EVALUATION OF ENZYMATIC SARCOSINE OXIDASE METHOD AND COMPARISON WITH MODIFIED KINETIC JAFFE'S REACTION ANALYTICAL METHOD FOR QUANTITATIVE ANALYSIS OF CREATININE

SWALEH BAKARI KULA

MASTER OF SCIENCE

(Medical Laboratory Sciences - Clinical Chemistry)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2022

Evaluation of Enzymatic Sarcosine Oxidase Method and Comparison with Modified Kinetic Jaffe's Reaction Analytical Method for Quantitative Analysis of Creatinine

Swaleh Bakari Kula

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Masters of Science in Medical Laboratory Sciences (Clinical Chemistry) of the Jomo Kenyatta University of Agriculture and Technology

2022

DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University

Signature…….. Date……

Swaleh Bakari Kula

This thesis has been submitted for examination with our approval as University Supervisor.

Signature…….. Date……

Prof. Joseph Kangangi Gikunju
JKUAT, Kenya

Signature…….. Date……

Dr. Stanely King'e Waithaka
MKU, Kenya

DEDICATION

This thesis is dedicated to my parents, wife and children for their moral support in the completion of this thesis document for the award of the masters degree.

ACKNOWLEDGEMENT

I would like to offer my acknowledgement to all those who supported me in achieving this great goal. My sincere acknowledgement goes to my supervisors, Prof. Joseph Kangangi Gikunju and Dr. Stanley King'e Waithaka for properly guiding me through the whole process of carrying out the research and write up of this thesis. My employer, the Technical University of Mombasa (TUM) for giving me the financial assistance and the opportunity to further my studies. The University, JKUAT, for offering me the chance to study my masters degree and the Kenyatta National Hospital (KNH) where the specimens were collected at the blood donation section, renal unit and medical ward and Biochemistry laboratory where the laboratory analysis of the specimens was carried out. I cannot also forget to thank all the study participants for their cooperation in the conduct of this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CLSI	Clinical and Laboratory Standards Institute
CKD	Chronic Kidney Disease
eCrCl	Estimated creatinine clearance
ERC	Ethical Review Committee
GFR	Glomerular Filtration Rate
HPLC	High Pressure Liquid Chromatography
IDMS	Isotope – dilution Mass Spectrometry
Kg	Kilogram
KNH	Kenyatta National Hospital
MDRD	Modification of Diet in Renal Disease
min	Minute
ml	Millilitre
QC	Quality Control
rpm	Revolution per Minute
SD	Standard Deviation
SPSS	Statistical Package for Social Science
Std	Standard
µl	Microlitre
µmol/L	Micro – mole per Litre
UoN	University of Nairobi

ABSTRACT

Kidneys are body organs located on the right and left side of the abdomen. Millions of nephrons are found in a kidney and are both structural and functional units of the organ. They remove waste products like creatinine from blood and passes it out through urine. Creatinine as a waste product of metabolism is a breakdown product of creatine phosphate found in body muscles. Most of the creatinine is excreted via the kidneys, therefore serum creatinine estimation is part of the renal function tests which determine the kidney function. Methods of creatinine estimation are grouped into chemical, enzymatic and spectrometry methods. The objective of this study was to evaluate the enzymatic sarcosine oxidase analytical method and compare it with the modified kinetic Jaffe's method for quantification analysis of creatinine. It was a cross – sectional study with a population of 384 participants grouped into 213 blood donors, 104 renal disordered patients and 67 liver disordered patients randomly selected from blood transfusion unit, renal unit and medical ward of the Kenyatta National Hospital respectively. The study was in 3 phases. The evaluation phase included individual specimen precision test using 10 specimens of extremely low and high serum creatinine levels. Then precision on within run, between run and between days modes of analyses where two specimens, one with low serum creatinine of 30 µmol/L and another with high serum creatinine of 4769 µmol/L were analysed. 67 icteric specimens were analysed for total bilirubin and serum creatinine using the two methods for substance interference. Phase 2 was to compare the quantitative serum creatinine results of the 232 specimens analysed using the two creatinine analytic methods. Reference ranges phase for serum creatinine and estimated creatinine clearance using serum creatinine results of 213 (male: 118; female: 95) healthy subjects was performed and determination of glomerular filtration rate done using same number of participants. The enzymatic sarcosine oxidase method had a good precision and performance compared to the modified kinetic Jaffe's method with standard deviation (SD) of <1. The three modes of analyses, the SD variation was < 1.6 µmol/L for the low serum creatinine level and < 4 µmol/L for the high serum creatinine level. Bilirubin levels affected the sensitivity of creatinine analysis using the modified kinetic Jaffe's reaction with no effect on the enzymatic sarcosine oxidase method, with $p = 0.013$ at 95% Confidence Interval (CI). The two serum creatinine analytical methods compared significantly different with $p=0.04$ at 95% CI. Reference ranges for serum creatinine using the enzymatic sarcosine oxidase method for male and female were [40.12 – 108.48] µmol/L and [31.1 – 93.5] µmol/L respectively with $p = 0.002$ at 95% CI while estimated creatinine clearance were [63.92 – 135.88] ml/min and [71.36 – 130.64] ml/min for male and female respectively with $p = 0.03$ at 95% CI. The glomerular filtration rate values for the normal adults were estimated at ≥ 99.9 ml/min and ≥ 101.0 ml/min for male and female respectively with $p = 0.021$. Therefore the enzymatic sarcosine oxidase method was recommended as a method of choice for estimation of serum creatinine and determination of glomerular filtration rate for accurate diagnosis of kidney disorders and appropriate categorization of chronic kidney disorders for the Kenyan population.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Chronic kidney disease (CKD) is a major public health problem in the world today. The incidence and prevalence of end-stage renal disease, management of kidney failure by dialysis, and transplantation have more than quadrupled over the last two decades. In United State of America the estimated number of people with earlier stages of CKD is 19 million, in Sub-Saharan Africa, the number is at least 3-4 times more frequent than in developed countries and in Kenya, the prevalence of CKD is 10% (Saraladevi, 2009, Coresh *et al*., 2003). Reliable creatinine measurements in both serum and plasma which are used in glomerular filtration rate (GFR) estimation are critical to ongoing global public health efforts to increase the diagnosis and treatment of chronic kidney disease (CKD).Understanding by laboratorians worldwide of the importance of reliable serum creatinine measurements in GFR estimation and of factors that may affect creatinine measurement have been widely emphasized (Arogundade *et al*., 2008).

The methods most widely used to measure serum/plasma and urine creatinine are alkaline picrate methods, enzymatic or partially enzymatic assays, and high pressure liquid chromatography (HPLC) methods. Isotope-dilution mass spectrometry (IDMS) high-order reference methods have been developed for assignment of reference materials but are available in only a few highly specialized laboratories worldwide. Today, the Jaffe's reaction using alkaline picrate remains the cornerstone of most current routine methods, after continuous refinements attempting to overcome inherent analytical interferences and limitations (Miller *et al*., 2005). The kinetic method decreases interference caused by Jaffe-reactive pseudo-creatinine of non - creatinine chromogens compared with earlier protocols. Other methods have been used to improve the specificity of the Jaffe's reaction, but they are not suitable in automated procedures.

Coupled enzymatic reactions have been developed which reduce or eliminate problems with most interfering substances that the cornerstone method has not successively eliminated. Inorganic chemical–based methods that have been developed as alternatives to the alkaline picrate methods have not been widely implemented clinically because they have not demonstrated improved performance compared with the various adaptations of the Jaffe's method. The only alternative methods that have been widely adopted for routine clinical laboratory use are enzymatic creatinine methods specifically the enzymatic sarcosine oxidase method. Although the enzymatic methods have been reported to have generally fewer interferences than the Jaffe's methods, there have been reports of various substances that do interfere (Myers *et al.*, 2006). It is also worth noting that non renal factors such as age, sex, and race also affect the measurement of serum creatinine concentration (Cabarkapa *et al.,* 2012).

Cockcroft and Gault and Modification of Diet in Renal Disease (MDRD) formulas have been used to estimate glomerular filtration rate of the kidney. The creatinine value used in either of these equations is determined using the routinely used creatinine determination methods either kinetic Jaffe's or enzymatic method. Because no systematic differences between serum and plasma measurements have been reported, serum and plasma results are considered as equivalent (Peake and Whiting, 2006). There is now ongoing activity to promote world-wide standardization of methods to measure creatinine concentrations, together with the introduction of a revised estimated glomerular filtration rate equation appropriate for use with standardized creatinine methods (Panteghini, 2008).

The proposed study was undertaken using modified kinetic Jaffe's reaction and enzymatic sarcosine oxidase creatinine determination methods. Enzymatic sarcosine oxidase method was evaluated since it was to be used for the first time in clinical chemistry laboratory of Kenyatta National Hospital (KNH). The two creatinine determination methods were compared and creatinine reference ranges for serum and creatinine clearance based on enzymatic sarcosine oxidase method established and

glomerular filtration rate for healthy Kenyan population using the enzymatic sarcosine oxidase method was determined. The analytical work was done using clinical chemistry automated analyzer, Mindray 380.

1.2 Statement of the Problem

Cases of kidney diseases in Kenya and the world are increasing everyday hence posing a public health problem. In Kenya, the prevalence of CKD is 10% (Levey *et al*., 2007), Africa is 13.9% (Cherono *et al.*, 2017) and the world is 13% (Mwenda *et al.*, 2019). The modified kinetic Jaffe's reaction method being used for analyzing creatinine values as one of the parameters of renal function tests is faced with limitations and analytical interferences. Therefore, creatinine values from this method cannot be used to offer accurate diagnosis and proper medical management of kidney diseases.

1.3 Justification

Investigation of creatinine concentration in serum and plasma carried out in clinical chemistry laboratory of Kenyatta National Hospital has been achieved using modified kinetic Jaffe's reaction method. Most recently the enzymatic sarcosine oxidase creatinine analytical method was introduced to be used alongside the modified kinetic Jaffe's reaction method. Evaluation of an analytical method is very critical whenever a new method is being introduced in a diagnostic laboratory. The study intends to carry out quantitative analysis of creatinine with modified kinetic Jaffe's reaction analytical method and compare it with the enzymatic sarcosine oxidase method as a part of the evaluation process of the enzymatic creatinine analytical method to determine its analytical suitability and reliability.

1.4 Hypothesis

Serum creatinine quantitative analytical results (values) produced by enzymatic sarcosine oxidase method and modified kinetic Jaffe's reaction analytical method are not similar.

1.5 Research Questions

1. What is the precision and performance of the enzymatic sarcosine oxidase analytical method?
2. What interference effect does icteric serum specimen has on serum creatinine analysis using enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods?
3. Do enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods produce similar results for quantification of serum creatinine?
4. Does enzymatic sarcosine oxidase method produce same creatinine reference ranges for male and female?
5. Can enzymatic sarcosine oxidase method be used to determine glomerular filtration rate for healthy Kenyan population?

1.6 Objectives

1.6.1 General Objective

The major objective of this study was to evaluate enzymatic sarcosine oxidase creatinine analytical method and compare it with the modified kinetic Jaffe's reaction method for quantitative analysis of creatinine.

1.6.2 Specific Objectives

1. To evaluate enzymatic sarcosine oxidase creatinine analytical method.
2. To determine interference effect icteric serum specimen has on analysis of creatinine using enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods.
3. To compare quantitative serum creatinine analytical reports produced using enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods.
4. To establish reference ranges of creatinine based on gender using enzymatic sarcosine oxidase method.
5. To determine glomerular filtration rate for healthy Kenyan population using the enzymatic sarcosine oxidase method.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background Information

Creatine is majorly synthesized in the liver and partly in pancreas and kidneys. It is then transported for storage through blood to muscles and other organs. Creatinine is a waste product of muscle metabolism and is excreted by the kidneys mainly via glomerular filtration method. Conversion of creatine to phosphocreatine catalyzed by creatine kinase yields creatinine as a by – product and a high level energy for use in the body (Kashani *et al*., 2019).

2.2 The Kidney Structure and Functions

The human body has two kidneys each located on either side of the body and appear bean shaped (Figure 2.1). Structurally, a kidney has two regions, an outer cortex and inner medulla. The cortex region is supplied with more blood capillaries and it is where the nephrons, the functional units begin. Glomerulus and convoluted tubules are also found here. Tubules and collecting ducts are found in the medulla region (Figure 2.2).
The functions of the kidney include excretion of waste products of metabolism which include creatinine and blood urea nitrogen, regulation of body's electrolytes which include Potassium, sodium, chloride and bicarbonates, maintenance of water and acid – base balance and secretion of body hormones (Murray *et al.,* 2020).

2.3 Renal Function Tests

Renal function tests (RFTs) are tests performed as a profile to determine the viability and functionality of the kidney. They show the efficiency of kidneys in performing their functions.

These parameters of the renal function tests include serum creatinine (Scr), blood urea nitrogen (BUN), electrolytes (mostly potassium and sodium), estimated creatinine clearance (Ecrcl), estimated glomerular filtration rate (eGFR) and albuminuria and Proteinuria (Gounden *et al*., 2021).

