EVALUATION OF RENAL DYSFUNCTION AMONG CHILDREN IN THE DEPARTMENT OF CHILD HEALTH, KORLE-BU TEACHING HOSPITAL, GHANA

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DECLARATION

This thesis is a presentation of my original research work. Wherever contributions from other persons are involved, every effort is made to indicate that clearly, with due reference to literature and acknowledgement of collaborative research and discussions.

The experimental work was carried out at the Korle-Bu Teaching Hospital and Mamprobi Polyclinic respectively under the guidance of Dr W.K.B.A Owiredu, Dr Victoria May Adabayeri and Dr Victor Etwire. This work has not been submitted for any other degree.

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ABSTRACT

There is an upsurge in the prevalence of kidney dysfunction among adults and children in many countries. Various screening modalities have been used in various jurisdictions but with poor reproducibility. This study therefore sought to evaluate the validity of using selected biomarkers in combination with the predictive equations for early detection of kidney dysfunction among children. In all 90 study participants' comprising 45 patients with varied medical conditions within the case group and another 45 patients within the control group were enrolled into the study. Participants were age matched with ages ranging from 2–12 years. Blood samples, urine samples and anthropometric data were obtained from each of the study participants. eGFR was estimated using two creatininebased equations (Schwartz and Counahan-Baratt) and four Cystatin C-based equations (Rule, Zappittelli, Larsson and Le Bricon). Respondents within the control group were significantly heavier (24.5 ± 9.8 kg) when compared with their counterparts within the case group (19.8 ± 11.3 kg; p=0.041). Age, BMI, %BF, systolic and diastolic pressures showed no statistically significant differences (p>0.05). There was significant increases in urea (5.6 ± 5.4 vs 3.1 ± 1.5 mmolL^{-1}; p=0.004), creatinine (74.2 ± 52.4 vs 49.3 ± 10.5 µmolL^{-1}; p=0.003) and Cystatin C (1062 ± 274.3 vs 854.7 ± 109.9 µgL^{-1}; p<0.000) in the case group when compared to the control group. Contrarily, serum protein (56.7 ± 14.3 vs 69.5 ± 6.7 gL^{-1}; p<0.000) and albumin (30.7 ± 10.0 vs 39.2 ± 5.0 gL^{-1}; p<0.000) concentrations in the case group were significantly decreased when compared to the control group. Urinary albumin (3.0 ± 4.5 gL^{-1}) and protein (4.7 ± 5.6 gL^{-1}) concentrations for the case group were significantly higher compared with that estimated in the control group (0.2 ± 0.1 g L^{-1}; 1.6 ± 0.9 g L^{-1}) respectively. Urinary creatinine concentration was however decreased in the case group (3059 ± 2812 mgdL^{-1}) compared with the control group (6971 ± 3764 mgdL^{-1}). Proteinuria was assessed by estimating urine protein–creatinine ratio (Upr/cr). In the control group 4.4% (2/45) of respondents were normal whilst 40.0% (18/45) and 55.6% (25/45) were within the significant and nephrotic proteinuria range respectively. None of the respondents in the case group were normal, however 17.8%(8/45) and 82.2%(37/45) fell within the significant and nephrotic ranges respectively. Prevalence of CKD determined with the Schwartz and Counahan-Baratt equations within the case group was 35.6% respectively while the Larsson, Rule and Zappitelli formulae yielded a CKD prevalence of 20.0% respectively. Le Bricon, yielded a CKD prevalence of 13.3%. There was an inverse relationship between Upr/cr and GFR as estimated with the renal function estimating equations. UACR against GFR also showed an inverse relationship. Weight, age, height and proteinuria (defined by urine proteincreatinine ratio) were identified as significant predictors of progression to CKD. The Schwartz and Counahan-Baratt equations showed the best agreement

with the predictors and showed a natural spread of the respondents within the case group across the five stages of CKD as defined by KDOQI guidelines.

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LIST OF ABBREVIATIONS

AIDS – Acquired Immune Deficiency Syndrome

ANOVA – Analysis of variance

CAKUT – Congenital anomalies of the kidney and urinary tract

CCR – Creatinine clearance

CHRPE – Committee on human research, publication and ethics

CI – Confidence interval

CKD – Chronic kidney disease

CRCL – Creatinine clearance CVD –

Cardiovascular disease eGFR – Estimated

glomerular filtration rate

EQC – External quality control

ESKD – End stage kidney disease

ESRD – End stage renal disease

GFR – Glomerular filtration rate

GDP – Gross domestic product

GLDH – Glutamate dehydrogenase

HIV – Human immunodeficiency virus

ISO – International standardization organization

K/DOQI – Kidney disease outcome quality initiative

KATH – Komfo Anokye Teaching Hospital

KBTH – Korle-Bu Teaching Hospital

KDIGO – Kidney disease improving global outcome

LBW – low birth weight

LVH – Left ventricular hypertrophy

NADP – Nicotinamide adenine dinucleotide phosphate

NAPRTCS – North American Paediatric Renal Trials and Collaborative Studies

NKF – National kidney foundation

PKD – Polycystic kidney disease

PMLH – Princess Marie Louis Hospital

PUV – Posterior urethral valve

RBC – Red blood cell

ROC – Receiver operator characteristics

ROD – Renal osteodystrophy

SD – Standard deviation

SRNS – Steroid resist and nephrotic syndrome

UACR – Urine albumin creatinine ratio

UPCR – Urine protein creatinine ratio

UTI – Urinary tract infection

VUR – Vesicoureteral reflux

WHO – World Health Organization

Chapter 1 INTRODUCTION

1.1 GENERAL INTRODUCTION

There are more than a million children in Ghana with long-term or even lifelong illness that will need medication for the foreseeable future. All children are likely to have different health problems during infancy and childhood with most of these problems being mild, such that they do not interfere with their daily life and development. For some however, chronic health conditions may affect everyday life throughout childhood. Chronic health conditions are normally defined as health problems which last for over three months and might affect a child's normal activities requiring hospitalization and/or home health care and/or extensive medical care. Children with chronic illnesses may be ill or well at any given time, but they always live with their condition. Some examples of chronic conditions include (*but not limited to*) diabetes, cancer, AIDS, epilepsy, congenital heart problems etc.

There are studies which suggest that families of chronically ill children experience denial, disbelief, distress, depression, guilt, burden of responsibility, family conflict and resentment in response to the child's illness (Judson, 2004). Prevalence studies in the United States have suggested that 15 to 18% of all children suffer from a chronic condition (Judson, 2004).

