COMPARISON OF MEASURED GFR BY TC-99M-DTPA WITH ESTIMATED GFR IN POTENTIAL KIDNEY DONORS AT KENYATTA NATIONAL HOSPITAL

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE FELLOWSHIP IN CLINICAL NEPHROLOGY OF THE UNIVERSITY OF NAIROBI

December 2018
DECLARATIONS

This proposal is my original work and has not been presented for any award in any other university.

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This proposal has been presented with my full approval as supervisor.
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This proposal has been presented with my full approval as supervisor.

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Department of Medicine

Kenyatta National Hospital
DEDICATION

I dedicate this book to my dear friend and wife, Wambui, who has tirelessly and selflessly stood with me and supported me throughout my Nephrology Fellowship training and during this study;

And to Joel, Caleb and Charity; our Triplets; who frequently endured the absence of their dad, yet inspired me to finish up quickly to finally get time to ride a bike with them.
ACKNOWLEDGMENTS

My acknowledgment first goes to God Almighty for giving me life, unceasing strength and hope as I laboured through the programme and the study;

To Prof. Kayima, Prof. McLigeyo, Dr.Were and Dr.Ngigi: My dedicated and insightful supervisors who have patiently guided me throughout the study;

To my teachers who have consistently taught me the principles of Nephrology; And held my hand in this delicate hands on specialisation;

To Dr.Kagereki, my statistician;

To Nancy Wang’ombe for great assistance at the transplant clinic;

To Florence and other records officers for helping trace the files for the participants;

To my colleagues for having made this journey of learning interesting.
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<tr>
<td>APOL1</td>
<td>Apoliporotein 1</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>Ccr</td>
<td>Creatinine clearance</td>
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<td>CG</td>
<td>Cockcroft–Gault</td>
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<td>CKD</td>
<td>Chronic Kidney Disease</td>
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<td>CKD-EPI</td>
<td>Chronic Kidney Disease Epidemiology Collaboration</td>
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<td>CRP</td>
<td>C Reactive Protein</td>
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<td>DTPA</td>
<td>Diethylenetriaminepentaacetic acid</td>
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<td>EDTA</td>
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<td>estimated Glomerular Filtration Rate</td>
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<td>Hispanic Health and Nutrition Examination Survey</td>
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<td>Kilodalton</td>
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<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Disease</td>
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<td>mGFR</td>
<td>Measured GFR</td>
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<tr>
<td>Tc-99m</td>
<td>Technetium 99m</td>
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ABSTRACT

Background: Glomerular filtration rate (GFR) is key in assessing the renal function of an individual. GFR cannot be measured directly, but as urinary clearance of an ideal filtration marker. Estimated GFR is vital in detection, evaluation and management of kidney disease. While there are many methods and substances of measuring GFR, nuclear imaging through DTPA is considered to be accurate and more convenient compared with other methods. There is no local study outlining the measured GFR of the kidney donors and there is no local study comparing the variation of DTPA GFR with other GFR estimating formulae like Cockcroft-Gault, CKD- EPI or MDRD.

Objective: The aim of the study was to determine the measured GFR by DTPA of the potential kidney donors and compare the measured GFR with three GFR estimating methods among potential kidney donors at KNH.

Study design: Retrospective descriptive study.

Methodology: The Study population included all potential live kidney donors, aged eighteen years and above, from 2010 who had pre donation work up DTPA done. The participants serum creatinine was obtained from the file and eGFR calculated through the three formulae. This eGFR was then compared with the DTPA mGFR.

Data analysis and presentation: Data analysis was done using the Statistical Package for Social Science (SPSS) version 20.0. For continuous variables like age, mean, mode, median, range and standard deviations were calculated. Frequencies of categorical variables e.g. sex were calculated. The Bland-Altman method was used to evaluate the agreement between the measured GFR and the estimated GFR for each of the equations. Data is presented as graphs pie-charts and tables.

Results: A total of 130 records were retrieved from the renal and central filing area. Eight had missing data and were therefore excluded from the study. A total of 122 records were reviewed and included. Seventy (57.4%) were male. The average age was 33.9yrs ± 9.2 with a range of 19-58 years. Almost 50% of all donations were from siblings. Forty percent of the right kidneys had a higher GFR than the left. Conversely 60% of the left kidneys had a higher GFR than the right kidneys. The median GFR in the right was 48.67ml/min/1.73m² and the left was 49.2 ml/min/1.73m². The means of estimated GFR by the 3 equations were within 10% of mGFR, but variation was marked. Seventy eight (86%) of the nephrectomies were left sided. Out of these, 44(48.9%) were kidneys that had less GFR compared to the contralateral kidney.

Conclusion: Most of the donors were young, male and siblings to the recipients. A hundred percent of the donors met the international recommended mGFR of 80 ml/min/1.73m². The mean and median of split renal function was comparable. The online donor GFR calculator has a potential role in transplantation though needs more validation.
CHAPTER ONE
GLOMERULAR FILTRATION RATE MEASUREMENT

1.1 Introduction and background information
Glomerular filtration rate (GFR) is very important in evaluating kidney function in an individual. It is key in detection, assessment and treatment of kidney illnesses. It is determined by several factors including sex, body size and age. In young men its about 130 ml/min/1.73 m^2 and in young women it is 120 ml/min/1.73 m^2 (1). GFR cannot be directly measured. It can only be through urine clearance of an indefectible filtration substance. The substance can be either endogenous or exogenous(1)(2).