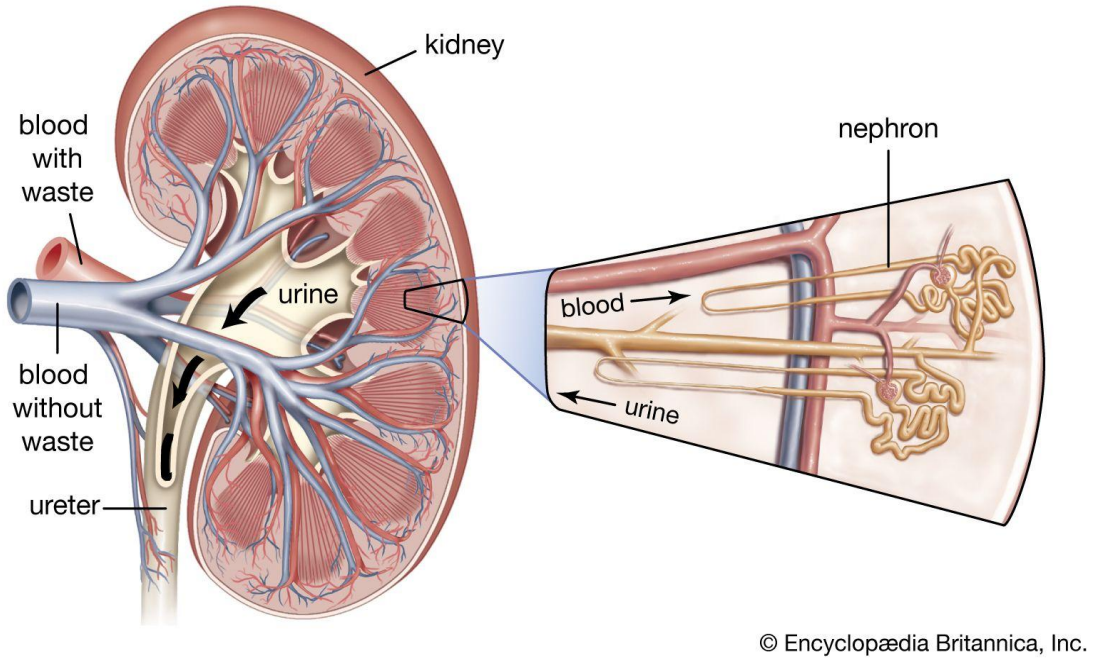


Figure 2.1: Shape and structure of the kidney

Source: https://www.britannica.com/science/nephron

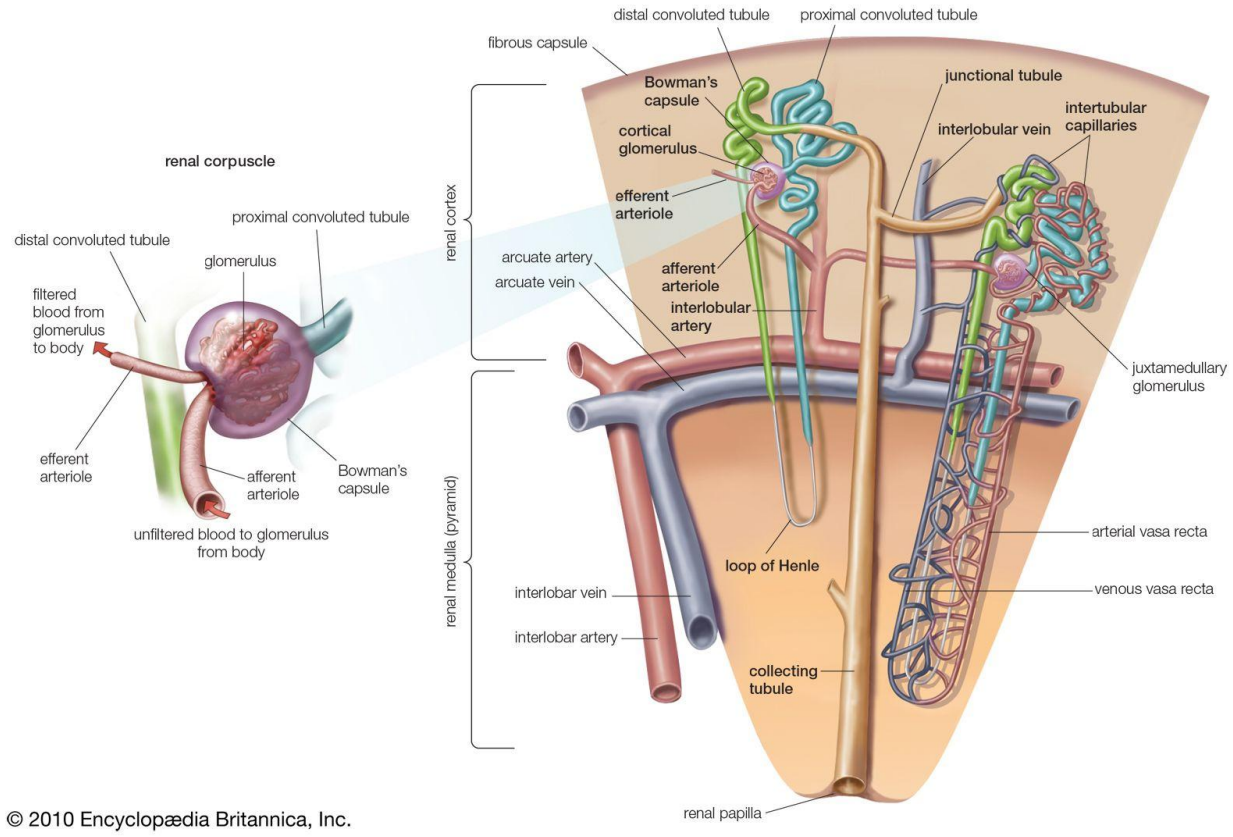


Figure 2.2: Structure of the nephron

Source: https://www.britannica.com/science/nephron

The concentration of creatinine in plasma or serum of a healthy individual is fairly constant, independent from water intake, exercise and rate of urine production. Therefore, increased plasma creatinine values always indicate decrease in excretion, i.e. impaired kidney function (McKillop *et al.*, 2006). In this regard, serum creatinine (Scr) estimation forms a major integral part of the diagnostic renal function profile together with other parameters. The estimated creatinine clearance (Ecrcl) is a measurement to estimate the clearance of creatinine through the kidneys. This measurement enables a quite good estimation of the glomerular filtration rate (eGFR), which reflects the

efficiency and sufficiency of the kidney and allows better detection of kidney impairments (Killeen *et al.,* 2013).

2.4 Methods of Laboratory Analysis

Modified kinetic Jaffe's reaction and enzymatic sarcosine oxidase are chemical and enzymatic methods respectively and are used to measure serum creatinine in routine diagnostic laboratories.

In laboratory medicine, the first method to be used for serum creatinine analysis was the Neubauer method applying Neubauer's reaction. This method involved the reaction of creatinine with alcoholic zinc chloride followed by Weyl's test to produce a precipitated compound whose absorption was read colorimetrically. This was a breakthrough in serum creatinine determination but the method had analytical challenges as it was non - specific to creatinine because other organic compounds gave similar reaction (Wikipedia, the free encyclopedia, 2022). In 1886, Max Jaffe introduced the Jaffe's method which used Jaffe's reaction to replace Neubauer's method. The Jaffe's reaction involved creatinine with picric acid under alkaline medium provided by sodium hydroxide. This was a colorimetric method and the formed compound, creatinine picrate which is reddish orange was read absorptiometrically. It also had its analytical challenges with interfering substances. The Jaffe's method was then automated to improve it to kinetic Jaffe's method where the kinetic readings where obtained at 520 nm spectrometrically. More developments were made and improved to modified kinetic Jaffe's reaction method to minimize the analytical interfering substances (Delanghe *et al*., 2011).

Analytical non – specificity for substances found in individual patient samples affects the accuracy of estimated glomerular filtration computed from serum creatinine concentrations for any alkaline picrate method, including the so – called "compensated" Jaffe's methods (Waithaka *et al.,* 2010).

The inter-laboratory variation of measurement of serum creatinine is due to use of Jaffe's method in analysis of serum creatinine and also results to downgrading of the chronic kidney disease patients into lower categories (Drion *et al.,* 2012).

Enzymatic methods were introduced for serum creatinine determination which are more specific to creatinine than the modified kinetic Jaffe's reaction method. Enzymatic Sarcosine oxidase method is one of those enzymatic methods of creatinine analysis. The reaction involves a series of coupled enzymatic reactions including conversion of creatinine into creatine, and the product creatine is converted to sarcosine by creatine amidinohydrolase (Creatinase). Sarcosine under oxygen and water environment is acted upon by sarcosine oxidase enzyme to produce glycine, formaldehyde and hydrogen peroxide. The hydrogen peroxide is then mixed with phenol and 4 – Aminoantipyrine to produce quinoneimine dye/colour which is measured spectrometrically (https://www.yumpu.com/pointe-scientific-inc, 2013).

The use of the enzymatic methods traceable to the standardized method, the Isotope dilution mass spectrometry (IDMS) have reduced the analytical errors, both the precision and bias errors in serum creatinine analysis (Bargnoux *et al.,* 2018). The enzymatic methods for serum creatinine analysis have more analytical advantages compared to the modified kinetic Jaffe's method. Several studies have concluded that enzymatic methods in serum creatinine measurement show improved specificity, are free from most of substance interferences and small sample volumes are applicable compared to the modified kinetic Jaffe's method. The enzymatic methods however suffer from high cost of operation. The recommendation of use of enzymatic analytical methods for serum creatinine analysis in routine laboratory work is in accordance with the Laboratory Working Group of the National Kidney Disease Education Program which recommends the use of a method which has high specificity and accuracy in serum creatinine measurement (Marakala *et al.,* 2012).

Enzymatic techniques are widely used in medical laboratories in the developed countries to appropriately estimate the glomerular filtration rate. They reduce the serum creatinine measurement variability which affect the creatinine based formulas for glomerular filtration rate estimation. This widely use of estimated glomerular filtration rate has put more focus on the accuracy of serum creatinine measurement with appropriate analytical technique (Drion *et al.*, 2012).

Estimation of glomerular filtration rate on the basis of serum creatinine concentration measurements using equations is critical to ongoing global public health efforts to improve the diagnosis and treatment of chronic kidney disease (Panteghini M., 2008). There is now ongoing activity to promote world – wide standardization of methods to measure creatinine concentrations, together with the introduction of a reversed estimated glomerular filtration equation appropriate for use with standardized creatinine methods. Standardization of calibration, i.e. implementation of calibration traceable to higher – order reference measurement procedures and reference materials, do not, however, correct for analytical interferences of field methods non – specificity bias (Komenda *et al.*, 2008).

The estimated glomerular filtration rate is an important diagnostic tool for diagnosing and categorization of chronic kidney diseases. The serum creatinine based equations appropriately involved in accurate estimation of glomerular filtration rate are the Cockcroft – Gault and Modification of Diet in Renal Disease (Botev *et. al.*, 2011).

Supporting the choice of more specific assays by clinical laboratories is one of the main tasks in achieving the ultimate clinical goal, which is to routinely report an accurate estimated glomerular filtration in all pertinent clinical situations. Measured plasma or serum creatinine concentration is a primary component of equations used to calculate estimated glomerular filtration rate (Waithaka *et al.*, 2010).

The performance of the creatinine – based equations allowing the estimation of glomerular filtration rate and the bias of the creatinine measurements is, more than ever,

a crucial issue. Today, only some enzymatic methods can prove that they are traceable to the reference method (Badious *et al.*, 2003).

High Performance Liquid Chromatography (HPLC) was introduced in 1980s and recommended by the American Association for Clinical Chemistry (AACC) as a reference method for serum creatinine analysis. This method increased the specificity and sensitivity in creatinine determination but it had its limitations of being labour intensive, high cost and therefore not suitable for routine analysis of creatinine in a diagnostic laboratory. In 2006, Isotope Dilution Mass Spectrometry (IDMS) was introduced to replace the HPLC as the reference method. There after Standard Reference Material 967 (SRM 967) was adopted as a reference material to achieve standardization in calibration for serum creatinine determination. Currently, the use of both IDMS and SRM 967 has been recommended for use by the National Institutes of Health (NIH) (Wikipedia, the free encyclopedia, 2022).

Stabilized diazonium salt method is a recommended method for determination of levels of bilirubin. In this method, bilirubin (Total or Direct) couples with diazo reagent to form a coloured compound, azobilirubin which is pink to reddish purple and it is read at 550nm spectrometrically. The intensity of the coloured azobilirubin formed is directly proportional to the bilirubin concentration. Many substances have shown interferences to the Jaffe's based methods in serum creatinine assays and bilirubin is one of them. Bilirubin interferes with the reaction of creatinine with alkaline picrate which is a major step in formation of a coloured compound to be read absorptiometrically (Gencheva *et al.*, 2015). Creatinine determination for neonates with evidence of neonatal jaundice characterized by hyperbilirubinaemia has produced a big challenge in clinical laboratories using Jaffe's reaction method of creatinine determination. Current studies have identified the use of other methods of creatinine determination especially the enzymatic methods which are not affected by such interferences (Christa *et al.,* 2009).

Reference values in laboratories are used for interpretation of laboratory results to state whether the obtained value is normal or abnormal. Recommendation by the Clinical and Laboratory Standards Institute (CLSI) states that reference values should be derived from a specific population for the purposes of serving that population and should be verified often to make it useful throughout (Samaneka *et al.,* 2016). The physiological healthy status of an organ together with its normal reference ranges in laboratory analyses of its functioning, assist in determination of disease status of the organ. Proper renal functioning is normally assessed by estimation of its glomerular filtration rate. Decreased estimated glomerular filtration rate below the lower limit of 60ml/min for at least three months indicates renal insufficiency resulting to impaired renal function and if not early and accurately diagnosed leads to manifestation of chronic kidney disease (CKD) which mostly develops asymptomatically (Delanaya *et al.,* 2012).

Diagnosis and management of various pathological disorders is achieved through proper clinical history, physical examination and use of quality clinical laboratory results. Proper interpretation of quality laboratory results is based on the use of reference ranges established from healthy individuals. Several factors such as gender, age, sample type, analytical procedures, instruments and geographical location of the healthy individuals are known to influence the clinical laboratory parameters. It is therefore recommended that clinical laboratories are used to establish reference ranges for laboratory parameters based on local healthy population (Waithaka *et al.,* 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted in the Department of Laboratory Medicine, Clinical Chemistry Laboratory of Kenyatta National Hospital (KNH). The hospital is located in Nairobi, the capital city of Kenya in Nairobi county. It is one of the largest referral hospital in the country. Patients from different parts of the country are referred here for specialized treatment. It is also a teaching hospital where the school of medicine of University of Nairobi is based.

3.2 Study Design

This was a cross – sectional study on healthy population, renal patients and liver disordered patients to evaluate the enzymatic Sarcosine oxidase creatinine analytical method and compared it with the modified kinetic Jaffe's reaction method. Specimens for the study were taken once from the clinical based study participants who were randomly selected for analytical processing and measurement to produce data for the study.

3.3 Study Population

The study population comprised of 384 (Donors: 213, Renal patients: 104 and liver disordered patients: 67) subjects. The blood donors in the age bracket (18 – 55) years and weight bracket (48 – 97) kg were randomly recruited from the Blood Transfusion Unit. The renal patients were randomly recruited from the renal unit and the liver disordered patients were randomly recruited from the medical ward.

3.3.1 Inclusion Criteria

The healthy blood donor individuals were randomly selected and met the criteria that they were Kenyan citizens, falling in the age bracket of 18 – 55 years, they were non obese (BMI chart in appendix VI), were not involved in any excessive exercise, were not on any medication, not involved in any drug abuse and were non pregnant and not on any oral contraceptives for female subjects.

Those patient participants from Kenyatta National Hospital were randomly selected and met the criteria that they were admitted in the renal unit and on dialysis for the renal patients and be admitted in the medical ward for those patients with liver disorders.

3.3.2 Exclusion Criteria

Those with the following criteria were excluded from participating in the study; children, obese adults, those with body weight less than 48 kg (<48 kg) and those declined to sign the consent form (Appendix IV).

