do you have any of these complications? (Y/N)

1. Heart failure
2. Renal failure
3. Stroke
4. Sub-clinical Atherosclerosis

Medications currently on (Y/N)

1. ACE-inhibitors
2. Anti-hypertensives
3. Oral hypoglycemic agents
4. Antibiotics (specify)
5. Analgesics
6. Others (specify)

Family History

1. Do any of your relatives have similar symptoms? YES / NO
2. Have they been diagnosed with similar disease or any other renal disease?
YES / NO
3. If YES, relation to you is....

1° degree..... [Mother, father, brother, Child]

2° degree.....

PHYSICAL EXAMINATION

1. Height (m)

--	--	--

2. Weight (kg)

--	--	--

--	--

3. BMI

--	--

4. BP reading
Systolic
mm/Hg

BP

BP

--	--

 mm/Hg

Diastolic

LABORATORY RESULTS (Renal Patients)

1. Serum Cystatin C (mg/L)

--	--	--

eGFR

cystatin

c

--	--	--

(mL/min/1.73m²)

2. Serum creatinine (μmol/L)

--	--	--

eGFR_{scr} (mL/min/1.

--	--	--

Appendix III: Study Explanation for Renal Patients

COMPARATIVE STUDY BETWEEN SERUM CYSTATIN C AND CREATININE LEVELS IN RENAL PATIENTS AT KENYATTA NATIONAL HOSPITAL

Introduction and objectives of the study:

I am Dr. Mwaludindi D. M., a masters student in Human Pathology at the University of Nairobi and conducting a study on the status of kidney function. The kidneys are a pair of organs that carry out 3 keys functions: removal of body waste, maintain volume and composition of body fluid and hormone production. In a number of disease processes, the functions of the kidney are impaired progressively with eventual death unless intervened. A number of body compounds can be used to diagnose kidney failure. My interest is in 2 compounds found in blood: creatinine and cystatin C. The study aims to:

- i. Compare cystatin C and serum creatinine levels in renal patients at Kenyatta National Hospital.
- ii. Determine the reference range for serum cystatin c among healthy blood donors in Kenyatta National Hospital and in outreach campaigns by the National Blood Transfusion Services teams.

Benefits and risks of the study to you:

By participating in it, you will benefit by:

- Having examinations and laboratory tests done on you at no added cost.
- A report on your renal function status being sent to your physician
- Receiving appropriate advice and intervention measures undertaken to stop/ reverse progression of Chronic Kidney Disease.

Risk: You will be withdrawn venous blood 5mls from the antecubital vein. The needle prick will be painful.

If you consent to participate as case, you will:

- Sign a consent form (Appendix IV).
- Answer a number of questions contained in the screening and study questionnaire (Appendices I/II).
- Undergo a physical examination

Participation in this study is voluntary and you can withdraw at any time. Any information given to us will remain confidential. You may ask me any questions regarding this study now and at any time during the study.

In case you have questions relating to the study, kindly contact..

1. Dr. Mwaludindi D. Mchana 0722821192 (PI) or
2. Dr. Marybeth C. Maritim 0733729963 (Co-Investigator)
3. The Secretary to the Ethical Committee KNH Tel. Nos. 2727260 Ext. 44102

Appendix IV: Consent Form for Renal Patients

COMPARATIVE STUDY BETWEEN SERUM CYSTATIN C AND CREATININE LEVELS IN RENAL PATIENTS AT KENYATTA -NATIONAL HOSPITAL

I after reading and being explained to on the study purpose by Dr. Mwaludindi D.M., do hereby give informed consent to participate in the COMPARATIVE STUDY BETWEEN CYSTATIN C AND CREATININE IN RENAL PATIENTS AT KNH.

I am aware that i can withdraw from this study without having any benefits or quality of management of my medical condition interfered with.