Estimated GFR has many potential applications including, assessing response to treatment, unmasking of CKD in risky populations, grading the severity of CKD, evaluating complications of CKD, informing risks of potentially nephrotoxic agents, renal adjustments of agents and drugs excreted by glomerular filtration and qualification of living donors(3).

1.2 Endogenous filtration markers
Endogenous filtration markers are used by calculating timed urine sample and a single serum concentration. While 24 hour urinary creatinine clearance is inexpensive, it is inaccurate and inconvenient. To overcome the challenge of inaccurate twenty four hour urinary measurements, shorter durations of urine collection have been suggested(4). Pickering et al (n=484) observed that 4 hour creatinine clearance was more accurate than plasma creatinine in monitoring kidney function. The magnitude of the GFR is usually inverse to the measure of an endogenous substance. The endogenous marker can therefore project the magnitude of GFR with no urine acquisition. Unfortunately , blood amounts of endogenous filtration substances can also be influenced by elements like generation, extra renal elimination, tubular reabsorption and tubular secretion (5). These are called non GFR factors. Endogenous GFR markers include creatinine, urea and cystacin C.

Creatinine is a 113D amino acid, a breakdown product from muscle. It’s easily undergoes glomerular filtration. At the proximal tubular cell, creatinine undergoes tubular secretion into the tubular fluid, therefore, urinary clearance exceeds GFR(1).Tubular secretion has both intra and inter-individual variability. The generation of creatinine depend on muscle mass and
dietary intake. Inhibition of tubular secretion increases the serum creatinine level without affecting GFR. Drugs including cimetidine and trimethoprim inhibit secretion(6). At reduced GFR, extra renal creatinine elimination increase through intestinal flora degradation(7, 8).

A second glomerular filtration marker is Cystatin C. It is synthesized from nucleated cells. All nucleated cells make this protein. It’s a 13 KDa protein that easily undergoes glomerular filtration. It then undergoes proximal tubular reabsorption, where it undergoes epithelial catabolism. Only very small amounts of cystatin C undergo urinary excretion, hence its clearance in the urine is not measurable. Its generation has less inter-individual variability in comparison to creatinine. It’s produced at a regular rate and its amount has been shown to be consistently reciprocal to the GFR. Separately, Dharmidharka et al still supported this fact in a meta-analysis(9, 10). While Inter-individual variation of creatinine biological activity is 93%, its only 25% for cystatin C. Plasma cystatin C rises early compared with plasma creatinine hence the potential to be a better biomarker in early renal disease detection(11).

While there are reports suggesting that cystatin C could replace creatinine, recent studies show substantial variability between its values and GFR. Lesley et al (n=3418) found cystatin C to be influenced by diabetes, measures of body size, and inflammation(12). Knight et al in Netherlands, in a study with 8058 participants found that cystatin C is associated with males, older, heavier, taller, current cigarette smokers and higher serum CRP levels (13). Estimated GFR formulae using cystatin C have been developed. They include Larsson, Hoek and Filler methods(14).

Creatinine based GFR estimating equations can be combined cystatin C based equations to estimate the GFR. These combined formulae have been reported to be superior to using either of the equations. Wang et al in a Chinese study (n=376), using DTPA as the standard, showed that compared with the MDRD and a cystatin based equation, combining plasma creatinine and cystatin C improved the accuracy of the estimated GFR (2).
1.3 Exogenous filtration markers

Exogenous GFR markers include inulin, diethylenetriaminepentaacetic acid (DTPA), iothalamate, ethylenediaminetetraacetic acid (EDTA) and iohexol(1). Measurement of GFR with exogenous markers is complex, costly, lengthy and therefore not utilised routinely in clinical practice(14)(15).

Inulin clearance is the benchmark for quantification of GFR(1). It undergoes full glomerular filtration. It undergoes no metabolism, synthesis, reabsorption nor secretion. Therefore, the quantity of glomerular filtration is equivalent to urinary excreted inulin. The downside of inulin is being expensive, complex to assay, requires continuous infusion and multiple blood samples(16).

Diethylenetriaminepentaacetic acid (DTPA) is the most frequently used agent used for GFR calculation. It’s the least expensive radiopharmaceutical. Its extraction fraction is 20% therefore less useful with worsening renal function. For the renal scintigraphy it has to be Technetium 99m labelled(17). Twenty four hour urinary creatinine clearance has been shown to correspond well with inulin clearance and also DTPA (18). In 1982, Gates et al, showed a strong correlation between DTPA and 24 hour urinary clearance among 31 hospitalised patients (19). In 2003, Itoh also proved Tc-99m-DTPA renography predicted creatinine clearance (20).

Maioli et al compared DTPA with CG, MDRD and CKD EPI formulae and observed that in participants less than seventy years old, no significant dissimilarity was obtained between GFR assessed by DTPA and GFR assessed by the other methodology. In participants above seventy years, GFR measured using CG formula had no statistically difference with the DTPA GFR, however, creatinine only use in CKD-EPI and MDRD over approximates the GFR(21).

As opposed to serum creatinine that is used to approximate the GFR. Measured GFR by the DTPA gives the actual measurement. It’s through this measured GFR that other GFR estimating methods i.e. Cockcroft Gault, CKD EPI and MDRD are compared. The study therefore evaluates the validity of eGFR based on various equations when compared with measured GFR.
CHAPTER TWO

GLOMERULAR FILTRATION RATE ESTIMATION

2.1 Serum creatinine as a marker
Creatinine is the commonest used endogenous GFR marker. Use of creatinine alone has various limitations including factors affecting its generation and elimination. Factors influencing creatinine levels include age, gender, race, body habitus e.g. muscular, amputation, chronic illness, malnutrition, inflammation, reconditioning, neuromuscular diseases, and diet whether it has meat or not. The use of body size, sex, age and race in addition to serum creatinine as proxy for muscle mass reduces the limitations of use of creatinine alone as a marker of GFR. Pérez-Stable et al carried a study from HHANES to analyze creatinine levels in the serum among Mexican and Cuban Americans and Puerto Ricans. The creatinine measurements differed significantly among Latino subgroups, suggesting a role of nationality in renal disease evaluation(22).