3.4 Sample Size Determination

The following formula for sample size determination by Bartlett *et al*. (2001), was used to determine the minimum number of study subjects involved

$$n = [(t^2)\ (p)\ (q)] / d^2$$

"n"	=	Calculated sample size
"t"	=	1.96
"p"	=	Reported prevalence in literature (If no data available or for new study, assumption of "p" = 50% is made)
"q"	=	(1 – p)
"d"	=	Error at 95% CI, which is (0.05)

Therefore:

$$n= [(1.96)^2 (0.5) (1 – 0.5)] / (0.05)^2$$

n = 384 study subjects

3.5 Sampling Method

The study subjects were randomly selected from the blood transfusion unit who came to donate blood, from renal unit patients who were admitted and on dialysis and from liver disordered patients admitted in the medical ward of the Kenyatta National Hospital. The procedure of recruiting the 384 sample size for the study is described in appendix V where the average number of patients in each unit per month was established, a ratio for these numbers was calculated and the systematic random sampling method was used to pick participants in each unit within the study period until the required sample size was achieved.

3.6 Data Tool

A questionnaire in appendix III was administered to the randomly recruited participants in the study to collect some of the demographic data including gender, age (year), weight (kg), blood pressure, BMI and other health status.

Other data in the study like levels of serum bilirubin, serum creatinine, estimated creatinine clearance and glomerular filtration rate were collected step by step in the study phases as the serum specimens were analysed in the laboratory.

3.7 Study equipments and consumables

3.7.1 Mindray 380

The machine which was used for the samples analysis of the serum creatinine levels and bilirubin levels was the clinical chemistry auto analyzer Mindray 380. This machine is a discrete, random access clinical analyzer capable of performing a wide range of clinical

chemistry tests in a single run, upto 450 tests per hour. The auto analyzer is manufactured by Mindray company in Shenzhen, China.

3.7.2 Reagent Preparation

Reagents for the machine were commercially prepared. Reconstitution of the reagents was done in the laboratory according to the manufacturer's instructions to fit the required volumes and concentration. The reagents were in specific containers referred to as "reagent cartridges". The reagent cartridges were bar-coded for the identification by the machine. The modified kinetic Jaffe's reaction and enzymatic sarcosine oxidase analytical methods were used for the analysis of serum creatinine samples. The stabilized diazonium salt method was used for analysis of samples for bilirubin estimation.

3.8 Laboratory Procedures

3.8.1 Collection of blood specimens

Healthy blood donors: Three millilitres of blood was drawn from the pilot tube of the blood bag using a sterile needle connected to a 10ml syringe. Some amount of blood was allowed to flow down the pilot tube so as to clear any anticoagulant which might be along the walls of the pilot tube then the specimen was collected. Venous blood was collected from those consented volunteers not necessary donating blood for transfusion purposes. Specimen bottles were labelled correctly with the subjects' name and the study number. These collected specimens were involved in the establishment of reference ranges for serum creatinine and estimated creatinine clearance values and also determination of glomerular filtration rate for healthy Kenyan population using the enzymatic sarcosine oxidase method.

Renal patients: Three millilitres of venous blood was collected from consenting renal patients. Plain vacutainer tubes were labelled correctly with the subjects' name and the study number. Specimens from the renal patient subjects were used in the evaluation of

the enzymatic Sarcosine oxidase method for precision and performance determination and also for comparison of the modified kinetic Jaffe's reaction and enzymatic Sarcosine oxidase methods using the serum creatinine quantitative results.

Liver disordered patients: Three millilitres of venous blood was collected from consenting liver disordered patients. Plain vacutainer tubes were labelled correctly with the subjects' name and the study number. The specimens from these liver disordered patient subjects were used in the evaluation of the enzymatic Sarcosine oxidase method for the substance interference effects in serum creatinine analysis on the enzymatic and the modified kinetic Jaffe's reaction method.

Those specimens collected from the study subjects in the blood transfusion unit, renal unit and medical ward of KNH were transported to the Clinical Chemistry laboratory within one hour after collection. The specimens were centrifuged using a speed of 3000 rpm for five minutes. The separation of serum was done using a pasteur pipette for each specimen and transferred into specific vials which also had donor's identification number for donors and patient number for renal and liver disordered patients which also served as the research subject's number.

The serum collected from the healthy donors, renal and liver disordered patients was analyzed for serum creatinine levels using the enzymatic sarcosine oxidase and the modified kinetic Jaffe's reaction methods. Serum bilirubin levels were also obtained from the analysis of the liver disordered patients' serum using the stabilized diazonium salt method.

3.9 Calibration of the test

The purpose of calibration of the procedure was to determine the relationship between measured absorbance to known concentration of the same analyte contained in calibrator solutions (e.g. Mindray multical). Calibration factors were desired once the relationship was achieved.

3.10 Quality Control Materials

The assayed multisera normal was used for the quality control (QC) of the analytical work during the study period. The QC multisera was supplied in lyophilized form and was reconstituted as per the manufacturer's preparation method. For internal quality control assessment, the prepared QC multisera was analyzed daily or any other time samples for the study subjects were being analyzed.

3.11 Methods for the tests

Methods were programmed and stored in the microprocessor of auto analyzer, Mindray 380. The auto analyzer proportioned the required amount of reagent and sample using the reagent and sample probes respectively. Reagent volumes was 50 µl and the sample volumes was 2 µl. The various reactions took place in the reaction compartment of the auto analyzer at 37 degrees centigrade. After the reaction, results were obtained from the data manager either printed out or read from the screen.

3.12 Study Phases

The study was divided into three phases. Phase one was evaluation of the enzymatic sarcosine oxidase method. Phase two was the comparison of the two serum creatinine analytical methods on quantification of serum creatinine. Phase three was the establishment of reference ranges using enzymatic sarcosine oxidase method for serum creatinine and estimated creatinine clearance together with determination of glomerular filtration rate for the healthy Kenyan population.

3.12.1 Phase One: Evaluating Enzymatic Sarcosine Oxidase Method

The following was the step by step activities which was used in the evaluation step of the study.

Step 1: Determination of precision for individual specimens

This was done by analyzing ten serum specimens which were comprised of 5 serum specimens with extremely low known serum creatinine concentrations from the consented healthy donors and other 5 serum specimens with extremely high known serum creatinine concentrations from the consented renal patients. Each of these ten serum specimens were analyzed twenty times for serum creatinine to determine individual specimen precision.

Step 2: Determination of precision based on three modes of analyses.

The three modes of analyses which included within run, between run and between days were used to determine precision of the enzymatic sarcosine oxidase method.

Two serum specimens, one with known low serum creatinine concentration from a healthy blood donor and another with known high serum creatinine concentration from a renal patient on dialysis were each analyzed 20 times for serum creatinine using the three modes of analyses for determination of precision of those modes of analyses.

Step 3: Test the Interference of icteric serum specimen on enzymatic sarcosine oxidase and modified kinetic Jaffe's methods on serum creatinine analysis.

Specimens with serum bilirubin levels above the upper limit of the normal range are referred to as icteric specimens. Icteric specimens were obtained from patients with liver disorders in the medical ward. Interference of bilirubin on quantitative analysis of serum creatinine by modified kinetic Jaffe's reaction and enzymatic sarcosine oxidase methods was determined by analyzing 67 icteric serum specimens for both serum bilirubin and creatinine concentrations.

3.12.2 Phase Two: Comparison of modified kinetic Jaffe's reaction and enzymatic sarcosine oxidase methods Phase

Quantitative serum creatinine analysis was done on randomly selected 232 serum specimens (selection procedure in Appendix V) using enzymatic Sarcosine oxidase method and the modified kinetic Jaffe's reaction method. These specimens were obtained from both the healthy donors and renal patients. The results of the two methods were compared.

3.12.3 Phase Three: References Ranges

Establishment of Serum Creatinine Reference Range.

Serum creatinine results for the 213 (male: 118; female: 95) healthy subjects from blood transfusion unit were obtained using the auto analyzer Mindray 380 by principle of the enzymatic Sarcosine oxidase analytical method and were used to establish serum creatinine reference ranges for the same method.

Establishment of Estimated Creatinine Clearance Reference Range.

The following Cockcroft and Gault formula was used for estimation of creatinine clearance (ml/min)

Male: [140 – Age (yr)] x [Weight (Kg)] x [Factor: 1.23] ÷ [Serum Creatinine (μmol/L)]

Female: [140 – Age (yr)] x [Weight (Kg)] x [Factor: 1.04] ÷ [SerumCreatinine(μmol/L)]

The parameters required for this formula included: Age (yr), weight (kg) and serum creatinine level (μmol/L). These data were collected from the 213 (male: 118; female: 95) healthy subjects from blood transfusion unit. The age and weight of these subjects were captured in the questionnaire (Appendix III) while the serum creatinine levels were obtained by analyzing serum specimens using the enzymatic Sarcosine Oxidase method. The Cockcroft and Gault formula was used to get the estimated creatinine clearance

values for establishment of the reference ranges.

Determination of Glomerular Filtration Rate for Healthy Kenyan Population Using the Enzymatic Sarcosine Oxidase Method.

Determination of glomerular filtration rate (GFR) for healthy Kenyan population was done by analyzing the estimation of clearance of serum creatinine (eCrCl) through the glomerulus of the kidney. Blood specimens were collected from 213 (male: 118; female: 95) healthy subjects from blood transfusion unit for serum creatinine analysis using the enzymatic sarcosine oxidase method and together with age (yr) and weight (kg) parameters of the subjects captured in the questionnaire (Appendix III) the estimated creatinine clearance values were calculated. Thereafter the corresponding estimated glomerular filtration rate from the estimated creatinine clearance were determined.

3.13 Statistical Analysis

3.13.1 Statistical Methods Used in the Establishment of Reference Ranges.

In order to produce unbiased reference ranges based on Sarcosine oxidase enzymatic method of creatinine analysis for the adult Kenyan population, the data from 213 study subjects was statistically treated using the following steps; (1) Partitioning of reference values, (2) Inspection of data distribution, (3) Detection and handling of outliers, (4) Determination of reference limits and (5) Selection of statistical method

3.13.1.1 Partitioning of Reference Values

This was done according to gender and age. The study subjects were divided into male and female groups. The data from each group was used to produce separate reference ranges for each gender. The reference ranges produced was used for comparison with the adult reference ranges for other populations as given in literature. The data was also used to categorize the study subjects in four age groups (18-28, 29-39, 40-50 and 51 - 61

years). By categorizing it was possible to get the effect of gender and age on the reference ranges.

3.13.1.2 Inspection of Data Distribution.

Histograms for parameters analyzed were prepared by the use of computer. The visual examination of the histograms (testing fit to Gaussian distribution) was to safeguard against the misapplication or misinterpretation of statistical methods and it also gave some valuable information about the data. The following characteristics of the data distribution was expressed:

a) Outliers (highly deviating values) were easily detected, and this represented erroneous values in the collected data, which could affect the production of reference ranges
b) The histograms were used to examine the shape of data distribution

3.13.1.3 Identification and Handling Erroneous Values.

The identification of erroneous values (outliers) was done by the visual inspection of the histograms. According to Solberg (1987), there is no other statistical test for the identification of outliers, which is more sensitive or more reliable than the simple visual inspection of a histogram. The data that remained after removing the values (outliers) of both tails of the Gaussian curve (representing 95 % normal reference population) was used for the construction of the reference ranges.

3.13.1.4 Determination of Reference Limits.

By definition, reference limit is a descriptive of reference distribution that tells us something about the observed variation of values in the selected set of reference individuals. In the proposed study, the lower and upper reference limits of each analyte was obtained by the formula: - mean plus/minus 1.960 multiplied by standard deviation (x +/- 1.960 SD). All the values in-between and including the two reference limits gave

us the reference range (interval) of the analytes. This reference interval was also defined as the central 95% interval bounded by 2.5 and 97.5 percentiles, that is, 2.5 % of the values which was cut off in both tails of the reference distribution. The confidence interval of the percentiles which showed the limits within which the true percentiles was located with a specified degree of confidence, for each analyte was also determined.

3.13.1.5 Selection of Statistical Method.

The data obtained from the randomly selected individuals was computed using parametric approach methods, whereby the lower and upper limits of the reference intervals was obtained using the following formula.

X – 1.960 SD, X + 1.960 SD where X = the mean and SD = Standard Deviation.

To test the significant difference, data obtained was analyzed using a statistical package for social science (SPSS Version 21). T-test was used for means comparison, while post-ANOVA-tests was used for multiple comparison of means. The tests were conducted at 95% confidence interval and significance level of 5%, every difference at ($p = 0.05$) was considered significant.

Analytical instrument comparison and methodology comparison was achieved by least squares regression method.

3.14 Ethical Approval

Ethical approval was sought from the following authorities: (1) Department of medical laboratory sciences of Jomo Kenyatta University of Agriculture and Technology (2) Kenyatta National Hospital / University Of Nairobi Ethics and Research Committee (KNH/UoN – ERC). The study was approved and given the study reference No. (P459/08/2013), this is found in appendix I. Study subjects were also requested to give consent (Appendix IV) to allow their blood specimens to be used for the study.

3.15 Limitations of the Study

There were challenges concerning time period of the research and finance for implementation of the budget. The time was adjusted accordingly while the Kenyatta National Hospital laboratory department of clinical chemistry assisted in allowing the specimens analysis of this study to be performed at their department therefore offering their machines and reagents.

CHAPTER FOUR

RESULTS

4.1 Internal Quality Control of the Analysis

An internal quality control (IQC) serum for specific parameter was included in each analytical session throughout the study period. The analytical sessions were 20 for each parameter under study. Quality control results for the analyzed parameters were within the specific assigned QC range of target value ± 2 standard deviations (SD). The parameters under study were direct bilirubin, total bilirubin and serum creatinine. Reports for both assigned and study quality control which included the mean values and quality control ranges were recorded as shown in Table 4.1 below.

Table 4.1: Internal quality control (IQC) for the studied parameters

Parameter (unit)	Assigned QC report			Study QC report			
	Session	QC range	Mean	QC range	Mean	1 SD	CV%
D-BILI (µmol/L)	20	7-17	12	9-17	13	2	16
T-BILI (µmol/L)	20	14-28	21	18.4-27.6	23	2.3	10
CREAT (µmol/L)	20	57-229	143	129-149	139	5	3.5

KEY:

SD = Standard Deviation QC = Quality Control D – BILI = Direct Bilirubin

CV = Coefficient of Variation CREAT = Creatinine. T – BILI = Total Bilirubin

4.2 Evaluation of Enzymatic Sarcosine Oxidase Creatinine Analytical Method

4.2.1 Determination of Precision for Individual Specimens (Extremely Low and Extremely High Serum Creatinine Concentration)

Evaluation of the enzymatic sarcosine oxidase creatinine analytical method was done first by testing of precision for individual serum specimens. Ten clinical serum specimens labelled A-J (Table 4.2) comprised of 5(50%) with low serum creatinine concentrations (A-E) and the other 5(50%) with high serum creatinine concentrations (F-J) were used for analysis.