Chronic kidney disease (CKD) which has been defined (National Kidney, 2002) as kidney damage with structural or functional abnormalities or a glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for three months or more is now being recognized as a major public health problem, threatening to reach epidemic level (Lysaght, 2002). A high proportion of persons presenting with CKDs are usually adults; however, there is an increasing percentage of children who develop CKD in their early stage of life (Warady and Chadha, 2007). In paediatric patients, development of chronic kidney disease is mostly secondary to congenital abnormalities in the urinary system as opposed to adults (Seikaly *et al.*, 2003).

Reports from the North American Paediatric Renal Trials and Collaborative Studies (NAPRTCS), suggests congenital causes, including congenital anomalies of the kidney and urinary tract (CAKUT) (48%) and hereditary nephropathies (10%) as the most common (Fivush *et al.*, 1998) with glomerulonephritis accounting for 14% of cases.

Preliminary investigations conducted at the Department of Child Health of the Korle-bu Teaching Hospital (KBTH), Ghana, suggest predisposition to CKD in children with posterior urethral valve (PUV), hydronephrosis, polycystic kidney disease, recurrent urinary tract infections (UTI), steroid resistant nephrotic syndrome (SRNS) and those on anti-hypertensives (*unpublished ward records*). However, there is paucity of information on the epidemiology of the early stages of CKD in the pediatric population; as such cases are most often asymptomatic and therefore under-diagnosed and under-reported.

In Ghana, data on the prevalence of CKD has been varied over the years with Bamgboye (2005) reporting a prevalence of 1.6% per million people. However, Addo *et al.* (2009) puts the prevalence among Ghanaian hypertensives at 4% and Osafo *et al.* (2011) reported a prevalence of 46.9% among hypertensives in Ghana in a recent publication.

Kidney function typically is measured by estimated Glomerular Filtration Rate (eGFR) and in clinical practice it is most frequently estimated using endogenous surrogate markers. Serum creatinine remains the most widely used endogenous marker. Serum cystatin C is a relatively new endogenous marker that offers the advantage of a constant production by all nucleated body cells and it is almost entirely catabolized at the proximal tubule (Laterza *et al.*, 2002). In clinical studies, serum cystatin C has been found to be a good marker for predicting GFR (Bokenkamp *et al.*, 1998). Cystatin C is neither affected by the muscle mass, gender, nutritional status or inflammatory state of an individual. It is usually excreted by filtration through the glomerulus and metabolized in the cells of the proximal tubules. Its measurement has been projected as an alternative and more precise

marker of GFR compared to creatinine especially among patients with slight to moderate reductions in GFR (Grubb *et al.*, 1985; Newman *et al.*, 1995).

Tests used for CKD screening in children are usually limited to urinary dipstick protein instead of urine albumin/creatinine ratio or creatinine-based calculation of estimated glomerular filtration rate (eGFR), as recommended for adults. However, there is a large variation in the methods used and approaches taken by different countries, and the findings have shown poor reproducibility. Timed urine collection is also flawed because of improper urine collection and very often overestimated due to renal tubular secretion of creatinine (Johnson *et al.*, 2004). This study therefore seeks to evaluate renal dysfunction in children and to further explore the applicability and clinical utilization of selected biomarkers in combination with the predictive equations for early detection of CKD in children.

1.2 MAIN OBJECTIVE/AIM

To explore the applicability and clinical utilization of selected biomarkers in combination with the renal function predictive equations for early diagnosis of CKD in children.

1.2.1 Specific objectives

1. To measure urinary proteins and explore their screening value.
2. To find the value of cystatin C in diagnosing renal dysfunction
3. To estimate GFR using cystatin C and creatinine respectively and further explore them as diagnostic tools for predicting risk of renal dysfunction in children.
4. To determine the prevalence of chronic kidney disease in children with renal dysfunction.
5. To determine the applicability of the predictive equations in the measurement of GFR in children with increased risk of CKD in Ghana.

1.3 JUSTIFICATION

Chronic Kidney Disease (CKD) is a major public health problem and is becoming a growing concern in children with an increasing percentage of children developing CKD in their early stages of life (Warady and Chadha, 2007). Most epidemiological information on CKD in children has to do with data on End Stage Renal Disease (ESRD). The epidemiological studies that have been performed provide evidence to suggest that patients with earlier stages of the disease are likely to exceed those reaching ESRD by as much as 50 times (Warady and Chadha, 2007).

Late detection of CKD in children presents with bone disease, anaemia, poor nutrition and even death as well as increased financial burden on the family stemming from frequent dialysis and ultimately renal replacement therapy.

Unlike adults where albumin/creatinine ratios together with eGFR are clinically exploited for the diagnosis of CKD, often times in children, testing is limited to urinary dipstick method for protein detection. Variations exist in the methods used and approaches taken by different countries and as such the findings have shown poor reproducibility. This observed gap in the early diagnosis and prediction of CKD in children therefore warrants the need to determine the predictive ability and possible clinical utilization of selected biomarkers in the early detection of CKD in children.

Chapter 2 LITERATURE REVIEW

2.1 CHRONIC KIDNEY DISEASE

Chronic Kidney Disease (CKD) is a major public health problem worldwide and extensive epidemiological research in the adult population is available. In contrast, little is known about the epidemiology of CKD in the pediatric population. ESRD is a devastating disorder associated with excessive mortality and cardiovascular morbidity, and specific problems occur in children, such as impaired growth and psychosocial adjustment, all of which severely impact on the quality of life (Greenbaum *et al.*, 2009; Shroff and Ledermann, 2009).

The magnitude of the problem is poorly described by the number of people that will initiate renal replacement therapy (haemodialysis, peritoneal dialysis and renal transplantation), as the incidence of 1-3 per 10,000 per year in the general population may seem small (Lysaght, 2002; Dor *et al.*, 2007; Hsu *et al.*, 2007). However, chronic dialysis treatment and transplantation have an enormous impact on the life of individual patients and their families, and renal replacement therapy is very costly (Lysaght, 2002; Dor *et al.*, 2007). The annual worldwide costs are estimated at 70 to 75 billion US dollars to maintain the renal replacement therapy of the roughly 1.1 million worldwide dialysis patients in 2001. In Ghana, renal replacement therapy (mainly haemodialysis and peritoneal dialysis) is available only in two of the teaching hospitals and the estimated cost of dialysis is approximately $44,300 per patient per annum. This amount is rather high for a country with a per capita income of $1500 and a GDP of 6.3%. The first renal transplant in this country was performed at the end of 2008 by a combined team of Ghanaian and British surgeons. Moreover, the number of patients requiring renal replacement therapy is increasing globally, by up to 7% annually according to some reports (Gansevoort *et al.*, 2004; Jones *et al.*, 2005; Muntner *et al.*, 2005).