Signed:

Thumb print: Date:

Signature of questionnaire administrator (Dr Mwaludindi)

Witness: Date:

Appendix V: Study Explanation for Blood Donors

COMPARATIVE STUDY BETWEEN SERUM CYSTATIN C AND CREATININE LEVELS IN RENAL PATIENTS AT KENYATTA NATIONAL HOSPITAL

Introduction and objectives of the study:

I am Dr. Mwaludindi D. M., a masters student in Human Pathology at the University of Nairobi and conducting a study on the status of kidney function. The kidneys are a pair of organs that carry out 3 keys functions: removal of body waste, maintain volume and composition of body fluid and hormone production. In a number of disease processes, the functions of the kidney are impaired progressively with eventual death unless intervened. A number of body compounds can be used to diagnose kidney failure. My interest is in 2 compounds: Creatinine and cystatin C. The study aims to:

- i. Compare cystatin C and serum creatinine levels in renal patients at Kenyatta National Hospital.
- ii. Determine the reference range for serum cystatin C among healthy blood donors in Kenyatta National Hospital and in outreach campaigns by the National Blood Transfusion Services teams.

Benefits and risks of the study to you:

Your participation will help improve the care of patients with impaired renal function and screening of at risk population.

There shall be no extra procedure over and above what the NBTS team does. Two needle-pricks, one to find out the level of blood in your body (Hemoglobin), and the other for drawing blood for transfusion. Your blood will also undergo routine screening test (HIV, VDRL and HBsAg) as per blood donor card.

I humbly request you to join the study and allow for your pre-donation blood sample of 10mls to have the cystatin C and creatinine levels established.

Participation in this study is voluntary and you can withdraw at any time. Any information given to us will remain confidential. You may ask me any questions regarding this study now and at any time during the study.

In case you have questions relating to the study, kindly contact..

1. Dr. Mchana 0722821192 (PI) or
2. Dr. Maritim C. Marybeth 0733729963 (Co-Investigator)
3. The Secretary to the Ethical Committee KNH Tel. Nos. 2727260 Ext. 44102

Appendix VI: CONSENT FORM FOR HEALTHY BLOOD DONORS

I after reading the consent explanation form as explained by Dr. Mwaludindi D.M., do hereby give informed consent to participate in the COMPARATIVE STUDY BETWEEN CYSTATIN C AND CREATININE IN RENAL PATIENTS AT KNH. The specimen obtained from me shall be used to establish the reference range for the local population. Prior to that, routine pre-transfusion blood test for Hepatitis B, Syphilis and HIV will be carried out and the results communicated to me.

I am also aware that i can withdraw from this study without having any compromise to my care at KNH now and in future.

Signed:

Thumb print: Date:

Signature of questionnaire administrator (Dr Mwaludindi):

Witness: Date:

Appendix VII: Blood Donors Screening/Study Questionnaire

(Adapted from **KNH BLOOD DONOR RECORD CARD.**)

Date of birth (dd/mm/yy)

Family Name:

Other Names:

Physical Address:

P.O. Box:

Town: Married (Y/N)

Sex (M/F) . Age Years

Phone numbers:

Home: Work:

MEDICAL HISTORY- Please tick appropriately if you have ever suffered from any of the following:

- | | | | |
|---------------|---------------------|----------|----------|
| Allergy | Anaemia | Diabetes | Epilepsy |
| Heart disease | High blood pressure | Jaundice | |

• If you have had any major operation in the previous two years, please state

which

- If you suffer from any other disease NOT listed above, please state which

.....

- Remarks:

Date	Centre	Tube no.	

Are you willing to participate in this study? YES / NO. If Yes, recruit and issue study number.

FOR OFFICAL USE:

RECRUITED (encircle)

YES

NO

STUDY NUMBER:

--	--	--

LABORATORY RESULTS (Healthy Blood Donors)

1. Serum Cystatin C (mg/dL)

--	--	--

eGFR

cystatin

c

--	--	--

(mL/min/1.73m²)

2. Serum creatinine (µmol/L)

--	--	--

eGFR_{scr} (mL/min/1.73m²)

--	--	--

Appendix VIII: Methodology for Serum Creatinine

auto-CREATININE liquicolor

Jaffé-Reaction

Photometric Colorimetric Test for Kinetic Measurements of Creatinine

Package Size

REF ⁵	10052	250 ml	Complete kit
IVD			

Method^{1,2}

Creatinine forms in alkaline solution an orange-red coloured complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.