Serum specimens A-E had known low serum creatinine concentrations of 50µmol/l, 75µmol/l, 63µmol/l, 85µmol/l, and 80µmol/l respectively. The mean serum creatinine concentration after analyzing each specimen (A-E) 20 times were: 51.665 µmol/l, 75.045µmol/l, 63.055µmol/l, 85.68µmol/l and 80.975 µmol/l respectively as shown in the same table 4.2 below.

Precision was also tested using five clinical serum specimens (F-J) with known high serum creatinine concentrations of 301µmol/l, 410µmol/l, 551µmol/l, 402µmol/l and 310µmol/l respectively as shown in the same table below. These specimens were treated in a similar way as those with low serum creatinine concentrations by analyzing each specimen twenty times and the mean serum creatinine concentration obtained were as follows: 301.64 µmol/l, 410.955µmol/l, 551.005 µmol/l, 402.15 µmol/l and 310.54 µmol/l respectively as shown in the same table below.

Table 4.2: Precision performance of specimens with known extremely low and extremely high serum creatinine concentrations using enzymatic sarcosine oxidase method.

SPECIMEN (Creatinine Conc)	N	Minimum	Maximum	Mean	SD
A (50µmol/l)	20	50.80	52.60	51.6650	.51122
B (75µmol/l)	20	73.90	75.60	75.0450	.47736
C (63µmol/l)	20	62.70	63.80	63.0550	.29105
D (85µmol/l)	20	85.00	86.20	85.6800	.35924
E (80µmol/l)	20	80.00	81.90	80.9750	.54664
F (301µmol/l)	20	301.00	302.50	301.6400	.42352
G (410µmol/l)	20	410.00	411.80	410.9550	.49786
H (551µmol/l)	20	550.00	552.20	551.0050	.69772
I (402µmol/l)	20	401.00	402.80	402.1500	.47184
J (310µmol/l)	20	309.00	312.50	310.5400	.99282
Valid N (list wise)	20				

KEY:

N = Number of sessions SD = Standard Deviation Conc = Concentration

4.2.2 Precision Performance Based on Within Run, Between Run and Between Days.

Precision was also performed based on three modes of analyses namely within run, between run and between days. Two serum specimens, one with low serum creatinine concentration of 30 µmol/l from a healthy donor and the other with high serum creatinine concentration of 4765 µmol/l from a renal patient on dialysis were each analyzed twenty times using the three modes of analyses and the results were as shown in table 4.3 below.

Within run analysis meant that an identified clinical serum specimen with low serum creatinine concentration of 30 µmol/l was divided 20 times and each of these specimens placed in specific positions among other serum specimens for biochemical analysis in a single run. The mean serum creatinine concentration obtained was 30.9 µmol/l with the SD of 1.07. The same procedure was performed on a clinical serum specimen with high serum creatinine concentration of 4765 µmol/l and the mean serum creatinine concentration achieved was 4769.65 µmol/l with the SD of 3.86 as shown in the same. table 4.3 below.

Serum specimens are analyzed for various requested biochemical parameters and the analysis is done as per session. Between run analysis meant that an identified clinical serum specimen with low serum creatinine concentration of 30 µmol/l was divided 20 times and each of these serum specimens was analyzed with other serum specimens for biochemical analysis in a single run, therefore, the 20 divided portions were analyzed in 20 sessions. The mean serum creatinine concentration obtained in these 20 sessions was 30.55µmol/l with the SD of 1.54. The same procedure was performed on the clinical serum specimen with high serum creatinine concentration of 4765 µmol/l and the mean serum creatinine concentration achieved was 4769.25 µmol/l with the SD of 1.33 as shown in the same table below.

Serum specimens for biochemical analysis are requested and analyzed every day in a routine clinical chemistry laboratory. Between days analysis meant that a clinical specimen with a low serum creatinine concentration of 30 µmol/l was divided 20 times and each specimen analyzed every day for 20 days together with other serum specimens for biochemical analysis. The mean day serum creatinine concentration of the 20 serum specimens was 30.55µmol/l with the SD of 1.39. A clinical serum specimen with high serum creatinine concentration of 4765 µmol/l underwent the same procedure and the mean serum creatinine concentration obtained was 4770.5 µmol/l with the SD of 1.28 as shown in table 4.3 below.

Table 4.3: Precision performance based on within run, between run and between days.

Modes of analysis	N	Minimum	Maximum	Mean	SD
Between run low	20	29.00	33.00	30.55	1.53811
Within run low	20	29.00	33.00	30.9	1.07115
Between days low	20	29.00	33.00	30.55	1.39454
Within run high	20	4765.00	4781.00	4769.65	3.85630
Between run high	20	4768.00	4772.00	4769.25	1.33278
Between days high	20	4768.00	4773.00	4770.5	1.27733
Valid N (list wise)	20				

KEY:

N = Number of sessions SD = Standard Deviation

Precision Modes Comparisons

Comparison of the various modes of precision analysis was statistically tested using paired t test to determine whether there was any difference between them. The statistical analysis did not show any significant difference among the various modes of precision testing of the enzymatic sarcosine oxidase method on serum creatinine analysis. The statistical comparison results were as follows: within run low and between run low ($p = 0.601$), within run low and between days low ($p = 0.506$), between run low and between days low ($p = 1.000$), within run high and between run high ($p = 0.694$), within run high and between days high ($p = 0.351$) and finally between run high and between days high ($p = 0.352$) as show in table 4.4 below.

Table 4.4: Paired samples test for statistics on the precision modes comparison

Modes of analysis		Paired Differences					t	df	Sig. (2-tailed)
		Mean	SD	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	WRL - BRL	.3500	2.09950	.46946	-.7326	1.2326	.533	19	.601
Pair 2	WRL - BDL	.3500	1.65036	.36903	-.5224	1.0224	.677	19	.506
Pair 3	BRL - BDL	.0000	2.29416	.51299	-1.0737	1.0737	.000	19	1.000
Pair 4	WRH - BRH	.4000	4.47684	1.00105	-1.6952	2.4952	.400	19	.694
Pair 5	WRH - BDH	-.8500	3.97724	.88934	-2.7114	1.0114	-.956	19	.351
Pair 6	BRH - BDH	-1.2500	1.55174	.34698	-1.9762	-.5238	-.603	19	.352

KEY: Within run low (WRL), Between run low (BRL), Within run high (WRH), Between run high (BRH), Between days low (BDL), Between days high (BDH),

SD = Standard Deviation, df = Degree of freedom, Sig=Significance.

4.3 Determination of the Interference Effect of Bilirubin on Serum Creatinine Analysis Using the two Creatinine Analytical Methods.

Sixty-seven icteric serum specimens were used to determine the effect of interference substance in the analysis of serum creatinine using both enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods. Total bilirubin concentration of the sixty-seven serum specimens were analyzed using the stabilized diazonium salt method. The mean bilirubin concentration was 310 μmol/L (Figure 4.1). The same icteric serum specimens were also analyzed for serum creatinine using the two creatinine analytical methods and mean serum creatinine concentration using modified kinetic Jaffe's reaction and enzymatic sarcosine oxidase methods were 39.5 μmol/L and 78.7 μmol/L respectively. The distribution of bilirubin (using stabilized diazonium salt method), serum creatinine (using enzymatic sarcosine oxidase method) and serum creatinine (using modified kinetic Jaffe's reaction method) are presented in figure 4.1, figure 4.2 and figure 4.3 respectively. The mean difference between serum creatinine analyzed using enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods was 39.2 μmol/L which was statistically significance p= 0.013, Table 4.5.

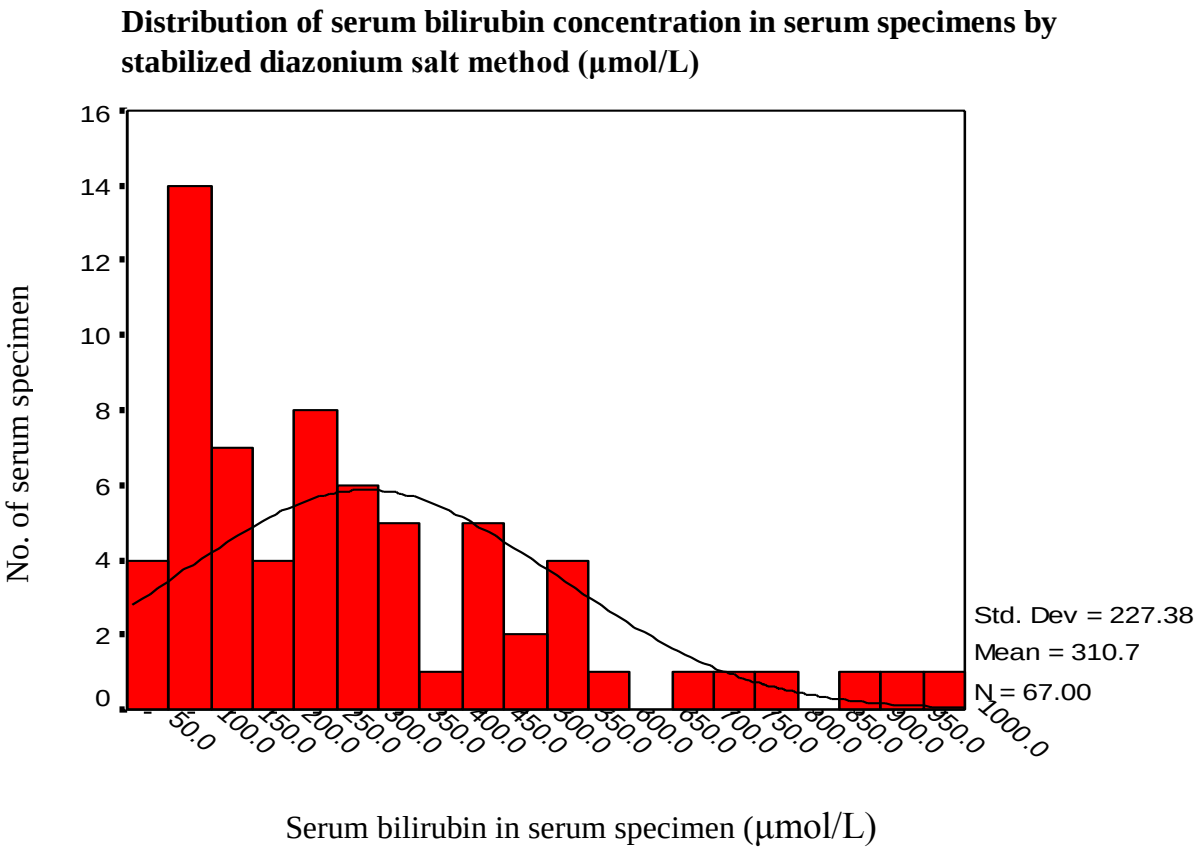


Figure 4.1: Distribution of bilirubin concentration (µmol/L)

Distribution of serum creatinine concentration (µmol/L) by enzymatic sarcosine oxidase method

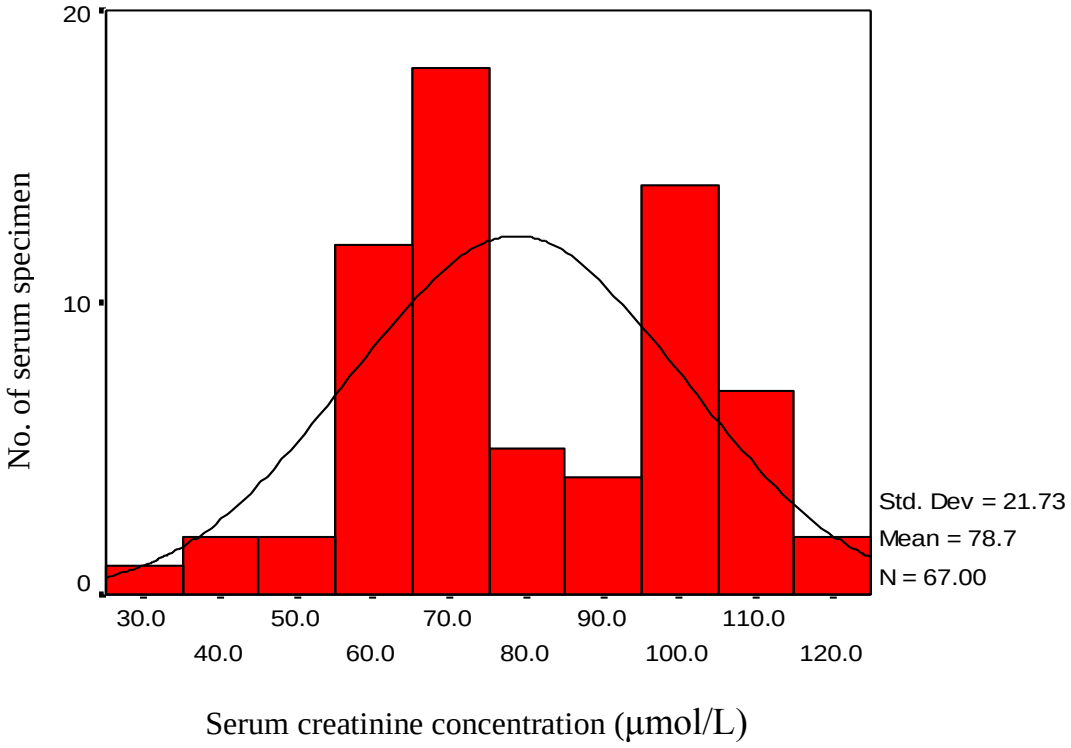


Figure 4.2: Serum creatinine concentration analyzed by enzymatic Sarcosine Oxidase method

Distribution of serum creatinine concentration (µmol/L) by modified kinetic Jaffe's method