There is no GFR estimating equation that can prevail over the constraints of creatinine in GFR evaluation, despite considerable growth in the exactness of creatinine based estimating equations. The equations cannot work well in subjects with unusual amounts of creatinine synthesis, such as huge or minute individuals, subjects who have undergone amputation, subjects with muscle diseases, or people with significantly higher or reduced levels of meat intake(1). The approximating equations provide improvement on the creatinine by adding clinical and social variables as proxies for the untested factors other than GFR that affect the serum creatinine levels(2)(23).

2.2 Estimation equations
They are usually derived by the usage of regression methods that relate the observed amount of serum substance and the actual measured GFR. Most GFR estimating equations have been generated mainly in subjects with reduced GFR and with CKD. An equation generated in particular inhabitants’ needs to be tested again if intended to be utilised in a different population to prove the generalizability. (24)(25)(26).

There are at least 25 creatinine based equations to estimate GFR. However most are not in common use due to complexity of the formulae, lack of validation in different populations,
underestimation or overestimation of the GFR among others. The commonest used clinical equations are Cockcroft–Gault, Modification of Diet in Renal Disease study and Chronic Kidney Disease Epidemiology Collaboration formulae. These three will be the focus of this study (23, 25, 27)(28)(29).

2.3 The Cockcroft–Gault (CG) equation

This equation was derived by Donald Cockcroft and Henry Gault in 1973. It was derived from comparison of relationship of 24 hour urine creatinine excretion per kilogram of 249 patients. Their ages were from 18 to 92 years. It gave a coefficient correlation of 0.83 between predicted and mean measured creatinine clearance.

The CG equation is,

\[
\text{Creatinine clearance (mls/min)} = \frac{(140 - \text{age (yrs)} \times \text{weight (Kgs)})}{\text{Serum creatinine}}
\]

If the subject is female multiply the value with a ratio of 0.85

This formula has various limitations. Firstly, it is not precise for subjects with GFR above 60 ml/min. Secondly, creatinine undergoes tubular secretion, since the equation estimates creatinine clearance, tends to overestimate GFR. Thirdly, with weight being a numerator, it overestimates GFR in obese and oedematous persons. Finally, it was developed by ancient methods for serum creatinine measurement; these can no longer be standardized in the modern machines and methods. Subsequently today, it will lead to 10-40% overestimate of GFR. (30)(31, 32).

2.4 The Modification of Diet in Renal Disease (MDRD) equation

While the CG formula greatly improved GFR estimation, the need to have weight reduced its practicability in widespread clinical use. In 1999, Levey and others developed a new more precise formulae for estimating GFR based on ethnicity, sex, age, serum creatinine, ethnicity, urea and albumin using a six variable MDRD (6-v MDRD) equation. However, albumin and urea were a bottleneck because of analytical variation and the added cost. subsequently, one year later, Levey et al published a four variable (4-vMDRD) equation that removed albumin and urea variables with no influence on accuracy(33, 34).
The estimating equation is,

\[ \text{GFR} = 30849 \times \text{Standardized Scr (micromol/l)}^{1.154} \times \text{age}^{-0.203} \times 0.742 \times (\text{if female}) \times 1.210 \times (\text{if black}) \]

This equation had better accuracy and precision than the CG formula. The equation underrates GFR in subjects with good to high levels of GFR, for instance, potential kidney donors. No validation has been done in children, expectant women and the elderly exceeding 85 years (33, 35).

2.5 The Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) equation

Levey et al in 2009 revisited the MDRD formula to address the systematic its deficiencies. After analysis of Data from ten studies (n=5504), CKD-EPI equation was developed. These studies correlated serum creatinine with iothalamate clearance. Validation of the new equation was done with data amalgamated from 16 studies (n=3896). Though the equation uses similar variables as MDRD, it was developed from a larger database and participants with more characteristics than the MDRD study equation. It’s as good as MDRD for GFR estimation if less than 60ml/min/1.73m² and still accurate for higher GFR. While there is no perfect GFR estimation equation, CKD EPI appears to be the best of all so far for routine clinical use (27).

2.6 The challenge of reproducibility

While these equations have improved GFR estimations concerns remain about their reproducibility amongst various racial and ethnic groups. In the initial MDRD study, Asian subjects were grouped with white subjects despite making only one percent of the sample. The GFR outcomes of MDRD equation in a Chinese population improved by the addition of an ethnic coefficient. The bias-eliminating coefficient was calculated at 1.233 (36, 37). Lesley et al carried out a multi-racial evaluation of the CKD-EPI equation. Races included were Black, Asian and Native American. The conclusion of the study was that a multilevel variable for race developed in one geographic region may not be applicable in other regions (38, 39). There are no studies to validate the exactness of these estimation equations in the African subjects.
2.7 The role of measured GFR in kidney transplantation