CKD represents a progressive irreversible decline in the glomerular filtration rate (GFR). A common phenomenon in renal failure is progressive renal function loss irrespective of the underlying cause of the kidney disease. Most chronic nephropathies lack a specific treatment and progress relentlessly to end stage

kidney disease (ESKD), the prevalence of which is increasing worldwide (Locatelli *et al.*, 2003).

A better understanding of the epidemiology of CKD in children is essential in order to make a precise and early diagnosis, identify preventable or reversible causes of progression, predict prognosis, and aid the counseling of the children and their families. The most common screening test for CKD is the measurement of serum creatinine. However, it as an insensitive measure, since as much as 50% of the nephron mass may be lost before creatinine concentration increases and levels are influenced by several factors such as gender, age, body mass, and diet (Parmar, 2002).

2.2 FUNCTIONS OF THE KIDNEY

The kidneys play an important role in the maintenance of normal body function. The basic function of the kidneys has to do with the formation of urine through complex filtration, reabsorption and secretion mechanisms. In addition, the kidneys also excrete urea and uric acid which are the end products of protein and nucleic acid metabolism respectively. The kidneys regulate fluid, electrolyte and acid base balance of the body and create a steady environment for the metabolic processes of tissues and cells. This function is essential for life and it is realized by balancing solute and water transport, excreting metabolic waste products, conserving nutrients, and regulating acid-base balance in the body.

Additionally, the kidney also produces three (3) important hormones; erythropoietin which stimulates the production of red blood cells, renin which regulates blood pressure and calcitriol (the active form of vitamin D) which helps in regulation of plasma calcium levels (Henderson and Henderson, 1994).

2.3 DEFINITION OF KIDNEY DISEASE

Kidney disease results in the loss or reduction of functioning nephrons. Chronic kidney disease (CKD) a new terminology that has replaced chronic renal failure (CRF) was defined in 2002 by the National Kidney Foundation Kidney Disease

Quality Outcome Initiative (NKF/KDOQI) as structural damage or GFR<60 ml/min/1.73 m^2 for more than three months. Kidney damage is defined by the NKF/KDOQI as the presence of pathological abnormalities or markers of kidney damage, including abnormalities in urine or blood tests or abnormal imaging tests (National Kidney, 2002).

This new vocabulary provides a platform for healthcare professionals tasked with caring for CKD patients worldwide to speak a common language. In addition, it gives a simple definition of CKD and a staging system which distinguishes groups of patients (Levin, 2003).

2.3.1 Prevalence of CKD

The prevalence of CKD in the western societies (United States, Britain and parts of Asia) ranges between 5-15% in the adult population (Chen *et al.*, 2011). In 2009, Afolabi and his colleagues put the prevalence among Nigerians in a family practice population at 10.7% (Afolabi *et al.*, 2009). The prevalence of CKD in Ghana has varied over the years; from 1.6% per million people by Bamgboye (2005) to 4% among hypertensives in the Greater Accra region as documented in the study by Addo *et al.* (2009). Recently, a prevalence of 46.9% has been recorded among hypertensives in Ghana (Osafo *et al.*, 2011).

Not much is known about the prevalence of the early stages of CKD and most reports are imprecise and flawed with methodological differences (Warady and Chadha, 2007).

Most epidemiological information on CKD in children has to do with data on End Stage Renal Disease (ESRD). A study on the epidemiology of CKD conducted in several Latin American countries (Argentina, Brazil, Chile, Colombia, Mexico, Uruguay and Venezuela) showed a wide variation in incidence that range from 2.8-18.8% (Harambat *et al.*, 2012). Single-centre studies from sub-Saharan Africa have shown a very low incidence of CKD, estimated at 3% in Nigeria (Anochie and Eke, 2003) and 1-2% in South Africa (Bhimma *et al.*, 2008).

2.4 RISK FACTORS OF CKD

Clinical and epidemiological reports have provided a relationship between numerous factors and the initiation and progression of CKD. These have been grouped into two well defined classes: those that cause the CKD (risk factors) and those that are associated with CKD in the absence of established causal relations (risk markers).

2.4.1 Aetiology and risk factors

Progressive glomerulopathies are among renal diseases which cause a rapid permanent loss of kidney function. Most kidney diseases progress slowly over ten to fifteen years, initially without symptoms. This makes it very difficult to identify the aetiology. Indications are that environmental and lifestyle factors affect kidney function even though genetic factors also show some relevance (Bowden, 2003). Generally, kidney function reduces with age even among healthy subjects; this reduction or decline, however, is not similar but exhibits considerable individual variation. Furthermore, there is a significant variation in the incidence of kidney damage among persons at risk for CKD such as persons presenting with hypertension and diabetes mellitus.

In the paediatric population, certain renal conditions may increase the risk of progression to CKD; this includes patients presenting with hydronephrosis, congenital malformation of the kidneys, posterior urethral valve, polycystic kidney, proven recurrent UTI, steroid resistance nephrotic syndrome (SRNS) and patients on anti-hypertensives.

Finally, the rate at which kidney function is lost shows a high level of inter individual variation even among persons with the same underlying cause of kidney injury (McClellan and Flanders, 2003). Established or suspected risk factors associated with the occurrence or progression of CKD is as shown in Table 2.1.

Table 2.1Established or suspected factors associated with the occurrence or the progression of chronic renal failure.

Specific kidney diseases	Race and ethnicity

Hypertension	Hereditary factors
Diabetes	Low birth weight
Hyperinsulinaemia	Short stature
Chronic anaemia	Obesity
Proteinuria	Cigarette smoking
Oxidative stress	Illicit drug use
Older age	Analgesics
Male gender	High intake of proteins
Low socio-economic status	Lead, cadmium and other heavy metals
Dyslipidaemia	Organic solvent

2.5 PROGRESSION OF CKD

Virtually, all kidney diseases progress to terminal renal failure relatively independent of the initial disease. Diabetic nephropathy, chronic glomerular diseases and hypertensive nephrosclerosis are among the most widespread causes of CKD. In paediatric patients, there are a number of causes of chronic kidney disease (Seikaly *et al.*, 2003). As opposed to adults, many paediatric patients develop chronic kidney disease secondary to congenital abnormalities in the urinary system. These patients are much more likely to have been followed more carefully after birth. In recent report from the North American Paediatric Renal Trials and Collaborative Studies (NAPRTCS), congenital causes, including congenital anomalies of the kidney and urinary tract (CAKUT) (48%) and hereditary nephropathies (10%), were the most common. Glomerulonephritis accounted for 14% of cases (Seikaly *et al.*, 2003). The distribution of causes varied with age. Whereas CAKUT predominated in younger patients, glomerulonephritis was the leading cause in children older than 12 years of age. Causes of CKD vary across races, for example, focal segmental glomerulosclerosis, the main cause of glomerular disease, was three times more common in blacks than in whites (19 vs 6%) and especially among black adolescents (35%).