Principle

Creatinine + Picric acid → Creatinine-picric acid complex

Contents

NaOH	2 x 100 ml Sodium Hydroxide X _i (R36/38) (S 26-37/39-45)	160 mmol/l
PIC	1 x 50 ml Picric Acid	13.9 mmol/l
STD	1 x 25 ml Standard Creatinine	2 mg/dl or 176.8 µmol/l

Reagent Preparation

NaOH and PIC are ready to use on analyzers.

For a working reagent mix NaOH and PIC in the ratio 4 + 1.

STD is ready for use.

Reagent Stability

The reagents are stable, even after opening, up to the stated expiry date when stored at 15...25°C.

Contamination must be avoided.

The working reagent, protected from light, is stable for 4 weeks at 15...25°C.

Specimen

Serum, heparinised plasma or urine.

Avoid hemolysis!

Stability: 24 hours at 2...8°C

Dilute urine 1 + 49 with dist. water.

Assay

Wavelength: Hg 492 nm (490 - 510 nm)

Optical path: 1 cm

Temperature: 37°C

Measurement: against air (increasing absorbance)

Warm the reagents and cuvettes to the desired temperature and keep constant (± 0.5°C) for the duration of the test.

Pipetting Scheme

Analyzer settings (2-shot version)

Apply sample : NaOH : PIC in a ratio 1 : 10 : 2.5. Temperature: 37°C. Method: 2-point fixed time (see manual procedure).

Proposals to apply the reagents on analyzers are available on request. Each laboratory has to validate the application in its own responsibility.

Manual application

Pipette into cuvettes	Semi-micro	Macro
Sample / STD	100 µl	200 µl
Working reagent	1000 µl	2000 µl

Mix and start the stopwatch. After 30 sec. read the absorbance A₁. Read the absorbance A₂ exactly after 2 min. A₂ - A₁ = ΔA_{sample} or ΔA_{STD}

Calculation

1. Serum / Plasma

Please use only the standard supplied with the kit.

$$C = 2.0 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}} \quad [\text{mg/dl}]$$

$$C = 176.8 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}} \quad [\mu\text{mol/l}]$$

2. Urine

$$C = 100 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}} \quad [\text{mg/dl}]$$

Creatinine concentration in 24 h urine:

$$C = \text{mg/dl} \times \text{ml urine}/24 \text{ h} \times 0.01 \quad [\text{mg}/24 \text{ h}]$$

$$C = \text{mg}/24 \text{ h} \times 0.00884 \quad [\text{mmol}/24 \text{ h}]$$

$$\text{Creatinine Clearance} = \frac{\text{mg creatinine/dl urine} \times \text{ml urine}/24 \text{ h}}{\text{mg creatinine/dl serum} \times 1440} \quad [\text{ml}/\text{min.}]$$

Conversion of [mg/dl] into [µmol/l] and vice versa:

$$[\text{mg/dl}] \times 88.402 = [\mu\text{mol/l}]$$

$$[\mu\text{mol/l}] \times 0.0113 = [\text{mg/dl}]$$

Performance Characteristics

Linearity

The test is linear up to a creatinine concentration in serum of 15 mg/dl or 1326 µmol/l, in urine of 500 mg/dl or 44200 µmol/l.

Dilute samples with a higher concentration in serum, plasma or diluted urine 1 + 5 with physiological saline (0.9%) and repeat the assay. Multiply the result by 6.

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/su-acrea.pdf

www.human-de.com/data/gb/vr/su-acrea.pdf

Reference Values^{3,4}

Serum	[mg/dl]	[µmol/l]
Men	0.6 - 1.1	53 - 97
Women	0.5 - 0.9	44 - 80
Urine	1000 - 1500 mg / 24 hours	
Creatinine clearance:		
Men	98 - 156 ml/min.	
Women	95 - 160 ml/min.	

Quality Control

All control sera with creatinine values determined by this method can be employed. We recommend the use of our animal serum based HUMATROL or our human serum based SERODOS quality control sera.