Every potential renal donor undergoes GFR evaluation during pre-transplant evaluation. Guidelines recommend measured GFR (mGFR) rather than estimated GFR. Huang et al, utilizing data from Transplant Recipients Registry, observed that, fifty three percent of living donors had predonation eGFRcr high enough to ensure ninety five percent chance that predonation mGFR was 90 ml/min/1.73m², suggesting that mGFR may not be necessary in a significant portion of potential kidney donors. Consequently, a Web-based program to analyze the probability, based on eGFR was developed. It uses the measured creatinine to predict the probability of mGFR for a donor candidate being above or below a certain range for living donor assessment and election(40). The estimation equation can be accessed at [http://ckdepi.org/equations/donor-candidate-gfr-calculator/](http://ckdepi.org/equations/donor-candidate-gfr-calculator/). Gaillard et al, in 2016, in a study involving 354 potential renal donors undertook to validate the online prediction equation. The probability of mGFR<90 ml/min higher than 2% had 100% sensitivity for detection of actual mGFR<80 ml. This study confirmed the usefulness of the web-based application to identify potential donors who should benefit from GFR measurement(41, 42).
CHAPTER THREE

STUDY JUSTIFICATION

GFR estimations are used on a day to day basis to make critical decisions regarding patient diagnosis, staging, management and prognosis. The commonest used GFR estimation equations are Cockcroft Gault, MDRD and CKD EPI. Through this study it will reveal how estimated GFR compares with measured GFR. There is no study that has been done in our region to compare the performance of estimated GFR and measured GFR.

The level of GFR that is safe for kidney donation is widely discussed with different opinions and evidence which levels are safe. This study seeks to establish what are the levels of GFR in potential kidney donors and the differential contribution from each kidney.

The CKD EPI Web-based application tool to calculate the probability, based on eGFR that measured GFR for a potential donor is in or out of certain range has a potential to revolutionise living donor evaluation and selection. While it has been validated in some population, no known validation has been done in Africa.
CHAPTER FOUR

4.1 RESEARCH QUESTION

What is the level of measured GFR by DTPA and how does it compare with creatinine based estimated GFR in kidney donors at Kenyatta National Hospital?
CHAPTER FIVE

STUDY OBJECTIVES

5.1 Broad Objective

To determine the level of measured GFR by Tc-99m-DTPA and compare it with estimated GFR in potential kidney donors at Kenyatta National Hospital.

5.2 Specific Objectives

a) To describe the age, gender, relationship to recipient and level of education of the potential kidney donors at KNH.

b) To describe the weight, height, BMI and blood groups of the potential kidney donors at KNH.

c) To detail the total and differential GFR of potential kidney donors as measured by Tc-99m-DTPA at KNH.

d) To document the serum creatinine of the potential kidney donors and calculate the estimated GFR by Cockcroft Gault, CKD EPI and MDRD equations as creatinine based methods of estimating the GFR.

e) To compare the concordance of measured GFR (5.2c) and estimated GFR (5.2d).
CHAPTER SIX

METHODOLOGY

6.1 Study design
The study was a retrospective descriptive study

6.2 Study site
The study was carried out at the kidney transplant outpatient clinic at Kenyatta National hospital. This is the largest teaching and referral hospital in Kenya. Only two public hospitals offer kidney transplantation and KNH is the busier of the two. Renal unit is a busy centre under internal medicine department. Preparation of kidney donors and recipients is done under transplantation office in this unit.

6.3 Study population
All potential live kidney donors, aged eighteen years and above, who had done DTPA in their pre-transplant evaluation from the year 2010.

6.4 Inclusion criteria
Any kidney donor, 18 years and above.

6.5 Exclusion criteria
Incomplete documentation e.g. missing one or more investigation/results.

6.6 Sample size determination
The sample size includes all kidney donors in renal transplant clinic since the year 2010.

6.7 Sampling
No sampling was employed. All potential kidney donors who had done a DTPA as part of pre-transplant work up were included in the study.

6.8 Recruitment, Enrolment and Consenting
See figure 1.

Upon approval from the ethics and research committee, the list of all renal donors from 2010 was requested from the kidney transplant office. Socio-demographics data were obtained from the file including age, gender, weight, race, and level of education. Further details
Including serum creatinine, the date it was done, date of DTPA, measured GFR, which kidney was donated were obtained. See Appendix 1).

**Figure 1| Flow Chart Representing Recruitment and Enrolment**

**6.9 Study variables**

Socio-demographic variables are weight, height, age and highest level of education. Laboratory data include date of DTPA, total GFR, right kidney GFR, left kidney GFR, date of first creatinine level, date of second creatinine level, first creatinine level and second creatinine level. Derived variables include average serum creatinine levels, estimated GFR by the CG, MDRD and CKD EPI equations.
6.10 Data collection and Data Entry

Primary data collection was done using the data abstraction tool (Appendix 1). Data was collected by the primary investigator. Data entry was thereafter entered into a database in MS Access. Double data entry was done by two trained data clerks. The data was thereafter transferred to MS. Excel spreadsheet for data management. The protocol for DTPA scan takes one to two and half hours. The standard dose of DTPA is 111-185MBq 99mTc DTPA administered as intravenous injection. The patient is positioned supine. On the other hand, the creatinine is measured from a venous sample via Biolis 50i Superior (Tokyo BoekiMedisysinco.) chemianalyser.

6.11 Ethical consideration

The study was undertaken after approval by the East African Kidney Institute and the KNH/UON ERC. Consent from the KNH records officer was sought before file retrieval commenced. See appendix 2. The study is descriptive and does not involve physical engagement of the donors or performance of invasive procedures that would expose participants to risks. Information gathered from the files including data forms is kept confidential. No participant bore any cost of the study.