A primary disease eventually leads to secondary glomerular injury and nephron loss that is clinically characterized by proteinuria and hypertension, which leads

to inflammation or scarring which causes kidney failure and ultimately a gradual elevation in the plasma creatinine concentration and a progressive decline in GFR (Jacobson, 1991). Apparently, the excessive protein filtration, caused by the glomerular hypertension, might per se have toxic effects on the kidneys and increase the rate of progression (Remuzzi *et al.*, 1997; Tryggvason and Pettersson, 2003). Studies in rats have suggested that hyperfiltration and glomerular hypertension may play important roles (Brenner *et al.*, 1982). Hyperfiltration is observed in diabetes and obesity, but also in any condition associated with a reduced number of nephrons (Brenner *et al.*, 1996). To compensate for this nephron loss, the glomerular plasma flow rate and glomerular hydrostatic pressure increase in the surviving nephrons, thus raising the single nephron glomerular filtration rate. Initially, these changes are adaptive because they maintain the overall GFR. However, the glomerular hypertension has negative long term effects and causes progressive renal sclerosis in a self-perpetuating vicious cycle, whereby nephron loss due to sclerosis further increases flow and pressure in the remaining glomeruli leading to a gradual progress of CKD (Brenner *et al.*, 1996). The central mediator of this observed glomerular haemodynamic changes seems to be angiotensin II, but it also controls other factors that might be of importance in the progression of kidney disease, such as the production of reactive oxygen species, the regulation of cytokines and profibrotic growth factors, among others. Inappropriate activation of other systems, such as the sympathetic system, the endothelin system and of aldosterone, has also been implicated in the progression of CKD (Gross and Amann, 2004).

2.6 HYPERTENSION

Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated (Chobanian *et al.*, 2003). Blood pressure is associated with two measurements, systolic and diastolic, which depend on whether the heart muscle is contracting (systole) or relaxing between beats (diastole) and equate to a maximum and minimum pressure, respectively. Normal blood pressure at rest is

within the range of 100-140 mmHg systolic (top reading) and 60-90 mmHg diastolic (bottom reading). High blood pressure is said to be present if it is persistently at or above 140/90 mmHg (Lurbe *et al.*, 2009).

Failure to thrive, seizures, irritability, lack of energy, and difficulty breathing may be associated with hypertension in neonates and young infants. In older infants and children, hypertension can cause headache, unexplained irritability, fatigue, failure to thrive, blurred vision, nosebleeds, and facial paralysis (Dionne *et al.*, 2012).

There is compelling evidence from epidemiological studies that hypertension causes a decline in renal function (Ishida *et al.*, 2001; Young *et al.*, 2002) and increases risks of ESKD (Perry *et al.*, 1995; Klag *et al.*, 1996). However, some investigators have questioned whether non-malignant hypertension (in contrast to malignant hypertension) is an important initiator of kidney disease (Hsu *et al.*, 2002; Kincaid-Smith, 2004). Although evidence that hypertension accelerates the progression of already existing renal failure is overwhelming, there is lack of conclusive data from clinical trials that aggressive treatment of hypertension reduces risk of kidney disease onset.

2.6.1 Hydronephrosis

Hydronephrosis refers to distension and dilation of the renal pelvis and calyces, usually caused by obstruction of the free flow of urine from the kidney. Delay in diagnosis and treatment will lead to progressive atrophy of the kidney (Kumar *et al.*, 2014).

The signs and symptoms of hydronephrosis depend upon whether the obstruction is acute or chronic, partial or complete, unilateral or bilateral. Hydronephrosis that occurs acutely with sudden onset (as caused by a kidney stone) can cause intense pain in the flank area (between the hips and ribs). Historically, this type of pain has been described as "Dietl's crisis'' (Mergener *et al.*, 1997). Conversely, hydronephrosis that develops gradually will generally cause no pain or attacks of a dull discomfort.

Furthermore when the flow of the urine is blocked, urinary tract infections develop which will lead to formation of stones and development of fever, blood or pus in the urine. If complete obstruction occurs, kidney failure may follow as stated in the Merck Manuals Home Health Handbook.

2.6.2 Posterior urethral valve

Posterior urethral valves (PUV) occur in 1: 5000 (Zderic and Canning, 2007) live births and in approximately every 1/1250 fetal ultrasound screenings (Gunn *et al.*, 1995). Despite the high prevalence, the most common treatment for valves identified in utero is fetal termination (Farrugia and Woolf, 2010). The few children that survive do poorly, with over 50% progressing to ESRD in 10 years (Roth *et al.*, 2001).

The normal male urethra is anatomically divided into the prostatic and membranous portions (posterior urethra) and the spongy or anterior urethra. The urethral crest is a mucosal ridge that gives a specific form to the posterior urethra, and on either side of the ridge is the prostatic sinus. The urethral crest continues below the verumontanum and coalesces in a small midline bridge. This membrane, extending laterally and downward, eventually vanishes. The classic form of PUV is found in the prostatic urethra, below or proximal to the verumontanum. Although the precise embryologic mechanism of PUV remains unknown, four theories have been proposed to explain their development and include hypertrophy of the urethral mucosal folds, persistence and continuation of the urogenital membrane (Krishnan *et al.*, 2006), abnormal development of the Wolffian or Mullerian duct (Lowsley, 1914) and fusion of the verumontanum or the posterior urethral roof epithelium (Watson, 1922).

2.6.3 Proven recurrent urinary tract infection

A urinary tract infection (UTI) (also known as acute cystitis or bladder infection) is an infection that affects part of the urinary tract. When it affects the lower urinary tract it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract it is known as pyelonephritis (a kidney infection). Symptoms

from a lower urinary tract include painful urination and either frequent urination or urge to urinate (or both), while those of pyelonephritis include fever and flank pain in addition to the symptoms of a lower UTI. In the elderly and the very young, symptoms may be vague or non-specific.

Urinary tract infections (UTIs) are among the most common bacterial infections in childhood, and 2% to 5% of children experience ≥1 before puberty (Winberg *et al.*, 1974; Uhari and Nuutinen, 1988; Hellström *et al.*, 1991; Marild and Jodal, 1998). Childhood UTIs are found to cause kidney scarring and are claimed to lead to impaired kidney function later, especially in the presence of vesicoureteral reflux (VUR) (Jakobsson *et al.*, 1999; Lahdes-Vasama *et al.*, 2006; Swerkersson *et al.*, 2007). Therefore, children are subjected to radiologic imaging after the first UTI and longterm antibiotic prophylaxis if grade III to V VUR is found. The most severe consequence of childhood UTIs has been thought to be chronic kidney disease (CKD), but evidence showing the association between childhood UTIs, renal damage, and CKD in children with structurally normal kidneys is scarce (Hellerstein, 2000).