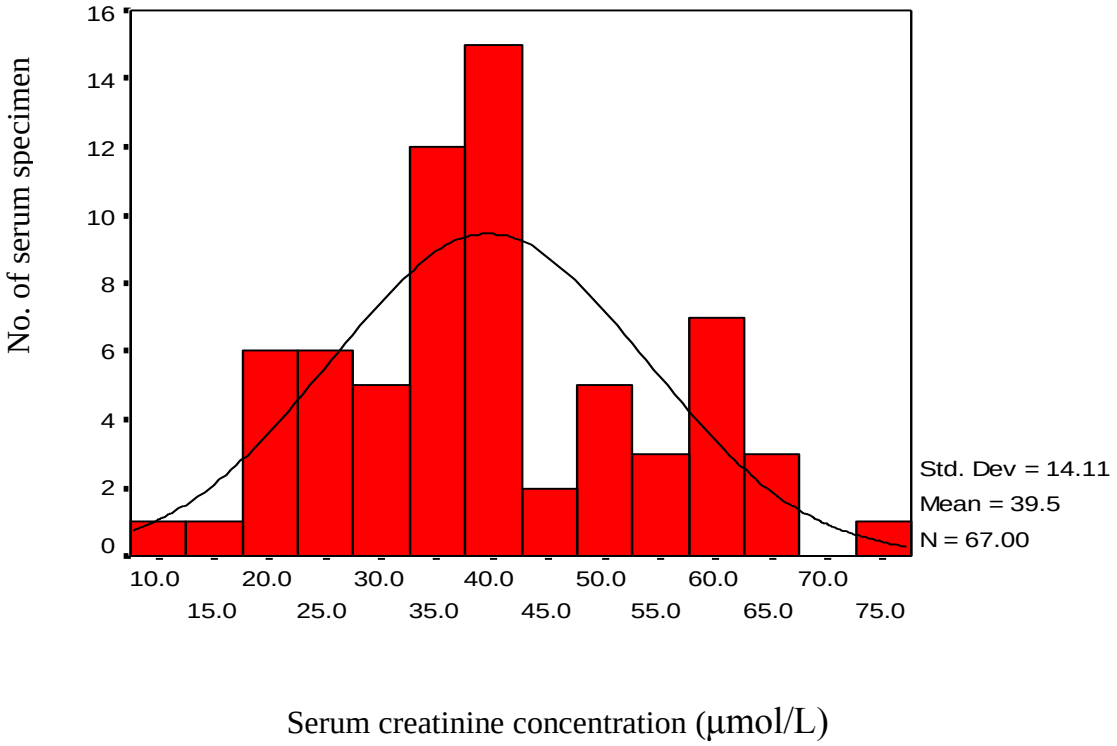


Figure 4.3: Serum creatinine concentration analyzed by modified kinetic Jaffe's method

Table 4.5: Statistical analysis on the effect of bilirubin in the analysis of serum creatinine using enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods.

Method of analysis	Mean creatinine concentration	Mean difference	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
			Lower	Upper			
Sarcosine	78.6067	39.2	73.3062	83.9073	26.2	66	0.013
Jaffe's	39.4873		36.0445	42.9301			

KEY: df = Degree of freedom, sig = Significance,

Sarcosine = Enzymatic Sarcosine oxidase method, Jaffe's = Modified kinetic Jaffe's method

4.4 Comparison of Quantitative Serum Creatinine Analytical Reports Produced Using Enzymatic Sarcosine Oxidase and Modified Kinetic Jaffe's Reaction Methods.

The two serum creatinine analytical methods, enzymatic sarcosine oxidase and modified kinetic Jaffe's reaction methods in this study were compared with each other using quantitative creatinine results. Two hundred and thirty-two specimens were analyzed for serum creatinine by each of the two quantitative creatinine analytical methods.

The mean serum creatinine value for the study subjects using modified kinetic Jaffe's reaction method was 265 µmol/L whilst that of enzymatic sarcosine oxidase method for the same population was 240 µmol/L. The mean difference of 25 µmol/L was found to be statistically significance at p = 0.04 as shown in Table 4.6 below.

Table 4.6: Comparison of quantitative serum creatinine analytical reports using enzymatic Sarcosine oxidase and modified kinetic Jaffe's reaction methods.

N	Method of analysis	Mean creatinine concentration (μmol/L)	Mean difference	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
				Lower	Upper			
232	Sarcosine	239.5517	25.4828	163.89	315.211	5.88	231	0.04
	Jaffe's	265.0345		197.512	333.781			

KEY:

df = Degree of freedom Sig = Significance N = No of subjects

4.5 Establishment of Serum Creatinine and Estimated Creatinine Clearance Reference Ranges for the Kenyan Adult Population Using Sarcosine Oxidase Method.

Establishment of serum creatinine reference ranges for the Kenyan adult population using enzymatic sarcosine oxidase method was performed using 213 blood donors comprising of 118 (55.4%) male and 95(44.6%) female. Serum creatinine concentrations with mean values of 74.3 μmol/L and 62.3 μmol/L were obtained for male and female respectively and the formula: Mean +/- 2 SD was used to calculate the reference range values. The ranges as per gender of (40.12 to 108.48) μmol/L and (31.10 to 93.50) μmol/L were obtained for male and female respectively.

Estimated creatinine clearance was calculated using the Cockcroft – Gault equation:

Male: [140 – Age (yr)] x [Weight (Kg)] x [Factor: 1.23] ÷ [Serum Creatinine (μmol/L)]

Female: [140 – Age (yr)] x [Weight (Kg)] x [Factor: 1.04] ÷ [Serum Creatinine (μmol/L)]

Same total samples and proportions as for the serum creatinine reference ranges establishment above were used. The subjects' age bracket was (18 – 54) years with a mean age for male as 32.8 years and female 31.1 years respectively while the subjects' weight bracket was (48 – 97) kg with a mean weight for male as 68.0kg and female as 67.0 kg respectively.

Estimated creatinine clearance mean values of 99.9 ml/min and 101.0 ml/min for male and female respectively were obtained and reference ranges calculated using formula: Mean +/- 2 SD where (63.92 to 135.88) ml/min and (71.36 to 130.64) ml/min were obtained for male and female respectively.

Two hundred and thirteen specimens from both male and female were analyzed for serum creatinine by enzymatic sarcosine oxidase method and the mean difference of 11.38 μmol/L was found to be statistically significance at p=0.002 as shown in table 4.7 below.

Table 4.7: Paired samples test for male and female serum creatinine values.

Gender	N	Paired Differences					t	df	Sig. (2-tailed)
		Mean Difference	SD	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Male: 118	213								
Female: 95		11.3803	23.38382	1.60223	-14.5386	-8.2219	-7.103	212	.002

KEY:

SD = Standard Deviation, df = Degree of freedom, Sig = Significance, N = No of subjects.

Two hundred and thirteen specimens from both male and female were analyzed for estimated creatinine clearance by enzymatic sarcosine oxidase method and the mean difference of 3.31 μmol/L was found to be statistically significance at p=0.032 as shown in table 4.8 below.

Table 4.8: Paired samples test for male and female estimated creatinine clearance values.

Gender	N	Paired Differences					t	df	Sig. (2-tailed)
		Mean Difference	SD	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Male: 118	213								
Female: 95		3.3072	22.42328	1.53642	-6.3358	-.2786	-2.153	212	.032

KEY:

SD = Standard Deviation, df = Degree of freedom, Sig = Significance, N = No of subjects

4.6 Determination of Glomerular Filtration Rate for Healthy Kenyan Population Using the Enzymatic Sarcosine Oxidase Method.

Glomerular filtration rate (GFR) for the Kenyan healthy population using the enzymatic sarcosine oxidase method was performed using 213 blood donors comprising of 118 (55.4%) male and 95(44.6%) female. First, the estimated creatinine clearance (eCrCl) of the participants were determined using the values of serum creatinine, age and weight in the Cockcroft – Gault equation.

Estimated creatinine clearance mean values of 99.9 ml/min and 101.0 ml/min for male and female respectively were obtained.

GFR is the period taken for a chemical or analyte to be excreted completely through the glomerulus and at same time the analyte is not reabsorbed to the blood through the nephrotic tubules. Serum creatinine is a suitable analyte to accurately estimate the GFR. Therefore the estimated creatinine clearance (ml/min) would accurately approximate the GFR (ml/min) of the healthy population. Hence the corresponding estimated glomerular filtration rate values for the health population were ≥ 99.9 ml/min and ≥ 101.0 ml/min for male and female respectively (Table 4.9) below.

Table 4.9: Glomerular filtration rate for healthy Kenyan population using the enzymatic sarcosine oxidase method.

N	Gender	Mean Values (ml/min)	Mean Difference (ml/min)	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
				Lower	Upper			
213	M: 118	99.9	1.1	94.6	105.2	8.33	212	0.021
	F: 95	101.0		97.5	104.5			

Key:

N = Number of subjects, df = Degree of freedom, Sig = Significance

CHAPTER FIVE

Discussion

The study evaluated the enzymatic sarcosine oxidase method for quantification of serum creatinine levels. Both the enzymatic sarcosine oxidase and the modified kinetic Jaffe's methods were analytically compared to each other and the reference ranges for serum creatinine and estimated creatinine clearance were also established based on the enzymatic sarcosine oxidase method. Glomerular filtration rate for the healthy Kenyan population was also determined using the enzymatic sarcosine oxidase method.

It was established in this study that the enzymatic sarcosine oxidase method had a good precision and performance compared to the modified kinetic Jaffe's method. It was also noted that bilirubin concentration in serum had interference effects on serum creatinine analyzed by the modified kinetic Jaffe's method and not on the enzymatic sarcosine oxidase method. The analytical comparison established that the two serum creatinine quantitative methods were significantly different and they gave different serum creatinine values. The reference ranges established for serum creatinine and estimated creatinine clearance based on the enzymatic sarcosine oxidase method emphasized that there should be different reference ranges on each analyte for each gender. Finally, the estimated creatinine clearance using the enzymatic sarcosine oxidase method accurately estimated the glomerular filtration rate for the healthy Kenyan population in the study.

For the evaluation process, the enzymatic sarcosine oxidase analytical method was analyzed for precision performance and substance interference. Based on serum creatinine levels of extremely low and extremely high concentrations, the precision for the evaluated method, enzymatic sarcosine oxidase creatinine method was good since the results standard deviation value for each analyzed serum specimen was small and less than one (<1).

All the three modes of analyses performed on the enzymatic sarcosine oxidase method, within run, between run and between days, their precision was good since the low serum creatinine concentration had a standard deviation variation of less than 1.6 μmol/L and the high serum creatinine concentration had a standard deviation variation of less than 4 μmol/L. The precision testing of the various modes of analyses on the enzymatic sarcosine oxidase method did not also show any statistical significant difference between them because all of the modes of precision comparison produced statistical significant values of greater than 0.05 ($p > 0.05$) at 95% confidence interval. Bargnoux *et al.* (2018) supported the above findings in their study as they stated that the enzymatic method had a better analytical precision than the Jaffe's method.

The analytical performance of enzymatic methods is superior to that of Jaffe's kinetic methods (Delanaye *et al*., 2014). In a study of performance characteristics and practicability of enzymatic method compared to Jaffe's kinetic method, Marakala *et al.* (2012) performed quality control assay for both levels ($n = 18$) on precision performance of the enzymatic method and obtained standard deviation values of 0.086 and 0.365. Both studies were in accordance with the present study which produced good precision with a results standard deviation of less than one (< 1).

Küme *et al.* (2018) supported this study as they found a better precision and performance with the enzymatic analytical method than with the Jaffe's method at a lower serum creatinine concentration levels. At the same concentration levels, their study also gave higher serum creatinine values with the Jaffe's method than the enzymatic method. Their serum creatinine reference range for Jaffe's method had higher values for the lower limit range than those of the enzymatic method as the Jaffe's method produced values which were 7% higher above the lower range of the enzymatic method.

Bilirubin was used to give the substance interference effect to the two creatinine analytical methods. The statistical significant value of $p = 0.013$ at 95% confidence

interval was achieved and showed that the two serum creatinine analytical methods work differently when subjected to bilirubin interference. The icteric nature of the specimen affected the sensitivity of serum creatinine analysis using modified kinetic Jaffe's reaction whilst it did not have any effect when serum creatinine was analyzed using the enzymatic sarcosine oxidase method. These results were supported by other studies where two enzymatic methods were free from bilirubin interference while three Jaffe's based methods showed significant bilirubin concentration dependent interference (Nah *et al*., 2016). The negative interference of serum creatinine with Jaffe's method was due to both conjugated and unconjugated bilirubin while the enzymatic methods were stated to be most accurate routine methods which produced serum creatinine results agreeable to the gold standard method for serum creatinine measurement, Isotope Dilution Mass Spectrometry method (Vaishya *et al*., 2010). Jaffe's method was affected by substance interference while enzymatic method was not according to Bargnoux *et al.* (2018) study in serum creatinine analysis. Schmidt *et al.* (2015) also concurred with this present study as they stated in their study that the Jaffe's analytical method had substance interference effects with misclassification rate of 4% than the enzymatic method.

The comparison phase of the two methods using serum creatinine analytical reports showed that the modified kinetic Jaffe's reaction method gave higher values than the enzymatic sarcosine oxidase method which gave lower values. The two methods showed significant difference, $p = 0.04$ at 95% confidence interval. Therefore, these are two different serum creatinine estimation methods whose results should be interpreted differently. Drion *et al*. (2012) supported this present study as in their study, the Jaffe's techniques overestimated serum creatinine target values 52, 73 and 94 µmol/L by 21%, 12% and 10% respectively while the same values by enzymatic method were 0%, -1% and -2% respectively. During method comparison between enzymatic and Jaffe's, the latter gave higher serum creatinine values than the enzymatic method (Marakala *et al*., 2012). Another study for staging of chronic kidney disease (CKD) using the modification of diet in renal disease (MDRD) formula and serum creatinine values

measured by both enzymatic and Jaffe's methods, the CKD categories of 45 – 60, 60 – 90 and > 90 ml/1.73 m^2 were downgraded by the Jaffe's techniques in 1 - 42%, 2 - 3% and 12 - 78.9% of patients respectively while only in 2 – 4% of the patients occurred with enzymatic method (Drion *et al*., 2012).Therefore, both studies supported the results of this present study in stating that the modified kinetic Jaffe's method and the enzymatic sarcosine oxidase method produced different serum creatinine measurements.

During reference ranges study, the data collection method was in accordance with the rigorous Clinical and Laboratory Standards Institute (CLSI) guidelines for determining laboratory reference ranges, which recommends a minimum of 153 subjects for a 95^{th} percentile clinical reference range determination with 95% confidence (Kibaya *et al*., 2008). Reference ranges for serum creatinine for male and female using the enzymatic sarcosine oxidase method in the study were [40.12 – 108.48] µmol/L and [31.1 – 93.5] µmol/L respectively. A statistical value, p = 0.002 at 95% confidence interval showed a statistical significant difference and supported the existence of two different reference ranges for serum creatinine for male and female using the enzymatic sarcosine oxidase method. These present study results were compared with the published studies evaluating reference intervals from serum concentrations in adults obtained with methods traceable to the reference measurement system for creatinine measurement. Results for male serum creatinine [59 – 104, 60 – 105 and 64 - 104] µmol/L and female [45 – 84, 46 – 92 and 49 - 90] µmol/L by Mazzachi *et al*, Rustad *et al* and Junge *et al* respectively (Ceriotti *et al*., 2008) were compared with the above results and the upper limits for both the study and published results were near similar but the lower limits of the study were lower than that in the published results. Another study of serum creatinine by Pokorna *et al.* (2002) obtained results of [40 – 160] µmol/L as a single interval for both gender which compared to the study intervals, the lower limit was matching with that of the male but the upper limits of the study results for both gender were lower than the published results.