6.12 Data management and Result dissemination

All the data manipulation and generation of the entire derived variables using the respective equations was done. A complete data set was archived and a copy is being used for the statistical analysis in SPSS. Firstly, descriptive statistics has been used for all the variables in the study. Proportions and actual figures will be used to describe the categorical variables. All the continuous variables have been described in terms of their measures of central tendency (mean, median and mode) and dispersion (SD and range). Secondly, Measures of diagnostic performance of the equations were assessed. The DTPA GFR measurement has been used as the gold standard. The Bland-Altman method was used to evaluate the agreement between the measured GFR and the estimated GFR for each of the equations. Finally, Measures of diagnostic performance (Sensitivity, specificity, positive predictive value negative predictive values and accuracy) were calculated using the GFR and the gold standard for every eGFR equation.
CHAPTER SEVEN

RESULTS

A sum of 130 records was retrieved from the renal and central filing area. Eight had missing data and were therefore excluded from the study. A sum of 122 records were reviewed and included. Ninety of the participants were post kidney donation. Thirty two were yet to donate, however they were advanced in the pre transplant work up and had already obtained radioisotope imaging with DTPA.

Seventy (57.4%) were male and the rest females. The average age was 33.9yrs ± 9.2 with a range of 19-58 years. See figure 2 below.

![Gender Distribution](image)

**Figure 2|** Gender distribution of potential kidney donors

The participants were young with an average age of 33 years. The 10 year age group of 21-30 years had the greatest number. Seventy five percent of all the donors were between 20-40yrs. See figure 3 below.

![Age Distribution](image)

**Figure 3|** Age distribution among kidney donors
Most of our donors were well educated with only 4% having no formal education. Sixty eight percent had secondary and tertiary education as depicted by figure 4 above.

The KNH has living related kidney donation program. However emotionally related individuals like spouses are allowed. Almost 50% of all donations were from siblings with child and parental donation following a far off. See figure 5 below.

After estimation of GFR, actual measurement of GFR was measured via technetium 99m labelled DTPA. The patterns and levels of the GFR are shown in the figure 6 below. The participants with GFR less than 80ml/min/1.73m$^2$ were dropped from donation. The highest number of participants had a GFR between 91-100ml/min/1.73m$^2$. The highest GFR was 142 ml/min/1.73m$^2$.

Figure 4| Level of education among kidney donors

Figure 5| Relationship of the donor to the kidney recipient
Figure 6| Level of GFR of the potential kidney donors

The surface area of the kidneys was assessed via the DTPA in cm$^2$. The median surface area of the right was 49.6 cm$^2$ and the left 51.5 cm$^2$. The mean surface area on the right was 51.0 cm$^2$ compared with 53.2 cm$^2$ on the left. See figure 7 below.

Figure 7| Comparison of right and left surface area of the kidneys
Forty percent of the right kidneys had a higher GFR than the left. Conversely, 60% of the left kidneys had a higher GFR than the right kidneys. The median GFR in the right was 48.67 ml/min/1.73m$^2$ and the left was 49.2 ml/min/1.73m$^2$. The mean GFR in the right was 47.81 ml/min/1.73m$^2$ and on the left 48.98 ml/min/1.73m$^2$.

The actual measured GFR was compared with three GFR estimating equations namely Cockcroft Gault, MDRD and CKD EPI. The comparison is shown in figure 9 below. The median GFR by DTPA was 99 ml/min/1.73m$^2$. DTPA had little variation and few outliers. The MDRD median GFR was 100 ml/min/1.73m$^2$. It had wider variation, slight skew to the upper side and a wider range compared to DTPA GFR. The Cockcroft Gault median GFR was 100 ml/min/1.73m$^2$. It had marked wide range, wide variation, skew to the third quarter and marked outliers. The CKD EPI median was 112, which was the highest of the methods. It had more variation than the DTPA GFR, but comparable with other methods. The range was less than Cockcroft Gault. It had no outliers.
Figure 9 | Comparison of measured GFR (DTPA) with estimated GFR

The CKD EPI based online eGFR calculator check the probability of GFR being below or above a certain threshold. Its results are shown in the table 1 below.

Table 1 | Correlation of online GFR calculator and DTPA measured GFR

<table>
<thead>
<tr>
<th></th>
<th>GFR &gt;80,100%</th>
<th>95% CI</th>
<th>GFR &gt;90,100%</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.79</td>
<td>(0.71- 0.86)</td>
<td>0.61</td>
<td>(0.51- 0.71)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.33</td>
<td>(0.01- 0.91)</td>
<td>0.50</td>
<td>(0.29- 0.71)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.98</td>
<td>(0.92-1.00)</td>
<td>0.83</td>
<td>(0.72- 0.91)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.04</td>
<td>(0.00-0.20)</td>
<td>0.25</td>
<td>(0.14- 0.40)</td>
</tr>
</tbody>
</table>

PPV; Positive predictive value, NPV; Negative predictive value

Seventy eight (86%) of the nephrectomies were left sided. Out of these, 44(48.9%) were kidneys that had less GFR compared to the contra lateral kidney. However in the rest (46), the GFR was higher than the harvested kidney. The reasons for this were, the GFR difference was less than 10 ml/min/1.73m$^2$ in 41 donors, 2 had abnormal vascular patterns and the reason was missing in three others.
CHAPTER EIGHT

DISCUSSION

All the participants in our study were Africans. Seventy (57.4%) were male. Most participants were young with average age of 33.9 years. Table 2 below summarises several studies.