2.6.4 Steroid resistant nephrotic syndrome

Nephrotic syndrome, or nephrosis is defined by the nephrotic- range proteinuria, edema, hyperlipidemia and hypoalbuminemia. Whiles nephrotic-range proteinuria in adults is characterized by protein excretion of 3.5g or more per day, in children it is defined as protein excretion of more than 40mg/m^2/h or a first morning urine protein/ creatinine of 2-3mg/mg creatinine or greater.

Over 80% of children presenting with an initial episode of nephrotic syndrome responds to steroids (steroid sensitive), whilst the remaining 20% do not respond and are considered as steroid resistant (Churg *et al.*, 1970). Their management is difficult since patients are, on one hand, at risk for complications of unremitting nephrotic syndrome and progressive renal disease and on the other, the side effects of treatment with immunosuppressive medications.

Why some children develops resistance to steroids is not well understood (Haack *et al.*, 1999). Studies have shown that specific genetic mutations constitute a

principal mechanism for steroid resistance. Mutation of NPHS1, NPHS2, ACTN4 and WTI genes are responsible for severe forms of SRNS in children, progressing to end- stage renal failure. Positional cloning has revealed defects in these four different genes as monogenic causes of SRNS in familial cases (Ruf *et al.*, 2004).

2.6.5 Birth weight

There are reports that low birth weight (LBW) is associated with increased risk of death from ischaemic heart disease (Barker, 1993). The elevated risk appears to be limited to those who had low birth weight as a result of growth retardation, rather than to those born prematurely. A considerable number of reports are now published relating intrauterine malnutrition to a number of diseases in adult life including cardiovascular disease (CVD), hypertension, diabetes and renal disease (Godfrey and Barker, 2000; Ingelfinger, 2004). The kidneys appear to be particularly sensitive to an unfavourable prenatal environment (Marchand and Langley-Evans, 2000; Ingelfinger, 2004). Studies of human fetuses and neonates have demonstrated an association between intrauterine growth retardation and nephron number and reduced renal volumes. Oligonephropathy or small number of nephrons may result in hyperfiltration and glomerular hypertension, which might lead to increased future risks of glomerulosclerosis, hypertension and renal failure (Brenner *et al.*, 1996).

2.7 COMPLICATIONS OF CHRONIC KIDNEY DISEASE

Complications associated with chronic kidney disease are as listed in table 2.2.

Table 2.2 List of Complications of CKD

Congestive heart failure	Bone, joint, and muscle pain
Coronary artery disease	Changes in blood sugar
Hypertension	Peripheral Neuropathy
Pericarditis	Dementia
Stroke	Pleural Effusion
Hyperphosphataemia	Heart and blood vessel complications
Hyperkalaemia	Miscarriages and infertility

Secondary hyperparathyroidism	Seizures
Increased risk of infections	Anemia
Liver damage or failure	Bleeding from the stomach or intestines
Malnutrition	Hypermagnesaemia

2.8 ANAEMIA

Anemia is defined when there is a decrease in more than one of the major red blood cell (RBC) indices; hemoglobin concentration, haematocrit, or red blood cell count. Anaemia is defined by the WHO as a hemoglobin <13 g/dL in men and post-menopausal women, <12 g/dL in pre-menopausal women (WHO, 1968). The NKF defines anaemia as haemoglobin <13.5 g/dL in men and <12.0 g/dL in women (NKF/DOQI™, 2002). On the other hand, both NKF and European best practice guidelines advocate assessment of anaemia when haemoglobin level is below 11 g/dL and ponders recombinant human erythropoietin if haemoglobin is constantly <11 g/dL to maintain target haemoglobin of >11 g/dL (National Kidney, 2002).

Normochromic, normocytic anaemia frequently accompanies progressive CKD (Besarab and Levin, 2000) and the general prevalence of CKD-associated anaemia is approximately 50% (McClellan *et al.*, 2004). Regardless of the stage at which anaemia is diagnosed in CKD; a strong correlation exists between the prevalence of anaemia and the severity of CKD. Twenty five percent (25%) of stage 1 CKD patients, fifty percent of those stratified to CKD stages 2, 3, and 4 and seventy five percent (75%) of CKD patients about to start dialysis reportedly have anaemia (McClellan *et al.*, 2004). Anaemia in CKD can result from various mechanisms (iron, folate, or vitamin B_{12} deficiency; blood loss due to-frequent blood sampling, haemodialysis and gastrointestinal bleeding; bone marrow suppression due to uraemic toxins and severe hyperparathyroidism, systemic/chronic inflammation, and shortened red blood cell survival; drugs-ACE inhibitors, angiotensin receptor blockers, theophylline; aluminium excess), decreased erythropoietin synthesis is the most important and specific aetiology causing CKD-associated anaemia. Erythropoietin, a glycoprotein, is secreted by the kidney interstitial fibroblasts

(McClellan *et al.*, 2004) and is vital for the differentiation and growth of red blood cells in the bone marrow. In CKD, tubular atrophy produces tubulointerstitial fibrosis, which compromises renal erythropoietin synthetic capacity and results in anaemia. The anaemia of CKD increases morbidity and mortality from cardiovascular complications (angina, left ventricular hypertrophy (LVH) and worsening heart failure) (Besarab and Levin, 2000), which may result in further decline of kidney function and the establishment of a vicious cycle known as the "cardiorenal anaemia syndrome". The presence of LVH is linked with reduced survival rate of patients on dialysis. In reality, end stage kidney disease patients with LVH have lower survival rates than individuals without LVH (Levin *et al.*, 1996). Additionally, anemia is an independent cause of death in steady coronary artery disease (CAD) patients with CKD (Muzzarelli *et al.*, 2006).

The anaemia of CKD is treated via recombinant human erythropoietin. This intervention has replaced transfusions as the basis of treatment and improved the survival of anaemic CKD patients (Fink *et al.*, 2001). The target level of haemoglobin in patients with CKD has varied as more findings have been reported. The major aim of treatment therefore is no longer to achieve normal levels of haemoglobin since these target levels have been linked with increased mortality (Besarab *et al.*, 1998).