The study reference ranges for estimated creatinine clearance using the enzymatic sarcosine oxidase method were [63.92 – 135.88] ml/min and [71.36 – 130.64] ml/min for male and female respectively. A statistical significant difference in the reference ranges was obtained with p = 0.032 at 95% confidence interval and this value agreed that there was a difference between values of male and female estimated creatinine clearance using the enzymatic sarcosine oxidase method. The study results were compared to the single interval [35 – 195]ml/min for both gender published by the same author, Pokorna *et al.* (2002) above, using the Cockcroft - Gault formula and found that the study lower limits were double that of the published and the upper limits were very low to that of the published results.

These observed differences of interval values in this study compared to the published results could be due to geographical location and mostly the racial variable because all of the published data cited in this study were from the white population as Ceriotti *et al.* (2008) stated that no literature data was available for serum creatinine reference intervals fulfilling their criteria of selection for either African or Asian population.

The estimation of creatinine clearance is done mainly using modern creatinine assays, and as per Delanghe (2008) the results from the modified kinetic Jaffe's method are less suitable while those from the enzymatic methods are more preferred for calculations of estimated creatinine clearance as they accurately measure the serum creatinine. The creatinine clearance estimated this way can also be used for classification of CKD especially in the early stages (Hermida *et al.*, 2014).

The determination of glomerular filtration rate using enzymatic sarcosine oxidase creatinine analytical method for the healthy population was accurately determined and the normal values were ≥ 99.9 ml/min and ≥ 101.0 ml/min for male and female respectively. According to Zitta *et al*. (2013), they stated in their study that people with impaired renal function results have poor estimated glomerular filtration rate value of < 60 ml/min and they also showed that the normal glomerular filtration rate for adults is

estimated at ≥ 90 ml/min. Therefore the above normal values arrived at in this study for both gender compared to the former study are within the normal.

The enzymatic methods recently have been commonly used for estimating the glomerular filtration rate. Serum creatinine is simply obtained and used to estimate creatinine clearance and eventually the estimated creatinine clearance used to accurately estimate the glomerular filtration rate in the laboratory. This estimated glomerular filtration rate is widely used to assess the function of the kidney (Zatko *et al.*, 2014). The renal function of both healthy and kidney disordered cases as stated by Fiseha *et al.* (2019) is accurately assessed by using the estimated glomerular filtration rate. Serum creatinine based equations which are mostly used in USA are recommended for appropriately categorizing the chronic kidney diseases, CKD (Earley *et al.*, 2012).

Therefore, the study has confirmed that the enzymatic sarcosine oxidase creatinine analytical method had a good precision and performance with no substance interference compared to the modified kinetic Jaffe's method. The two serum creatinine analytical methods produced different quantitative serum creatinine results. The enzymatic sarcosine oxidase method produced different reference ranges for serum creatinine and estimated creatinine clearance for each gender. The enzymatic sarcosine oxidase method also accurately determined the estimated glomerular filtration rate for the healthy Kenyan population. Therefore the enzymatic sarcosine oxidase creatinine analytical method should be a preferred method for measurement of serum creatinine levels and determination of glomerular filtration rate for accurate diagnosis of kidney diseases with appropriate categorization of chronic kidney disorders.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The study has concluded that the enzymatic sarcosine oxidase creatinine analytical method produced a good precision with consecutively good performance and no bilirubin substance interference compared to the modified kinetic Jaffe's reaction analytical method.
2. The enzymatic sarcosine oxidase creatinine analytical method and modified kinetic Jaffe's reaction method compared differently on the measured serum creatinine values with different reference ranges for serum creatinine and estimated creatinine clearance on age and sex.
3. The enzymatic sarcosine oxidase creatinine analytical method accurately determined the glomerular filtration rate for the healthy Kenyan population.
4. Therefore, the enzymatic sarcosine oxidase creatinine analytical method is recommended as a preferred method of choice for analytical estimation of serum creatinine and glomerular filtration rate in the biochemistry laboratory of Kenyatta National Hospital for accurate diagnosis of kidney diseases with appropriate categorization of chronic kidney disorders for the Kenyan population.

6.2 Recommendations

1. The enzymatic sarcosine oxidase creatinine analytical method was recommended to be used along side the modified kinetic Jaffe's reaction method in the biochemistry laboratory of Kenyatta National Hospital and by extension in Kenyan medical laboratories because of its good precision and performance, no bilirubin interference and accurately determines glomerular filtration rate for the healthy Kenyan population.
2. Another study should be carried out to check for more serum creatinine interference substances found in serum / plasma which were not studied in this research.
3. More study should be conducted for children serum creatinine and estimated creatinine clearance reference ranges as these values are also age dependent.

REFERENCES

Abe, Y., Shoji, M., Nishiva, Y., Aiba, H., Kishimoto, T., and Kitaura, K. (2017). The reaction mechanism of sarcosine oxidase elucidated using FMO and QM/MM methods. *Phys. Chem. Chem. Phys*, 19, 9811- 9822. doi.org/10.1039/C6CP08172J.

Arogundade, F. A. and Barsoum, R. S. (2008). CKD prevention in sub-Saharan Africa: a call for governmental, nongovernmental and community support. *American Journal of Kidney Diseases*, 51, 515–523.

Badious, S., Dupuy, A. M., Descomps, B. and Cristolead, J. P. (2003). Comparison between the enzymatic vitros assay for creatinine determination and three other methods adapted on the Olympus analyzer. *Journal of Clinical Laboratory Analysis*, 17 (6), 235 – 240.

Bargnoux, A.S., Kuster, N., Cavalier, E., Piéroni, L., Souweine, J. S., Delanaye, P. and Cristol, J. P. (2018). Serum creatinine: advantages and pitfalls. *J Lab Precis Med*, 3, 71.

Body Mass Index Chart. https://patient.info/doctor/bmi-calculator-calculator, 31st July 2022.

Botev, R., Mallié, J., Wetzels, J., Couchoud, C. and Schück, O. (2011). The Clinician and Estimation of Glomerular Filtration Rate by Creatinine-based Formulas: Current Limitations and Quo Vadis. *Clinical Journal of the American Society of Nephrology*,. 6 (4), 937-950. DOI: https://doi.org/10.2215/CJN.09241010.

Cabarkapa, V., Mijovi, R., Stosic, Z., Curic, N., Zeravica, R. and Ilincic, B. (2012).Estimation of Glomerular Filtration Rate from Serum Cystatin C and Creatinine in Patients with Thyroid Dysfunction. *J Med Biochem, 31(2), 88–93.* ISSN 1452-8258.

Ceriotti, F., Boyd, J. C., Klein, G., Henny, J., Queralto, J., Kairisto, V. and Panteghini, M. (2008). Reference Intervals for Serum Creatinine Concentrations: Assessment of Available Data for Global Application. *Clinical Chemistry*, 54 (3), 559–566.

Cherono, R.J., Role, E. and Owino,J. (2017). The prevalence and risk factors for chronic kidney diseases in Kericho county, Kenya. *Baraton Interdisciplinary Research Journal*, 7, 1 – 10.

Christa, M. C., Henk, B. and Cas, W. W. (2009). Prime time for enzymatic creatinine methods in pediatrics. *Clinical Chemistry*, 55 (3), 549 – 558.

Coresh, J., Astor, B. C., Greene, T., Eknoyan, G. and Levey, A. S. (2003). Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *American Journal of Kidney Diseases,* 41, 1–12.

Creatinine (Enzymatic) Reagent Set - Pointe Scientific, Inc. 24.03.2013 Retrived from https://www.yumpu.com/en/document/view/11522777/creatinine-enzymatic-reagent-set-pointe-scientific-inc, 30th July 2022.

Delanaye, P., Cavalier, E., Cristol, J. and Delanghe, J. R. (2014).Calibration and precision of serum creatinine and plasma cystatin C measurement: impact on the estimation of glomerular filtration rate. *J Nephrol*, 2014, 27, 467.

Delanaya, P., Schaeffner, E., Ebert, N., Cavalier, E., Mariat, C., Krzesinski, J. and Moranne, O. (2012). Normal reference values for glomerular filtration rate: What do we really know? *Nephrol Dial Transplant*, 27, 2664 – 2672.

Delanghe, J. R. (2008). How to Establish Glomerular Filtration Rate in Children. *The Scandinavian Journal of Clinical & Laboratory Investigation,* 68, 46–51. ISSN 0036-5513 print/ISSN 1502-7686 online.

Delanghe, J. R. and Speeckaert, M. M. (2011). Creatinine determination according to Jaffe- what does it stand for? *NDT Plus.* 2011 Apr; 4(2), 83-6. doi: 10.1093/ndtplus/sfq211. Epub 2011 Jan 27. PMID: 25984118; PMCID: PMC4421578.

Drion, I., Cobbaert, C., Groenier, K, H., Weykamp, C., Bilo, H. J. G., Wetzels, J. F. M. and NanneKleefstra, N. (2012). Clinical evaluation of analytical variations in serum creatinine measurements: why laboratories should abandon Jaffe techniques. *Biomed Central Nephrology,* 2012, 13, 133.

Earley, A., Miskulin, D., Lamb, E., Levey, A. and Uhlig, K. (2012). Estimating equations for glomerular filtration rate in the era of creatinine standardization: a systematic review. *Ann Intern Med,* 156(11), 785 – 795.

Encyclopaedia Britannica, Inc. Retrived from https://www.britannica.com/science/nephron, 28th July 2022.

Fiseha, T., Mengesha, T., Girma, R., Kebede, E. and Gebreweld, A. (2019). Estimation of renal function in adult out patients with normal serum creatinine. *BMC Res Notes, 12, 462.*

Gencheva, I. I. and Ruseva, A. L. (2015). Effects of glucose and bilirubin on the kinetic Jaffe's and the enzymatic methods for serum creatinine assay. *J Biomed Clin Res,* 8, 35 – 39.

Gounden, V., Bhatt, H., and Jialal, I. (2021). Renal Function Tests. ID: NBK507821 *PMID*: 29939598.

Hermida, F. J., Lorenzo, M. J., Pérez, A., Fernández, M., Sagastagoia, O. and Magadán, C. (2014).Comparison between ADVIA Chemistry systems Enzymatic Creatinine_2 method and ADVIA Chemistry systems Creatinine method (kinetic Jaffe method) for determining creatinine. *Scandinavian Journal of Clinical & Laboratory Investigation,* May 2014, 1 – 8

ISSN 0036-5513 print/ISSN 1502-7686 online.

Kashani, K., Rosner, M. H. and Ostermann, M. (2019). Creatinine: From physiology to clinical application. *European Journal of Internal Medicine,* 72, 9 – 14. DOI: https://doi.org/10.1016/j.ejim.2019.10.025

Kibaya, R. S., Bautista, C. T., Sawe, F. K., Shaffer, D. N., Sateren, W. B., Scott, P. T., Michael, N. L., Robb, M. L., Birx, D. L. and de Souza, M. S. (2008). Reference Ranges for the Clinical Laboratory Derived from a Rural Population in Kericho, Kenya. *Plos one. www.plosone.org, 3(10), 1 – 8.*

Killeen, A. A., Ashwood, E. R., Ventura, C. B. and Styer, P. (2013). Recent trends in performance and current state of creatinine assays. *Archieves of Pathology Laboratory Medicine,* 137(4), 496 – 502.

Komenda, P., Beaulieu, M., Seccombe, D. and Levin, A. (2008). Regional implementation of creatinine measurement standardization. *Journal of the American Society of Nephrology,* 19(1), 164 – 9.

Küme, T., Sağlam, B., Ergon, C. and Sisman, A. R. (2018). Evaluation and comparison of Abbott Jaffe and enzymatic creatinine methods: Could the old method meet the new requirements? *J Clin Lab Anal,* 2018, 32:e22168.

Levey, A.S., Atkins, R., Coresh, J., Cohen, E.P., Collins, A.J., Eckardt, K.-U, Nahas, M.E., Jaber, B.L., Jadoul, M., Levin, A., Powe, N.R., Rossert, J., Wheeler, D.C., Lameire, N. and Eknoyan, G. (2007). Chronic kidney disease as a global public health problem: Approaches and initiatives – a position statement from kidney disease improving global outcomes. *Kidney International,* 72(3), 247 259.

DOI:https://doi.org/10.1038/sj.ki.5002343.

Marakala, V., Avinash, S. S., Shivashankara, A. R., Malathi, M. and Kumar, A. (2012).Serum Creatinine Assay: Enzymatic vs Kinetic Jaffe's Method. *Journal of Evolution of Medical and Dental Sciences* 2012, 1(4), 328 – 334.

McKillop, D.J., Cairns, B., Ryan, M., Duly, E. and Van Drimmelen, M. (2006). The effect of serum creatinine method choice on estimated glomerular filtration rate determined by the abbreviated MDRD formula. *Annals of Clinical Biochemistry*, 43(3), 220 – 2.

Miller, W.G., Myers, G.L., Ashwood, E.R., Killeen, A.A., Wang, E. and Thienpont, L. M. (2005). Creatinine measurement: state of the art in accuracy and inter-laboratory harmonization. *Archives Pathology Laboratory Medicine*, 129, 297–304.

Murray, I. and Paolini, M, A. (2020). Histology, Kidney and Glomerulus. *PMID*, 32119431.

Mwenda, V., Githuku, J., Gathecha, G., Wambugu, B.M, Roka, Z.G. and Ong'or, W.O. (2019). Prevalence and factors associated with chronic kidney disease among medical inpatients at the Kenyatta National Hospital, Kenya, 2018: a cross – sectional study. *Pan Afri Med J,* 2019, 33, 321.

doi: 10.11604/pamj.2019.33.321.18114.

Myers, G. L., Miller, W.G., Coresh, J., Fleming, J., Greenberg, N., Greene, T., Hostetter, T., Levey, A. S., Panteghini, M., Welch, M. and Eckfeldt, J. H. (2006). Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. *Clinical Chemistry*, 52(1), 5-18.