Table 2|Summaries of socio-demographics of living kidney donors

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Country</th>
<th>Year</th>
<th>Sex (%)</th>
<th>Average Age (yrs)</th>
<th>Relationship</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Njogu et al</td>
<td>90</td>
<td>Kenya</td>
<td>2018</td>
<td>M-57F-43</td>
<td>33.9 (19-58)</td>
<td>Sibling, child</td>
<td></td>
</tr>
<tr>
<td>Johnson et al</td>
<td>-</td>
<td>USA</td>
<td>1999</td>
<td>M-39F-61</td>
<td>41(17-74)</td>
<td>Sibling, parent</td>
<td></td>
</tr>
<tr>
<td>Poggio et al(43)</td>
<td>1014</td>
<td>USA</td>
<td>2009</td>
<td>M 44 F 56</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sukanta et al(44)</td>
<td>610</td>
<td>India</td>
<td>2009</td>
<td>M 40 F 60</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young et al(45)</td>
<td>2057</td>
<td>Canada</td>
<td>2010</td>
<td>M 61 F 39</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdu et al(46)</td>
<td>230</td>
<td>SA</td>
<td>2011</td>
<td>M45 F55</td>
<td>35</td>
<td>Sibling, child</td>
<td>24% black</td>
</tr>
<tr>
<td>Gullaird et al(41)</td>
<td>311</td>
<td>France</td>
<td>2016</td>
<td>M 43 F 57</td>
<td>51</td>
<td></td>
<td>African, 42</td>
</tr>
<tr>
<td>Phillipa et al(47)</td>
<td>805</td>
<td>UK</td>
<td>2017</td>
<td>M 45 F 55</td>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Apart from the study by young et al in Canada(45), most of the studies showed a female preponderance in the male female ratio. Most of the donors in the several studies summarised in table 2 above were in their thirties and forties proving that most living kidney donors are young. It’s only the study by Gullaird et al (41) that the average age was 51 years. Still, in their study, the average age of Africans was forty two years. Most kidney donations were from siblings, parents or a child. This was similarly reflected by Johnson et al and Abdu et al(46).

The profile of our donors reflects population consisting of young African, males donating to their siblings. Kidney diseases are more common in Africans and in males. Africans are three to four times elevation of risk of ESRD correlated to Caucasians. The REGARDS study also showed that Africans were at an increased risk of renal disease in relation to Caucasians(48). APOL1 gene, chromosome 22 encodes apolipoprotein L1, which confers innate immunity against most strains of Trypanosoma brucei that causes African human trypanosomiasis. While the gene is protective against African trypanosomiasis, it increases
the risk of kidney disease in Africans (49). Coding variants in APOL1 are present only on African-ancestry haplotypes (50, 51). The risk of genetic disease is increased among siblings and parents/children this includes diseases like autosomal dominant polycystic kidney disease, autosomal dominant interstitial kidney disease, APOL1-related renal disease, Alport syndrome and some of the inherited podocytopathies (52). Young age of donors exposes them to long term risk of developing CKD. In our population, the donors are at risk of infections e.g. HIV, HBV, HCV, trauma, and non-communicable diseases including systemic hypertension and diabetes mellitus.

estimation of GFR is usually used as a preliminary test but insufficient to authorise a transplantation (41, 53). Therefore GFR has to be measured either via 24 hour urinary clearance or via exogenous marker each with its own unique shortcomings. The 24 hour urinary clearance has the disadvantage of cumbersome lengthy urine collections that may render the results inaccurate. The exogenous markers with DTPA, EDTA are costly and with limited availability (41, 54).

According to KDIGO 2017 transplantation guidelines GFR should be assessed via standardised creatinine measurement. Estimation of GFR via creatinine based equation preferably CKD-EPI equation. The estimated GFR can be enhanced by using cystatin C based equation. Actual GFR should be measured either through creatinine clearance or isotopic GFR measurement e.g. DTPA. The guidelines propose use of mCrcl routinely but use of mGFR when there is specific indication or need for split function (55). In 2007 A survey of 132 transplant centres 59% university, 40 % private, showed that 90% of the centres measured GFR via mCrcl and only 10 % used radioactive isotope (56). There is therefore a need to have a way of identifying the people who need actual GFR measurements. Huang et al, using large cohorts developed an online GFR calculator that can be used to estimate the probability the creatinine based estimated GFR is above or below certain limits (40).

Almost all potential donors had mGFR above 80ml/min/1.73m^2. The three potential donors below this GFR were dropped from proceeding with donation. Eighteen percent of the donors had mGFR of 80 to 90 ml/min/1.73m^2. Previously, the lower limit of recommended GFR was 80 ml/min/1.73m^2. The current practice guideline in KNH on donor GFR evaluation is in line with this level. A 2007 survey of practices by transplant programs in the United States showed that about sixty seven percent of transplant programs used a threshold of 80 mL/min or more to accept donors, while a quarter used a values based on sex and age (56). Others recommend GFR level within two standard deviations of normal for age and sex.
However, according to the 2017 KDIGO transplantation guidelines, the recommendation is to routinely accept donors with GFR above 90 ml/min/1.73m² and routinely decline those with GFR below 60 ml/min/1.73m² and to individualize for donors with GFR between 60-89 ml/min/1.73m² based on other risk factors (55). Eighty percent of our donors had a GFR of 90 ml/min/1.73m² and above. The highest mGFR obtained in our study was 143 ml/min/1.73m² in a 31 year old female. While there is much data on lowest GFR recommended for donation, it’s unclear whether there is a maximum recommended GFR, beyond which it’s not safe for the donor.

However as shown by the figure 10 and table 3 above, adapted from British transplantation society (57), the appropriate GFR is based on the age of the potential donor as the GFR has a gradual decline after the age of forty years. This is important as family sizes continue to reduce and older donors may be the only ones available for live donations. Older donors may also be at less long term risk of kidney donation (43).