2.8.1 CKD associated mineral and bone disorders

The term "CKD-associated mineral and bone disorders" connotes bone and mineral metabolism abnormalities and/or extra-skeletal calcification secondary to the consequences of CKD (Moe, 2006; Gal-Moscovici and Sprague, 2007). Renal osteodystrophy (ROD) is an array of histological changes, which arise in bone architecture of CKD patients. The primary site of phosphate excretion and 1-αhydroxylation of vitamin D is the kidney. CKD patients develop hyperphosphataemia as a result of reduced 1, 25 dihydroxy-vitamin D levels that indicate decreased synthesis as a result of parenchymal scarring. Moreover, excretion of phosphate by the kidney is reduced. Consequently, serum calcium levels fall resulting in an increase in the rate of production of parathyroid hormone

(secondary hyperparathyroidism). One prominent function of Parathyroid hormone is to increase phosphate excretion in the urine. In addition, it also increases plasma calcium levels by promoting bone resorption and increasing 1-αhydroxylation of 25-hydroxy vitamin D produced in the liver (limited effect because of reduced kidney reserve from scarring). Rising phosphate levels are geneally observed in stage 3 CKD patients. Conversely, bone architecture is distorted quite early by secondary hyperparathyroidism just before the serum phosphate level is noted to be abnormal. This gives an indication that treatment with phosphate binders should start when eGFR have declined below 50 mL/min per 1.73 m^2. A high or low bone turnover can result in changes in bone architecture. Four types of bone phenotypes of ROD can be diagnosed in CKD patients namely osteitis fibrosa cystica (with high bone turnover due to secondary hyperparathyroidism), osteomalacia (resulting in low bone turnover and inadequate mineralization, often associated with reduced synthesis of vitamin D), adynamic bone disorder (with low bone turnover due to over-suppression of the parathyroid glands), and lastly mixed osteodystrophy (with elements of both high and low bone turnover). The major type of ROD and CKD-mineral and bone disorder varies between pre-dialysis and end stage kidney disease patients. High bone turnover bone disease is most common in pre-dialysis patients. Conversely, low bone turnover is common in dialysis patients. Majority of incidents of ROD is found in CKD patients with low turnover bone disease (Joy *et al.*, 2007). This predominant condition is due to the over suppression of parathyroid hormone and high levels of calcium in the dialysis solutions (Hruska *et al.*, 2007). The ability of phosphate retention to stifle the renal synthesis of 1, 25 dihydroxyvitamin D, acidosis and the lack of the physiologic inhibitory effect of vitamin D on parathormone secretion also contribute, albeit small, to the low turnover of bone disease in CKD patients (Llach, 1995). CKD-associated mineral and bone disorders significantly increase mortality in patients with CKD. In reality, hyperphosphatemia has been identified as the most significant risk factor associated with cardiovascular disease in CKD patients (Lee *et al.*, 2007). The precise mechanism underlying this relationship is still unclear. It is believed to be related to hyperparathyroidism (El-Kishawi and El-Nahas, 2006) and vascular

calcification due to elevated phosphate levels (Hutchison, 2007). The use of calcium -based binders and excessive vitamin D therapy (Moe, 2006) influence vascular calcification and the associated cardiovascular mortality. Patients on haemodialysis with plasma phosphate level above the K/DOQI guideline objectives have a 40% higher rate of mortality compared to those having lower target levels (Noordzij *et al.*, 2005). The main objective of therapy of CKD-associated mineral and bone disorders is to reduce phosphate levels (Coresh *et al.*, 2007). When phosphate or parathyroid levels begin to rise, the primary therapy is to restrict dietary phosphate intake. Serum phosphate concentrations should be maintained between

2.7 and 4.6 mg/dL among patients with CKD stages 3 and 4, and between 3.5 and 5.5 mg/dL for those with stage 5 CKD according to KDOQI guidelines. Various groups of phosphate binders can be applied to achieve this goal. For the treatment of chronic conditions calcium-based formulations for the management of hyperphosphataemia due to CKD are the most widely used and have replaced aluminium binders since aluminum-associated toxicities have been established. However, calcium-based phosphate binders can induce hypercalcaemia, which increases the tissue calcium deposition, especially in the presence of hyperphosphatemia.

2.9 CLASSIFICATION AND STAGING OF CHRONIC KIDNEY DISEASE

The level of kidney function in all patients with chronic kidney disease can be uniformly measured regardless of the fundamental cause of the disease (Levey *et al.*, 2009). In the past, there has been a lack of agreement on how the progression of chronic kidney disease should be defined and classified. This may have contributed to under-diagnosis and under-treatment of early kidney disease resulting in lost opportunities for slowing or preventing disease progression (Levey *et al.*, 1999; Pereira, 2000). In literature, it is widely agreed that starting treatment at the right stage in the progression of CKD is essential in slowing disease progression and prevent adverse outcomes (Levey *et al.*, 1999; Pereira, 2000). In an attempt to reach a consensus and provide a common ground on which

to base future treatment and research, the American NKF/KDOQI work group developed a classification system that separated the period from very early kidney disease to ESKD into five stages (NKF/DOQI™, 2002). Definitions were based on renal function as measured by the GFR of the patient. Normal kidney function is said to equate to a glomerular filtration rate of 120-125 ml/min with deterioration in kidney function correlating with a reduction in the glomerular filtration rate. Table 2.3 describes the five stages of chronic kidney disease.

Table 2.3 CKD staging by KDOQI

Stage	Description	GFR (mL/min/1.73 m^2)
1	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with mildly decreased GFR	60-89
3	Moderate kidney disease	30-59
4	Severe kidney disease	15-29
5	Kidney failure	<15 or dialysis

Stage 1 of CKD is described as the very early period of the disease where only minor kidney damage has occurred. Usually, clinical symptoms are absent at this point, which make diagnosis very difficult. This is the ideal time to provide treatment for the underlying kidney disease, along with appropriate management of allied conditions like hypertension and diabetes.

Patients who are classified as having Stage 2 CKD have a glomerular filtration rate of between 60 and 89 ml/min/1.73 m^2 and suffer from a mild degree of kidney damage. Aggressive management of the underlying causes of the disease and emerging manifestations, for example, calcium and phosphate imbalance, hyperglycaemia and anaemia, are recommended (Silverberg *et al.*, 2003).

Stage 3 CKD indicates a further decline in kidney function with possibly some clinical signs beginning to appear. As mentioned previously, it is not uncommon for a patient to reach this stage of the disease without knowing that they have a problem. Again ongoing specialist treatment and follow up of these patients is

essential to try and maintain kidney function and prevent such complications as cardiovascular disease, anaemia, malnutrition and bone disease.

Stage 4 of CKD means that end stage failure is imminent and preparation for renal replacement therapy (dialysis or transplantation) is required.

Stage 5 CKD is defined as ESKD where dialysis or transplantation is mandatory to sustain life.

The need to provide a common language for communication among providers, patients and their families, investigators and policy-makers was the reason the American National Kidney Foundation developed the five-stage classification system. Defining chronic kidney disease this way provides opportunities to direct the most effective treatment at a particular stage of the disease process.