Notes

- The reaction is highly sensitive to temperature. The reaction temperature must be kept constant.
- PIC is harmful when inhaled, swallowed or in contact with the skin. If PIC comes into contact with the skin or mucous membranes wash with plenty of water. In case of sickness, contact a doctor.
- The assay can be affected by the presence of reducing compounds. The interference can be partially eliminated by boiling the urine for a short time.
- A slight precipitate in the sodium hydroxide solution is insignificant.

References

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- Sarre, H., Nierenkrankheiten, Thieme-Verl. Stuttgart. (1959)
- ISO 15223 Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied.

SU-ACREA
INF 1005201 GB
01-2004-1



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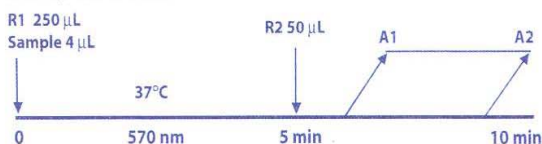
Diazyme Cystatin C

SUMMARY OF PERFORMANCE

Background

Cystatin C is a small, 13 kDa, protein that is produced by virtually all nucleated cells. Its production rate is constant and is unaffected by inflammatory process, gender, age and muscle mass. In the normal kidney, Cystatin C is freely filtered at the glomerular membrane and then nearly completely reabsorbed and degraded by the proximal tubular cells. Therefore, the plasma concentration of Cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), making Cystatin C an excellent indicator of GFR. Cystatin C has advantages over routine clinical measures of renal function. It is more accurate than plasma creatinine, the Cockcroft-Gault estimation of creatinine clearance and is more reliable than the 24-h creatinine clearance.ⁱ There is a growing body of evidence that suggests that Cystatin C can be used to detect kidney disease at earlier stages than serum creatinine, this may help facilitate prevention efforts in the elderly and those with diabetes, hypertension, or cardiovascular disease.ⁱⁱ

Assay Method



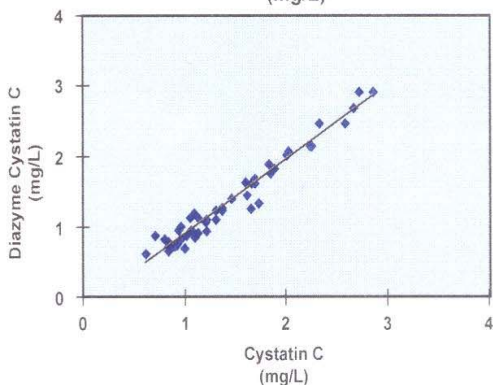
Diazyme Cystatin C assay is based on a latex enhanced immunoturbidimetric assay. Cystatin C in the sample binds to anti-Cystatin C antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of Cystatin C in the sample. The instrument calculates the Cystatin C concentration of a patient specimen by interpolation of the obtained signal on a 6-point calibration curve.

Performance

Accuracy

The Diazyme Cystatin C assay method (y) results generated on a Roche Hitachi 717[™] chemistry analyzer were compared with those obtained with a commercially available Cystatin C method (x). A total of forty five (45) patient samples with values ranging from 0.45 to 7.71 mg/L were analyzed over a period of 5 days. These studies yielded a correlation coefficient of 0.98 with a linear regression of $y = 0.093x + 0.1915$.

Method Comparison Diazyme Cystatin C Assay (mg/L)



510K Cleared

Precision

The precision of the Diazyme Cystatin C assay was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guidelines on a Roche Hitachi 717 chemistry analyzer. In the study, three serum specimens were tested with 2 runs per day with duplicates over 20 working days.

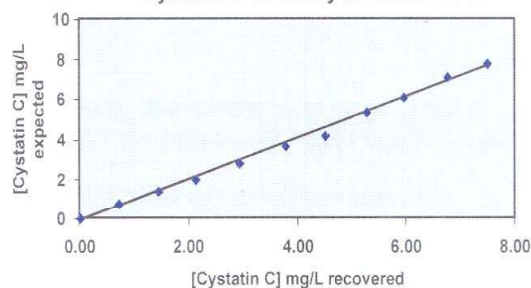
Within-run Precision	Level 1	Level 2	Level 3
Mean mg/L	1.00	4.63	5.95
SD mg/L	0.04	0.10	0.16
CV%	3.9	2.1	3.9

Between-run Precision	Level 1	Level 2	Level 3
Mean mg/L	1.00	4.63	5.95
SD mg/L	0.05	0.17	0.22
CV%	4.8	3.7	3.7

Linearity

Eleven levels of a commercial linearity set were prepared by diluting a serum containing 7.8 mg/L Cystatin C with saline according to Clinical and Laboratory Standards Institute (formerly NCCLS) EP6-A.