The comparison of the mGFR and the other eGFR equations is depicted in figure 9. The actual measured GFR was compared with three GFR estimating equations namely Cockcroft-Gault, MDRD and CKD EPI. The median GFR by DTPA was 99 ml/min/1.73m². DTPA had little variation and few outliers. The MDRD median GFR
was 100 ml/min/1.73m². It had wider variation, slight skew to the upper side and a wider range compared to DTPA GFR. The Cockcroft-Gault median GFR was 100 ml/min/1.73m². It had marked wide range, wide variation, skew to the third quarter and marked outliers. The CKD EPI median was 112 ml/min/1.73m², which was the highest of the methods. It had more variation than the DTPA GFR, but comparable with other methods. The range was less than Cockcroft-Gault. It had no outliers.

Table 4 | Correlation of measured GFR by DTPA compared to estimated GFR

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>CG</th>
<th>MDRD</th>
<th>EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natale et al(58)</td>
<td>1999</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ito et al(20)</td>
<td>2003</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ying et al(59)</td>
<td>2006</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poge et al</td>
<td>2006</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assad et al(60)</td>
<td>2008</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kearket et al</td>
<td>2011</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Xianglai et al(37)</td>
<td>2012</td>
<td>↓</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Andre et al(61)</td>
<td>2012</td>
<td>↓</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Yan Qi et al(62)</td>
<td>2015</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Massod et al(63)</td>
<td>2015</td>
<td>=</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In May 2012 to April 2015, Yan Qi et al in a Chinese population (n=38) compared the performance of measured GFR by ⁹⁹mTc-DTPA and eGFR by CKD-EPI in patients with horseshoe kidneys. The estimated GFR were consistently higher than the measured GFR(62). Masood et al compared the GFR by ⁹⁹mTc DTPA scan and the CG method. His study included 47 patients and Mean total GFR by DTPA Gates method was 73.6 ±18.6 ml/min and by the CG method was 79.8 ± 32.2 ml/min. The difference was statistically insignificant indicating an agreement between both the methods in measuring GFR(63). Similarly, Assadi et al proved a close correlation between the DTPA renogrphy and CG estimated GFR(60).

Contrary to this, Natale et al suggested that ⁹⁹mTc-DTPA clearance from the renogram is less precise than measured(inulin based clearance) and predicted creatinine clearance(58). Ito et al, also suggested that DTPA is less accurate than CG estimated GFR(20). As from these several studies, there was no single method of estimating GFR that consistently produced the same prediction when used by different investigators. While it’s possible that there were subtle differences in the methodology used by the investigators, eGFR is not reliable in predicting the actual GFR. From our data it is not advisable to use the eGFR to predict the mGFR.
Web-based application to compute the probability, based on eGFR was done. The estimation equation is available on the CKD-EPI website [http://ckdepi.org/equations/donor-candidate-gfr-calculator/](http://ckdepi.org/equations/donor-candidate-gfr-calculator/). From this study, we attempted to detect a 100% post test probability that eGFR is more than 80ml/min/1.73m². Our study showed a sensitivity of 79% and specificity of 33%. It had good positive predictive value of 98% but poor negative predictive value of 4%. When the probability of the GFR was set higher at 90 ml/min/1.73 m², the sensitivity of the test reduced 61% but the specificity improved to 50%. Consequently, from this data, the online eGFR calculator cannot be recommended for donor evaluation that requires a higher sensitivity and much better specificity.

Gullaird et al, on using the same calculator in a much larger study (n=311), showed good correlation of measured GFR and predicted probability of certain GFR threshold. This is depicted in the table 5 below.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Comparison of posttest strategies to detect mGFR lower than 80 mL/min/1.73m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posttest 90</td>
<td>Posttest 80</td>
</tr>
<tr>
<td>AUC (95% CI)</td>
<td>0.78 (0.72-0.85)</td>
</tr>
<tr>
<td>Maximum sensitivity (95% CI 96)</td>
<td>1 (0.92-1)</td>
</tr>
<tr>
<td>Specificity (95% CI)</td>
<td>&gt;2%</td>
</tr>
<tr>
<td>Reduction of GFR measurements</td>
<td>0.32 (0.26-0.38)</td>
</tr>
</tbody>
</table>

Similarly, in his study, the sensitivity to identify donors with GFR less than 80 reduced when the post test threshold was low but the specificity increased with low thresholds. He observed that measurement of GFR was only necessary if post-test probability of GFR less than 90 was less than 98%. With this approach there was reduction in measurements of GFR in 27% of potential kidney donors. This would save resources without compromising the safety of the donor. There is paucity of published studies using this calculator owing to the recent discovery of the same. More studies are needed to validate the online calculator; perhaps, it may change donor evaluation in a completely new way.

The right kidney is known to have smaller length and size compared to the left kidney. In 2009 Bernhard et al (n=1040), using a 64 slice MDCT, showed that the left kidney was longer and larger than the right kidney. Similarly Buchholz et al using ultrasound found the right kidneys to be slightly smaller (64, 65). A difference is considered significant if it is less than 10%. In this study 14 potential donors had significant GFR difference of more than 10ml/min/1.73m². Nine of them the left kidney
had more GFR with a GFR difference of up to 20.2 ml/min while in five the right had more GFR with up to 14.8ml/min. Our study demonstrated this fact as the mean and the median for the right kidney were consistently lower. GFR is known to correlate with the kidney volume/size\(^2\). Consequently, in our study the GFR in the right kidney was less compared with the left kidney. Defining the role of split function in kidney transplantation is still under discussion and different centres adopt different approaches. Most centres that use creatinine clearance to measure GFR do not measure split function\(^3\). KDIGO 2017 guidelines recommend measurement of split function if there is indication. Such indications include asymmetry in size and shape, parenchymal, vascular and urological abnormalities\((55, 56)\).