Nah, H., Lee, S., Lee, K., Won, J., Kim, H. O. and Kim, J. H. (2016). Evaluation of bilirubin interference and accuracy of six creatinine assays compared with isotope dilution–liquid chromatography mass spectrometry. *Clinical Biochemistry* 2016, 49(3), 274-281.

Panteghini, M. (2008). Enzymatic assays for creatinine: time for action. *Scandinavian Journal* Clinical Laboratory Investigations (Supplementary)., 241, 84-8.

Peake, M. and Whiting, M. (2006). Measurement of serum creatinine--current status and future goals. *Clin Biochem Revision,* 27(4), 173-84.

Pokorna, E., Schuck, O. Vitko, S. and Ekberg, H. (2002). Estimated and Measured Donor Creatinine Clearance are Poor Predictors of Long – Term Renal Graft Function and Survival. *American Journal of Transplantation,* 2, 373 – 380. ISSN 1600 – 6135.

Samaneka, W. P., Mandozana, G., Tinago, W., Nhando, N., Mgodi, N. M., Bwakura – Dangarembizi, M. F., Munjoma, M. W., Gomo, Z. A. R., Chirenje, Z. M. and Hakim, J. G. (2016). Adult hematology and clinical chemistry laboratory reference ranges in a Zimbabwean population. *PLoS One,* 11(11), e0165821.

Saraladevi, N. (2009). End stage renal disease in sub-Saharan Africa. Ethnicity and disease, *Spring,* 19, 13-15.

Schmidt, R. L., Straseski, J. A., Raphael, K. L., Adams, A. H. and Lehman, C. M. (2015). A Risk Assessment of the Jaffe vs Enzymatic Method for Creatinine Measurement in an Outpatient Population. PLoS ONE, 10(11), e0143205. Doi: 10.1371/journal. pone.0143205.

Vaishya, R., Arora, S., Singh, B. and Mallika, V. (2010). Modification of Jaffe's Kinetic Method Decreases Bilirubin Interference: A Preliminary Report. *Indian Journal of Clinical Biochemistry*, 2010, 25 (1), 64-66.

Waithaka, S. K., Njagi, E., Ngeranwa, J., Gatua, W. and Muturi, M. (2010). Reference ranges for creatinine related parameters for adult Kenyan population. *Asian journal of medical sciences*, 15(2), 234-240.

Wikipedia, the free encyclopedia. (2022). Jaffe reaction.

Retrived from https://en.wikipedia.org/wiki/Jaffe_reaction,

28th July 2022.

Zatko, T., Kolena, B., Petrovicova, I., Pilka, T. and Kolacan, A. (2014). Use of selected prediction equations (CG, MDRD4, CKD-EPI) in improving glomerular filtration rate assessment in clinical practice in Slovakia. *Cent Eur J Public Health*, 22 (1), 34–41.

Zitta, S., Schrabmair, W., Reibnegger, G., Meinitzer, A., Wagner, D., Estelberger, W. and Rosenkranz, A. (2013). Glomerular filtration rate (GFR) determination via individual kinetics of the inulin-like polyfructosan sinistrin versus creatinine-based population-derived regression formulae. *BMC Nephrol,* 14, 159.

APPENDICES

Appendix I: Ethical Approval letter

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/405 Link:www.uonbi.ac.ke/activities/KNHUoN 19th December 2013

Dr. Stanley Kinge Waithaka
Laboratory Medicine
Kenyatta N. Hospital

Dear Dr. Waithaka

RESEARCH PROPOSAL: EVALUATION OF ENZYMATIC (SARCOSINE OXIDASE) CREATININE METHOD AND COMPARISON WITH MODIFIED KINETIC JAFFES REACTION ANALYTICAL METHOD FOR QUANTITATIVE ANALYSIS OF CREATININE (P459/08/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 19th December 2013 to 18th December 2014.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

"Protect to Discover"

Appendix II: Publication

Asian Journal of Medical Sciences 8(1): 1-4, 2016
DOI:10.19026/ajms.8.2858
ISSN: 2040-8765; e-ISSN: 2040-8773

Submitted: June 24, 2015 Accepted: August 2, 2015 Published: March 25, 2016

Research Article

Interpretation Tool for Creatinine Related Parameters Established using Enzymatic Sarcosine Oxidase Quantitative Analytical Method

[1]Swaleh B. Kula, [2]Stanley K. Waithaka, [1]Joseph Gikunju and [3]Leonard J. Njagi
[1]Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya
[2]Mount Kenya University, Thika, Kenya
[3]Kenyatta National Hospital, Nairobi, Kenya

Abstract: The purpose of the study was to establish the reference ranges of serum creatinine and estimated creatinine clearance for adult Kenyan population using enzymatic sarcosine oxidase quantitative analytical method. A prospective study carried out in clinical chemistry laboratory of Kenyatta National Hospital involving 631 individuals between 18-61 years. Results for 316 study subjects (158 renal patients and 158 healthy subjects) were used to compare the difference between enzymatic sarcosine oxidase and modified jaffes reaction methods. Comparison of the two methods showed statistically significant difference (renal patient: $p = 0.024$, healthy subjects: $p = 0.038$). Parametric methods were used to construct reference ranges by estimating 2.5 and 97.5 percentiles of distribution as lower and upper reference limits. Results for 462 blood donor study subjects comprising of 249 male and 213 females were used for the establishment of reference ranges of:- serum creatinine and estimated creatinine clearance. Serum creatinine and estimated creatinine clearance reference ranges established were as follows:- serum creatinine {male: (41-109) µmol/L, female: (32-92) µmol/L} and estimated creatinine clearance {male: (36-98) ml/min, female: (31-81) ml/min}. The current study has established adult Kenyan reference ranges for serum creatinine and estimated creatinine clearance using enzymatic sarcosine oxidase analytical method. Enzymatic sarcosine oxidase method produced low creatinine concentration than the modified jaffes reaction method for the studied parameters.

Keywords: Adult Kenyan, estimated creatinine clearance, Kenyatta National Hospital, reference range

INTRODUCTION

Reliable creatinine measurements in both serum and plasma which are used in Glomerular Filtration Rate (GFR) estimation are critical to ongoing global public health efforts to increase the diagnosis and treatment of Chronic Kidney Disease (CKD). Understanding by laboratorians worldwide of the importance of reliable serum creatinine measurements in GFR estimation and of factors that may affect creatinine measurement have been widely emphasized (Arogundade and Barsoum, 2008). The methods most widely used to measure serum/plasma and urine creatinine are alkaline picrate methods, enzymatic or partially enzymatic assays and HPLC methods. Isotope-Dilution Mass Spectrometry (IDMS) high-order reference methods have been developed for assignment of reference materials but are available in only a few highly specialized laboratories worldwide. Today, the Jaffe reaction using alkaline picrate remains the cornerstone of most current routine methods, after continuous refinements attempting to overcome inherent analytical interferences and limitations (Miller *et al*., 2005). The kinetic method decreases interference caused by Jaffe-reactive pseudo-creatinine of non-creatinine chromogens compared with earlier protocols. Other methods have been used to improve the specificity of the Jaffe reaction, but they are not suitable in automated procedures. Coupled enzymatic reactions have been developed which reduce or eliminate problems with most interfering substances that the cornerstone method has not successively eliminated. Inorganic chemical–based methods that have been developed as alternatives to the alkaline picrate methods have not been widely implemented clinically because they have not demonstrated improved performance compared with the various adaptations of the Jaffe method. The only alternative methods that have been widely adopted for routine clinical laboratory use are enzymatic creatinine methods specifically the sarcosine-oxidase method.

Although the enzymatic methods have been reported to have generally less interference than the

Corresponding Author: Swaleh B. Kula, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200 Nairobi, Kenya

Jaffe methods, there have been reports of various substances that do interfere (Myers *et al*., 2006).

Cockcroft and Gault and Modification of Diet in Renal Disease (MDRD) formulas have been used to estimate glomerular filtration rate of the kidney. The creatinine value used in either of these equations is determined using the routinely used creatinine determination methods either kinetic jaffes or enzymatic method. Because no systematic differences between serum and plasma measurements have been reported, serum and plasma results are considered as equivalent, (Peake and Whiting, 2006). There is now ongoing activity to promote world-wide standardization of methods to measure creatinine concentrations, together with the introduction of a revised estimated glomerular filtration rate equation appropriate for use with standardized creatinine methods (Panteghini, 2008).

The current study was undertaken using modified kinetic Jaffes reaction and enzymatic sarcosine creatinine determination methods. Sarcosine oxidase enzymatic method was evaluated since it is to be used for the first time in clinical chemistry laboratory of Kenyatta National Hospital. The current study was to compare the two creatinine analytical methods and established reference ranges for serum creatinine and estimated creatinine clearance based on enzymatic sarcosine oxidase method. The Kenyatta National Hospital Research and Ethical Committee approved the study (P459/08/2013).

MATERIALS AND METHODS

Study site: The study was conducted in the department of laboratory medicine, clinical chemistry laboratory of Kenyatta National Hospital.

Study period: May-November 2014.

Study Population: Six hundred and thirty one individuals comprising of 328 males and 303 females between the 18-61years were recruited into the study. Four hundred and seventy three were healthy blood donors while the remaining 158 were renal patients. A questioner was administered to consenting study subjects (blood donors) to gather some socio-demographic data.

Specimen analysis: Three milliliters of blood was collected from each study subject. Serum was separated by centrifugation at 3000 g for 3 min. Studied creatinine levels were determined by modified jaffes reaction and enzymatic sarcosine oxidase methods using autoanalyzer Mindray 800 machine. Specific assayed normal and pathological sera were used for the quality control of analytical work during study period. The following formula was used for the determination of estimated creatinine clearance (Ecrcl):

Ecrcl = {(140-age) x weight (kg) x constant (male = 1.23, female = 1.04)} divide by Serum Creatinine (µmol/L)

The weight of each study subject was taken by weighing using a weigh balance.

RESULTS

The male study participants had a mean age and weight of 33 years and 68kgs respectively. On the other hand the female study subjects had a mean age and weight of 31 years and 67 kgs. Internal quality control report for both normal and pathological control sera were within the specific assigned QC range of target value±2 standard deviations as shown in Table 1.

Serum creatinine quantitative results for 316 study subjects (158 renal patients and 158 healthy subjects) were used to compare the difference between enzymatic sarcosine oxidase and modified jaffes reaction methods. Creatinine results for the renal patients using enzymatic sarcosine oxidase and modified jaffes reaction methods had a mean of 285 and 317 µmol/L, respectively. Paired means comparison test for the two methods showed statistically significant differences between the two creatinine quantitative methods ($p<0.024$) as shown in Table 2. Creatinine results for the healthy subjects using enzymatic sarcosine oxidase and modified jaffes reaction methods had a mean of 69µmol/L and 80µmol/L respectively. Paired means comparison test for the two methods showed statistically significant differences between the two creatinine quantitative methods ($p<0.038$) as shown in Table 2.

Paired means comparison test using results of equal number of male (n = 213) and female (n = 213) healthy

Table 1: Internal quality control report of the studied parameter

			Assigned QC report			Study QC report		
Method	Parameter	QC type	Session	Range	Mean	Mean	Sd	CV%
Jaffes reaction	Scr	n	25	81-135	108	105	4.5	4.2
		p	25	132-260	196	200	15	7.5
Sarcosine	Scr	n	67	56-98	77	79	7	8.9
Oxidase		p	25	67-133	100	102	5.1	5

Scr: Serum creatinine; QC: Quality control; N: normal control sera; P: Pathological control sera; SD: Standard deviation; CV: Coeficient of variation

Table 2: Comparison between enzymatic sarcosine oxidase and modified jaffes reaction methods for quantitative analysis of serum creatinine

Study subjects	N	Method	Mean	MD	S.E	95 C.I		*Sig.
Renal patients	158	Enzymatic sarcosine oxidase	285	32	5.9	L	U	0.024
		Modified jaffes reaction	317			20	44	
Healthy subjects	158	Enzymatic sarcosine oxidase	69	10	0.5	10	12	0.038
		Modified jaffes reaction	80					

*The mean difference significance at $p<0.05$ level; N: Number; MD: Mean difference; S.E: standard error; CI: Confidence interval

Table 3: Established reference ranges for serum creatinine and estimated creatinine clearance for studied Kenyan population using enzymatic sarcosine oxidase method

Parameter	Gender	n	Mean	MD	Mean±1.96SD	Rr	*Sig
Scr (µmol/L)	Male	249	75	13	75±34	41-109	0.014
	Female	213	62		62±30	32-92	
Ecrcl (ml/min)	Male	249	67	11	67±31	36-98	0.018
	Female	213	56		56±25	31-81	

Nb/Scr: Serum creatinine; Ecrcl: Estimated creatinine clearance; n: number; MD: Mean difference; Rr: Reference range; *Mean difference significance at $p<0.05$ level

Table 4: Comparision of enzymatic sarcosine oxidase and modified jaffes reaction method established reference ranges for creatinine related parameters for the Kenyan population

Parameter	Method	Sex	Number	Rr
Scr	Enzymatic sarcosine oxidase	Male	249	41-109
	Modified jaffes reaction	Male	106	68-128
	Enzymatic sarcosine oxidase	Female	213	32-92
	Modified jaffes reaction	Female	159	60-122
Ecrcl	Enzymatic sarcosine oxidase	Male	249	36-98
	Modified jaffes reaction	Male	106	54-118
	Enzymatic sarcosine oxidase	Female	213	31-81
	Modified jaffes reaction	Female	159	58-106

Scr: Serum creatinine; Ecrcl: Estimated creatinine clearance; Rr: Reference range

subjects showed gender difference which was statistically significant for serum creatinine ($p = 0.014$) and estimated creatinine clearance ($p = 0.018$). Therefore gender based reference ranges for serum creatinine and estimated creatinine clearance using enzymatic sarcosine oxidase creatinine analytical method were constructed as shown in Table 3.

Creatinine related reference ranges established in the current study were compared with reference ranges of creatinine related parameters established using modified jaffes reaction method for a similar Kenyan population. The results were as shown in Table 4.