In addition, classification seeks to provide a framework for developing guidelines for clinical practice, clinical performance measures, and improvement of continuous quality tasks. The classification of the stages of kidney disease by the American National Kidney Foundation has been integrated in some recent American and British literature in association with policies for prevention and early discovery of CKD (Parmar, 2002). As this classification system has only been available a little over decade it is difficult to predict the extent to which it will be utilized internationally. Kidney function and the outcome of kidney disease have been outlined along with a way of defining the loss of kidney function into stages. The five stages of chronic kidney disease, as described by the KDIGO (Kidney Disease, Improving Global Outcome) in table 2.4, were developed in an attempt to provide a common language for nephrology health care professionals to use to promote international best practice in the management of CKD.

Table [1].4 CKD staging using KDIGO

Stage	**GFR(mL/min/1.73 m [2])**	**Terms**
1	≥90	

[1].10 GLOMERULAR FILTRATION

		Normal or high
2	60-89	Mildly decreased
3a	45-59	Mildly to moderately decreased
3b	30-44	Moderately to severely decreased
4	15-29	Severely decreased
5	<15	Kidney failure

2.10.1 Clearance Methods

The idea behind renal clearance was proposed as a way of expressing the relationship between the excretion per unit time and the concentration in the plasma which is obviously an index of the kidney's ability to clear the blood of any substance. Measurements of GFR are by tradition based on the renal clearance of a plasma marker, expressed as the volume of plasma wholly cleared of the marker per unit time. If the marker has no extra-renal elimination, tubular reabsorption or secretion then the clearance is defined by the formula;

$$GFR = \frac{UV}{P}$$

Where

U = Urinary Concentration of the substance

Glomerular filtration rate (GFR) is defined as the rate at which filtered fluid flows through the kidney. Creatinine clearance (CCr or CrCl) refers to the amount of blood plasma cleared of creatinine per unit time and is a convenient measure for estimating the GFR. Together, GFR and CCr may well be accurately calculated by relative measurements of substances in the blood and urine, or calculated by formulas using only a blood test result (eGFR and eCCr). These test results are important in the assessment of the excretory capabilities of the kidney. For example, classification of CKD and dosage of drugs that are excreted mostly in urine are based on GFR (or creatinine clearance). Various methods of estimating GFR are briefly described below:

V = Urine flow rate (urinary volume/time)

P = Average plasma concentration

The perfect marker should be endogenous; in addition it must be filtered freely by the glomerulus. Furthermore it should neither be reabsorbed nor secreted by the renal tubule and eliminated solely by the kidney. A variety of markers used to measure GFR include exogenous (inulin, iothalamate) or endogenous (urea, creatinine) substances (Harvey, 1980).

2.10.2 Exogenous Substances

2.10.2.1 Inulin

Inulin, a polymer of fructose with a molecular weight of 5200 daltons is regarded as the gold standard for the estimation of GFR. It is filtered freely by glomerulus, and is neither secreted nor reabsorbed by the kidney tubules.

Metabolically, it is inert and excreted only by the kidney. It needs constant intravenous infusion to keep up plasma levels and once a balanced state has been achieved, plasma and timed urine specimen levels of the marker are measured. However, analysis of inulin is technically challenging, time consuming, labour intensive, expensive and unsuitable for outpatient use (Smith, 1951).

2.10.2.2 Non-radiolabelled contrast media

Besides inulin, non-radiolabelled contrast media infusion (iothalamate/iohexol) have also been used to measure GFR. The ability to perform urography and estimation of GFR in a single examination is a major advantage (Brown and O'Reilly, 1991). Cumbersome measurement makes it inappropriate for day to day clinical practice.

2.10.2.3 Radiolabelled compounds

A number of radiolabelled substances have been used to measure the GFR in humans, as very small non-poisonous amounts of the compound can be used and can be measured using conservative counters even at very low concentrations. Amongst these are [^{51}Cr] EDTA, [^{125}I] iothalamate, [^{99}Tcm] DTPA, and [^{131}I] Hippuran just to mention a few (Donker *et al.*, 1977; Apperloo *et al.*, 1996).

Disadvantages are that some radiation is administered, radiopharmaceuticals are very costly, Gamma camera and skilled personnel are required. Therefore it is impossible to use the chelates for the routine assessment of GFR.

2.10.3 Endogenous Substances

2.10.3.1 Urea

Urea (MW 60 dalton) was one of the first markers for assessing GFR (Chasis and Smith, 1938), however, presently is not used in clinical practice due to numerous reasons. Urea production is erratic and changes with protein intake. Readily it is reabsorbed by tubules and again amount of reabsorption is erratic. Hydration status of the individual also affects urea clearance clearly; in patients with depleted intravascular volume, high plasma levels accompany decreased urine flow. Also many substances may interfere with its estimation.

2.10.3.2 Creatinine

Creatinine (M.W 113 daltons) is produced through nonenzymatic dehydration of creatine and phosphocreatine. Muscle mass, therefore, is the main determinant (98%) of the creatinine pool. Dietary consumption of meat is another source of creatinine. Endogenous creatinine clearance gives as an estimate of GFR and is still highly patronized in clinical practice. However, its performance and interpretation present alarming difficulties: Changes in the rate of production of creatinine, accurate measurement of plasma creatinine, some level of secretion by the renal tubules and the difficulty of obtaining complete, accurately timed urine specimens (Payne, 1986; Spencer, 1986).

Creatinine is generally measured by the Jaffë colorimetric reaction used over the past century, using alkaline picrate with which it forms an orange red complex. Numerous substances such as ascorbic acid, uric acid, ketones and ketoacids, plasma proteins, bilirubin, fatty acids, urea, cephalosporins and glucose interfere with Jaffë's colorimetric assay for estimation of plasma creatinine resulting in erroneously high values.

Furthermore, tubular secretion and induction of true elevation of plasma creatinine is inhibited by drugs such as triametrine, spironolactone, amiloride, probenecid,

cimetidine, trimethoprim and high dose salicylates or pyrimethamine (Gerard and Khayam-Bashi, 1985; Weber and Van Zanten, 1991). Enzyme based assays have enhanced precision comparable to high performance liquid chromatographic techniques because they lack this interference (Gerard and Khayam-Bashi, 1985). As a result of tubular secretion, creatinine clearance (Ccr) usually overestimates GFR. This represents 10-40% of GFR in normal renal function with clear interindividual variability. In patients with decreased kidney function, tubular secretion can increase to above 100% especially among those with glomerulopathy and proteinuria (Shemesh *et al.*, 1985).