Most of the donor nephrectomies were left sided making up 87% of the total surgeries done. This is consistent with surgeries elsewhere\((66, 67)\). Almost 49% of the nephrectomies were done on the basis of having the lesser GFR. This is in agreement with the Amsterdam declaration that protects the short term and long term safety of the living kidney donor. Notably, of all the right nephrectomies done (n=12), all had the lesser GFR. Fifty one percent (n=46) of all nephrectomies were done in the kidney with the higher GFR. In forty one of all participants (45.6%), the GFR was less but considered not significant (GFR less than 10mls/min/1.73m\(^2\)). Two were because of vascular reasons, two were because of other surgical reasons and the other two the data was missing.

In a 6 year study by Stephen et al \((n=738)\) of consecutive live kidney donors in the University of Maryland, total left sided nephrectomies were 96%. Similarly, Abdekader et al over 6 year also, reviewed 168 donor nephrectomies and 97% were left sided\((66, 67)\). This raises a medical dilemma. Should transplant centres routinely perform left nephrectomy (unless lateralising issues)? Left sided nephrectomies are easier to perform, less complications especially after transplantation and there is comparable split function. Despite the high cost of DTPA and unavailability in most regions in our country, 87% of the grafts were harvested from the left. However, omitting donor split GFR raises the question of donor safety, since there are chances that the donor maybe left with the kidney with the less GFR.
1. Most of the donors were young, male and siblings to the recipients.

2. A hundred percent of the donors met the international recommended mGFR of 80 ml/min/1.73m$^2$.

3. The mean and median of split renal function was comparable.

4. The means of estimated GFR by the 3 equations were within 10% of mGFR, but variation was marked.

5. The Online donor GFR calculator has a potential role in transplantation though needs more validation.
CHAPTER TEN

LIMITATIONS AND RECOMMENDATIONS

10.1 Limitations

1. The participant population was composed of only Africans. This limits our study to one race as opposed to other studies that included more races in the GFR estimations and validations.
2. Inability to trace all patients files-few donors are on active follow up.
3. Incomplete documentation in the patients files.
4. Inability to use Inulin which is the gold standard in GFR measurement.

10.2 Recommendations

1. Expand the scope of our donors and consider older donors too.
2. Consider use of the GFR normogram as opposed to a single value for all.
3. Further validation of the online donor GFR calculator.
4. More rigorous follow up of donors, most files not active.
LIST OF REFERENCES

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62. Qi Y. Glomerular filtration rate measured by 99mTc-DTPA renal dynamic imaging is significantly lower than that estimated by the CKD-EPI equation in horseshoe kidney patients http://loginresearch4lifeorg/tacsgr1doi_org/101111/nep12663. 2015.


APPENDICES

APPENDIX 1

DATA COLLECTION FORM

I. BIO DATA

Study number________

Age_____Sex_____Weight(Kgs) Height__________Cm

BMI_____Blood group; Donor_____Recipient____

Highest Educational Attainment: ______
1= No formal Education; 2= Primary; 3= High School; 4= College / University

Relationship to recipient: ______
1=Husband; 2=Wife; 3=Daughter; 4=Son; 5=Father; 6=Mother; 7= Brother; 8= Sister; 9=cousin; 10=Nephew/Niece; 11=Uncle/Auntie; 12=Others.

II. GFR DATA

1. DTPA

Date  ____/____/____

GFR (mls/min/1.73m²): Total ________RT_________LT________

2. Serum Creatinine(Micromoles/L)

First Sample ________Date  ____/____/____

Second Sample ________Date  ____/____/____

Average___________

3. Nephrectomy: ___________ 1=Left  2=Right
APPENDIX 2

CONSENT INFORMATION DOCUMENT

Title of study: COMPARISON OF MEASURED GFR BY TC-99M-DTPA WITH ESTIMATED GFR IN POTENTIAL KIDNEY DONORS AT KENYATTA NATIONAL HOSPITAL

Primary investigator: Dr. Edward Njogu Maina

Aim: Request to access patients’ files and data for research purposes

Study Background: Glomerular filtration rate (GFR) is very important in evaluating kidney function in an individual. It is key in detection, assessment and treatment of kidney illnesses. While there are many methods and substances of measuring GFR, nuclear imaging through DTPA is considered to be accurate and more convenient compared with other methods. There is no local study outlining the measured GFR of the kidney donors and there is no study comparing the variation of DTPA GFR with other GFR estimating formulae like COCKROFT GAULT, CKD- EPI or MDRD.

Objective: To determine the measured GFR by DTPA of the potential kidney donors and compare the measured GFR with other GFR estimating methods among kidney transplant donors at KNH.

Study design and population: Descriptive study, Live Kidney donors at KNH.

Study benefits: The level of GFR that is safe for kidney donation is widely discussed with different opinions and evidence which levels are safe. This study seeks to establish what are the levels of GFR in our population and the differential contribution from each kidney. This information can influence the kind of investigations done on the patients during pre donation work up and may dramatically reduce the cost of transplant preparation. No participant will bear any cost of the study.

Risks: The study will only be undertaken after approval by the East African Kidney Institute and the UON/KNH ERC. The study is descriptive and does not involve physical engagement of the donors or performance of invasive procedures that would expose them to risks.

Confidentiality: Information gathered from the files including data forms will be kept confidential. No patient file number will be included in the study documents.