DISCUSION

Determination of creatinine in blood or urine specimen has been achieved by using either modified jaffes reaction or the enzymatic sarcosine oxidase methods. Clinical chemistry laboratory of Kenyatta National Hospital has been using the modified jaffes reaction method until recently when enzymatic sarcosine oxidase was introduced. It is a good laboratory practice to evaluate any new method being introduced for analytical purposes in a diagnostic laboratory in order to determine its critical reliability characteristics. The current study undertook the responsibility of establishing any analytical differences between these two creatinine analytical methods. Serum creatinine results of three hundred and sixteen study subjects which comprised of equal number of healthy individuals and renal patients were used for the comparison of the two creatinine analytical methods. The purpose of using creatinine of renal subjects was to express any analytical differences in analyzing elevated creatinine levels. On the other hand the use of healthy subjects was to express any differences in the analysis of normal creatinine levels. The study has established that when the two methods are used to determine the creatinine of the same individual, enzymatic sarcosine oxidase produces lower readings than the modified jaffes reaction method. The difference between the two methods was found to be statistically significant (renal patients: $p = 0.024$, healthy subjects: 0.038). Expression of this level of analytical differences requires the two methods to be considered independently as far as the interpretation of creatinine results is concerned. This expressed creatinine analytical methods difference suggests that the two methods cannot be interchangeably used. A similar study by Morimatsu *et al*. (2003), onion selective electrode and calorimetric methods of electrolytes estimation concurred with the current study findings.

The study established the interpretational tool for serum creatinine and estimated creatinine clearance reports produced using enzymatic sarcosine oxidase method. Gender differences were shown in serum creatinine ($p = 0.014$) and estimated creatinine clearance ($p = 0.018$). Therefore, specific male and

female reference ranges were established as shown in Table 3. A study by Waithaka *et al*. (2010), on a similar Kenyan study population established reference ranges for creatinine related parameters using modified jaffes reaction method. The information derived from the different reference ranges for the same parameters established using the two creatinine analytical methods indicates that the interpretation should be based on the specific reference ranges to avoid misdiagnosis of renal disorders. For example, a male serum creatinine test result of 125µmol/L produced by enzymatic sarcosine oxidase method and interpreted using jaffes reaction creatinine reference range would suggest that the client is healthy despite renal impairement clinical impression. To avoid misdiagnosis of renal disorders, it is important to interpret the results using creatinine reference ranges for the particular creatinine analytical method used.

CONCLUSION

The current study has established adult Kenyan reference ranges for serum creatinine and estimated creatinine clearance using enzymatic sarcosine oxidase analytical method. The established reference ranges are different from those of modified jaffes reaction method for the same studied adult Kenyan population. Enzymatic sarcosine oxidase method produced lower creatinine concentration than the modified jaffes reaction method for the studied parameters. Creatinine gender difference where males have higher concentration than females was evident in the current study. The two creatinine analytical methods can be used concurrently in the analysis of creatinine quoting specific reference range of the method used.

RECOMMENDATION

The established reference ranges can be used in the Kenyan health institution as opposed to literature based reference ranges. Authors recommend the establishment of reference ranges for other creatinine related parameters e.g., measured creatinine clearance not included in this study. Similar study is recommended to be carried out on children.

ACKNOWLEDGMENT

The authors would like to thank the study participants for their cooperation in the conduct of this study. The analytical equipments were provided by Kenyatta National Hospital (KNH). We thank the management of KNH for facilitating this study.

REFERENCES

Arogundade, F.A. and R.S. Barsoum, 2008. CKD prevention in sub-Saharan Africa: A call for governmental, nongovernmental and community support. Am. J. Kidney Dis., 51: 515-523.

Miller, W.G., G.L. Myers, E.R. Ashwood, A.A. Killeen, E. Wang and L.M. Thienpont, 2005. Creatinine measurement: State of the art in accuracy and inter-laboratory harmonization. Arch. Pathol. Lab Med., 129: 297-304.

Myers, G.L., W.G. Miller, J. Coresh, J. Fleming, N. Greenberg, T. Greene and T. Hostetter, A. S. Levey, M. Panteghini, M. Welch and J. H. Eckfeldt, 2006. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clinical Chemistry, 52(1): 5-18.

Morimatsu, H., J. Rocktaeschel, S. Uchino and R. Bellomo, 2003. Unmeasured anions in critically ill patients: Can they predict mortality? Critical Care Medicine 31(8): 2131-2136.

Panteghini, M., 2008. Enzymatic assays for creatinine: Time for action. Scand. J. Clin. Lab. Invest. Suppl., 241: 84-8.

Peake, M. and M. Whiting, 2006. Measurement of serum creatinine. Current status and future goals. Clin. Biochem. Rev., 27(4): 173-84.

Waithaka, S.K., E.N. Njagi, J.N. Ngeranwa, B.M. Chiuri, J.N. Njagi, W.K. Gatua and D.M. Muturi, 2010. Glomerular filtration rate assessment using creatinine related parameters for healthy adult Kenyan population. Asian J. Med. Sci., 2(3): 141-145.

Appendix III: Questionnaire

Title of the Study:

"Evaluation of enzymatic sarcosine oxidase method and comparison with modified kinetic jaffe's reaction analytical method for quantitative analysis of creatinine".

In respect to the above study, kindly answer this questionnaire by filling in where appropriate.

Study Number …… (DATE)…….

Name (optional)……..

Gender (tick): Male/Female

Age: …… (Years)

Weight ……. (Kg)

Height…… (Metre)

BMI…… (Kg/M^2)

Blood pressure…….. (Mm/Hg)

Occupation……..

Country……..

Health status for the last six months (tick): WELL/UNWELL

(If unwell, please state the nature of your illness in the space provided below)

……

……

Are you on any medication (tick): YES/NO

If YES, please specify: …….

Signature / Thumb print……..

Appendix IV: Consent form

Title of the Study:

"Evaluation of enzymatic sarcosine oxidase method and comparison with modified kinetic jaffe's reaction analytical method for quantitative analysis of creatinine".

The principal investigator, Mr. Swaleh Bakari Kula, hereby requests your participation in the above mentioned proposed study. Therefore you are requested to grant permission for the use of your blood specimens for the purposes of the proposed study. Please sign the consent statement below as an indication that you have granted permission for your specimens to be used for the proposed study.

I…… (Study number), have given consent for my blood specimen to be used ONLY for the proposed study.

Signature / Thumb Print……..

Date…….

Appendix V: Sampling Method

Total sample size of 384

The sample size was 384 participants recruited from three units which were blood transfusion (donor), renal and liver disordered units of the study site, Kenyatta National Hospital.

Procedure

The study site was visited prior to participants' recruitment to study the number of patients admitted or handled in each unit/ward as per the medical records per month. This was to be used to calculate the number of participants to be drawn from each unit to make the final study sample size. The average number of donors per month in the blood transfusion unit was 600 donors, the average number of patients in the renal unit was 300 patients and the average number of patients in the liver disordered unit was 150 patients and this is according to the medical records from those respective wards.

The ratio of the average numbers of patients from the three units/wards was calculated as:-

Donors unit	:	Renal unit	:	Liver unit
600	:	300	:	150
4	:	2	:	1

Therefore, the ratio of donors to renal to liver units was 4: 2: 1. To arrive to the sample size of 384 from the three units, this ratio was used and the following ratio of respective samples was achieved:-

213	:	104	:	67

Then, the systematic random sampling method was used to recruit all the required participants from each unit.

The formula, k=N/n

Where:-

k = Sampling interval

N = Average number of patients per month in each unit

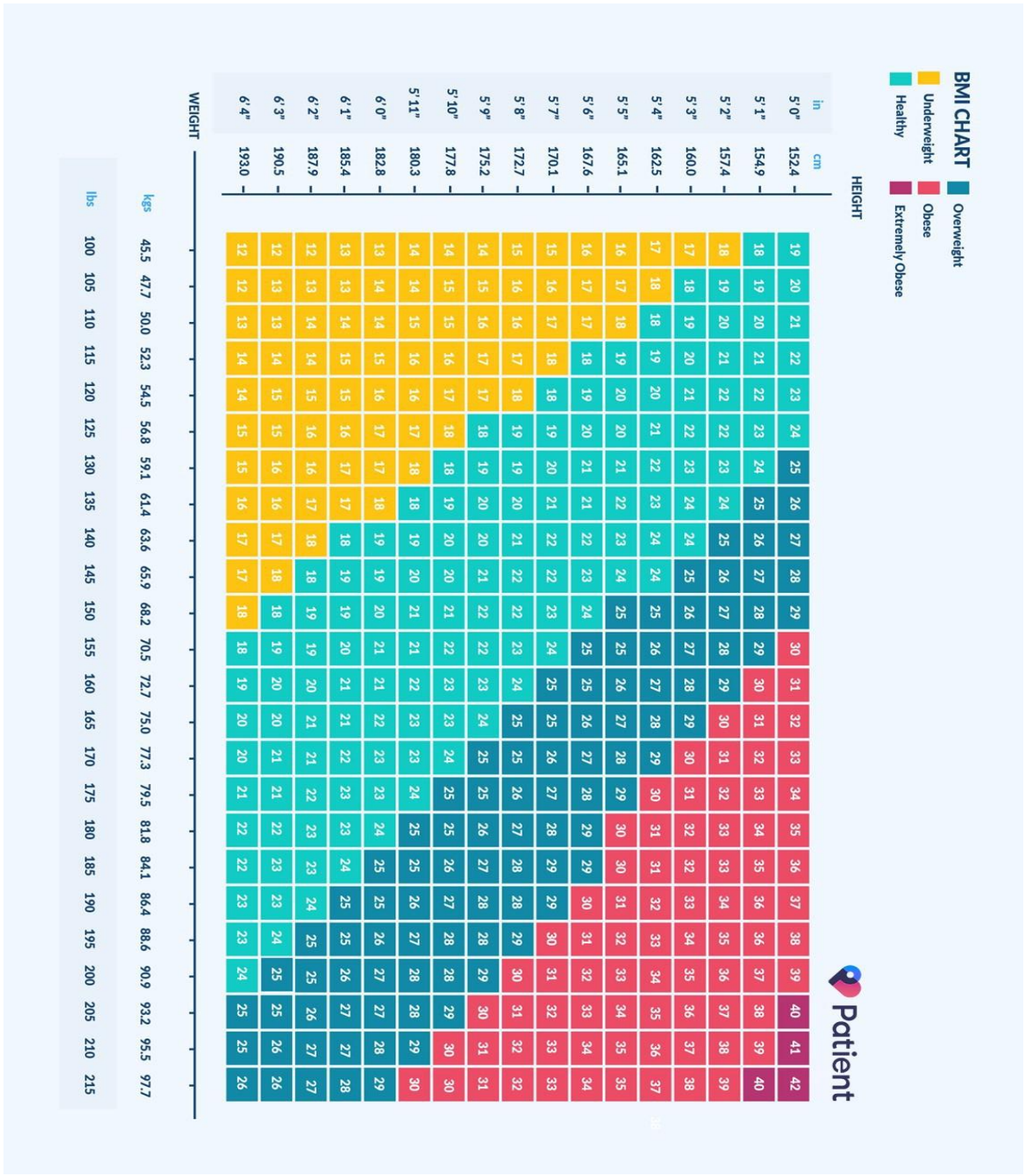
n = The desired sample size

Was used to calculate the interval for systematic sampling. At every beginning of the day, the first participant was picked randomly from the unit medical register, then the sample interval was applied to pick the next participant (k^{th}) until the desired samples from each unit was achieved. At the end, when adding all the recruited samples from the three units, the total sample size of 384 was achieved.

232 samples for comparison of enzymatic sarcosine oxidase and modified kinetic Jaffe's methods.

These samples were drawn from already randomly recruited heaithy donors and renal patients. As the ratio of donors to renal patients was 4:2, calculation gave 156 donors and 76 renal patients. The first participant was picked randomly from the donors' group, then the sample interval was applied to pick the next participant (k^{th}) until the desired sample of 156 was achieved. The same procedure was used to pick 76 renal patients. At the end, when adding all the recruited samples from the two groups, the total sample size of 232 was achieved.

Appendix VI: Body Mass Index Chart



https://patient.info/doctor/bmi-calculator-calculator

Appendix VII: Raw data for precision performance on modes of analyses

S/No.	A	B	C	D	E	F
1	30	29	29	4769	4769	4768
2	31	30	31	4765	4769	4769
3	31	31	30	4768	4769	4771
4	31	30	31	4769	4768	4771
5	30	31	31	4767	4771	4771
6	32	29	29	4768	4769	4772
7	29	32	29	4769	4768	4771
8	31	33	30	4769	4770	4772
9	30	31	33	4770	4769	4773
10	32	30	30	4769	4768	4770
11	31	29	32	4768	4771	4771
12	33	29	31	4769	4769	4770
13	30	31	33	4780	4768	4771
14	30	33	29	4781	4768	4770
15	32	30	32	4769	4769	4769
16	30	31	30	4768	4768	4768
17	31	33	29	4768	4770	4771
18	31	32	31	4769	4772	4771
19	30	30	32	4769	4772	4771
20	33	29	30	4769	4768	4770

KEY: (Modes of analyses)

A = Within Run, Low Serum Creatinine Concentration (30 µmol/L)

B = Between Run, Low Serum Creatinine Concentration

C = Between Day, Low Serum Creatinine Concentration

D = Within Run, High Serum Creatinine Concentration (4765 µmol/L)

E = Between Run, High Serum Creatinine Concentration

F = Between Day, High Serum Creatinine Concentration

Appendix VIII: Raw data for the values of serum bilirubin and serum creatinine concentrations (µmol/L)

S/No.	A	B	C	S/No.	A	B	C
1	45	34	58	**31**	68	35	39
2	112	69	48	**32**	102	96	62
3	98	58	41	**33**	258	114	47
4	156	112	35	**34**	301	69	40
5	211	98	58	**35**	214	110	36
6	235	78	52	**36**	98	68	35
7	189	65	41	**37**	102	59	24
8	236	102	41	**38**	114	79	34
9	358	98	41	**39**	369	107	35
10	405	115	36	**40**	258	89	26
11	211	39	10	**41**	123	56	36
12	299	87	56	**42**	147	56	40
13	100	104	36	**43**	258	75	30

14	98	56	51
15	302	95	64
16	288	102	38
17	568	114	25
18	489	96	21
19	569	98	24
20	462	95	32
21	268	114	36
22	112	59	50
23	369	78	34
24	458	100	47
25	987	120	20
26	478	97	31
27	320	112	39
28	129	56	41
29	98	98	25
30	57	101	59

44	336	59	33
45	685	68	29
46	745	56	18
47	456	85	25
48	321	69	39
49	589	74	20
50	958	65	15
51	568	69	28
52	789	74	18
53	158	69	56
54	369	87	40
55	147	69	65
56	258	74	58
57	456	65	34
58	897	48	19
59	124	68	60
60	65	56	74
61	103	65	54
62	147	74	59
63	569	52	38
64	456	64	40

65	257	56	39
66	136	66	50
67	109	75	64

KEY:

A = Serum Bilirubin Concentration (µmol/L)

B = Serum Creatinine Concentration by Enzymatic Sarcosine Oxidase Method

C = Serum Creatinine Concentration by Modified kinetic Jaffe's Method