Cystatin C Linearity on Hitachi 717



Interference

The following substances normally present in serum produced less than 10% deviation at the listed concentrations: triglyceride at 2,400 mg/dL (formadine), RF at 450 IU/mL, Bilirubin at 18.2 mg/dL, Bilirubin Conjugated at 19.6 mg/dL, and Hemoglobin at 460 mg/dL.

ⁱDiabetes Care 25:2004-2009, 2002

ⁱⁱAnnals of Internal Medicine 147:1, 21 2007

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Appendix X: Approval Letter KNH/UoN Ethics and Research Committee



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11th February 2009

Ref: KNH/UON-ERC/ A/148

Dr. Mwaludindi Dixon Mchana
Dept .of Human Pathology
School of Medicine
University of Nairobi

Dear Dr. Mchana

RESEARCH PROPOSAL: "COMPARATIVE STUDY BETWEEN SERUM CYSTATIN C AND CREATININE LEVELS IN RENAL PATIENTS AT KENYATTA N. HOSPITAL" (P322/11/2008)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your above revised research proposal for the period 11th February 2009 –10th February 2010.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely


DR. L. MUCHIRI
AG. SECRETARY, KNH/UON-ERC

c.c. Prof. K.M. Bhatt, Chairperson, KNH/UON-ERC

The Deputy Director CS, KNH

The Dean, School of Medicine, UON

The Chairman, Dept. of Human Pathology, UON

Supervisors: Prof. C. Kigundu, Dept.of Clinical Chemistry, UON

Dr. Julius Kuria, Dept.of clinical Chemistry, UON

Dr. Marybeth Cheron, Dept.of Internal Medicine, UON

Appendix XI: Formulae for Estimation of Glomerular Filtration Rate

1. Estimated creatinine clearance rate (eC_{Cr}) using Cockcroft-Gault formula

When serum creatinine is measured in µmol/L:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times \text{Constant}}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$$

Where *Constant* is 1.23 for men and 1.04 for women.

2. Estimated GFR (eGFR) using Modification of Diet in Renal Disease (MDRD) formula

For creatinine in µmol/L:

$$eGFR = 186 \times 88.4^{1.154} \times \text{Serum Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times [1.210 \text{ if Black}] \times [0.742 \text{ if Female}]$$

3. Estimated GFR (eGFR) using the Mayo Quadratic formula

When serum creatinine is measured in mg/dL:

$eGFR = \exp(1.911 + 5.249/SCr - 2.14/SCr^2 - 0.00686 * \text{Age} - 0.205 \text{ if female})$. If SCr < 0.8 mg/dL, use 0.8 for SCr

Appendix XII: Clearance from KNBTS Director, Nairobi

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MINISTRY OF HEALTH



NATIONAL BLOOD TRANSFUSION
CENTRE (N.B.T.C.)
P.O. BOX 20750
NAIROBI

Ref. No. **ST/7/1/7 Vol. I**

16th February, 2009, 20

Dr. Mwaludindi Dixon Mchana
Reg. No. H58/7707/06
Department of Human Pathology
College of Health Sciences
University of Nairobi.

**RE: PERMISSION TO CARRY OUT COMPARATIVE STUDY BETWEEN
CYSTATIN C AND CREATININE AT THE NATIONAL BLOOD
TRANSFUSION SERVICES (NBTS)**

This is to let you know that permission is hereby granted to you to carry out the above study. We look forward to your sharing findings of this study with the NBTS as it will contribute immensely to the provision of effective patient management.

A handwritten signature in black ink, appearing to read 'Wilson Sugut'.

Dr. Wilson Sugut
Director, KNBTS

I'm big hearted
I give blood



GIVE BLOOD TODAY YOU MAY NEED IT TOMMORROW