2.10.3.3 GFR estimation using serum creatinine.

An estimate of bed side GFR is often obtained from plasma creatinine concentration alone in clinical practice though with some level of accuracy (Perrone *et al.*, 1992). A formula that will permit an immediate estimation of GFR from plasma creatinine has been considered by a number of researchers. Approximation of GFR from plasma creatinine may give erratic results because plasma creatinine is dependent on GFR as well as on muscle mass which varies with gender, age and weight. Cirrhosis and muscle wasting diseases lead to a reduction in plasma creatinine; conversely ingestion of high amounts of protein can result in increase in plasma creatinine levels of up to 10% (Hull *et al.*, 1981). Furthermore, a marked reduction in GFR can be present before it shows in the concentration of plasma creatinine beyond the upper limit of the reference range. The value of these formulae for GFR prediction is likely to increase when there is an accurate plasma creatinine measurement in addition to inhibition of tubular secretion of creatinine by cimetidine. To improve the estimation of GFR from plasma creatinine concentration, formulae have been derived which incorporate variables like weight, height, age, and gender.

2.10.4 GFR estimation by new endogenous markers

2.10.4.1 β2-Microglobulin β-microglobulin (M.W 11815 dalton) is filtered at glomerulus like water. Afterwards almost the entire substance is reabsorbed and

broken down in the renal tubule. The plasma concentration in health is often low because it is filtered so freely (average 1.5 mg/L). The plasma concentration increases as the glomerular filtration rate declines reaching about 40 mg/l in terminal uremia. Plasma βmicroglobulin concentration logarithm is linearly related to the logarithm of glomerular filtration rate through the whole range so that it serves as a good marker of renal dysfunction. The plasma concentration of β-microglobulin is neither affected by muscle mass nor by the sex of an individual. The estimation of this substance entails the use of expensive radioimmunoassays and this has limited its use in clinical practice. Rise in plasma concentration could be due to increased production rather than reduced clearance in patients with some tumors and inflammatory diseases.

2.10.4.2 Cystatin C

Cystatin C is a 13-KD protease inhibitor which is produced generally by nucleated cells. It is neither affected by the muscle mass nor sex of an individual. Its production, unlike β2-microglobulin is not affected by states of inflammation or malignant conditions. Cystatin C is usually excreted by filtration through the glomerulus and metabolized in the cells of the proximal tubules. Its measurement has been projected as an alternative and more precise marker of GFR compared to creatinine especially among patients with slight to moderate reductions in GFR (Grubb *et al.*, 1985; Newman *et al.*, 1995).

2.10.4.3 Measurement of GFR using predictive equations

Multiple formulae exist to accurately estimate kidney function by correcting for factors such as variations in muscle mass in men versus women or in African American versus white people and changes in muscle mass due to aging. The most commonly used equations are the Cockroft-Gault (CG) equation (Cockcroft and Gault, 1976) and the 4v and 6v Modification of Diet in Renal Disease (MDRD) (Levey *et al.*, 1999) equations. Recently, the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula, (published in May 2009), has been added. This was developed in a bid to create a formula more detailed compared to the MDRD formula, particularly when real GFR is > 60 mL/min per 1.73 m^2(Levey *et al.*, 2009). Rule *et al.* (2006) have maintained that, because these formulae are

derived from patients with kidney disease, they may not predict kidney function in patients without kidney disease. Mostly, clinicians use the MDRD equation because of its accessibility on the internet, where one can simply insert in values for age, weight, race, and sex to give an estimated GFR. It should be noted that all these formulae have large confidence intervals such that insignificant changes in actual GFR are difficult to distinguish by this method.

In the case of children, the most widely-used formula is the Schwartz-formula (Schwartz *et al.*, 1976) together with modifications for adolescent boys (Schwartz and Gauthier, 1985) and infants (Schwartz *et al.*, 1987). The Counahan-Barratt formula may be slightly more suitable for GFR estimation.

Chapter 3 MATERIALS AND METHODS

3.1 STUDY PERIOD/SITE

The study was conducted between June and November 2014 at the Korle-Bu Teaching Hospital with eligible participants being drawn from the Renal Clinic, Outpatient Department (OPD) and Wards within the Department of Child Health respectively. Korle-Bu Teaching Hospital is the premier health care facility in Ghana and the only tertiary hospital in the southern part of Ghana. The hospital was established in 1923 with an initial bed capacity of 200 but currently has a bed capacity of 2,000 with 17 clinical and diagnostic Departments/Units which includes Medicine, Child Health, Obstetrics and Gynaecology, Pathology, Laboratories, Radiology, Anaesthesia, Surgery, Polyclinic, Accident Centre and the Surgical/Medical Emergency as well as Pharmacy. Other Departments include Pharmacy, Finance, Engineering and General Administration. The hospital is currently the third largest hospital in Africa and the leading national referral centre with an average daily attendance of 1,500 patients and about 250 patient admissions. The Department of Child Health serves as a referral centre for children suffering from various types of infections and severe diseases including complicated malaria, diarrhoea, pneumonia, meningitis, tuberculosis, severe anaemia, childhood cancers, heart abnormalities and premature babies (***source: www.kbth.gov.gh***)

3.2 SAMPLING AND STUDY SUBJECTS

3.2.1 Sample size estimation

Sample size was determined by the Cochran's method using an estimated chronic kidney disease prevalence of 3% based on an average of prevalence rates quoted in published works done in Nigeria (5%) (Anochie and Eke, 2003) and in South Africa (1 – 2%) (Bhimma *et al.*, 2008).

$$n_0 = \frac{Z^2 \times p(1-p)}{e^2}$$

Where: n_0 = sample size

Z^2 = abscissa of the normal curve that cuts off an area (α) at the tails (1.96)

e = the acceptable sample error at 95% confidence interval (0.05) p = the estimated proportion of the attribute present in the population (3%)

$$n_0 = \frac{(1.96)^2 \times 0.03(1 - 0.03)}{(0.05)^2}$$

$$n_0 = \frac{(3.84) \times (0.029)}{2.5 \times 10^{-3}}$$

$$n_0 = \frac{0.1114}{2.5 \times 10^{-3}}$$

$$n_0 = 44.54 \cong 45$$

3.2.2 Study participants

A purposive sampling technique was utilized to select a total number of 90 study participants' comprising 45 patients in the case group and 45 patients in the control group based on set inclusion and exclusion criteria. Participants were age matched with ages ranging between 2 – 12 years.

3.2.2.1 Inclusion criteria

Patients with varied medical conditions ranging from polycystic kidney disease (PKD), posterior urethral valve hydronephrosis, steroid resistant nephrotic syndrome (SRNS), proven recurrent UTI to those on anti-hypertensive drugs. Some patients also had co-morbid conditions comprising PUV and UTI, hydronephrosis and UTI and Hydronephrosis and PUV. Patients from the same departments diagnosed with medical conditions other than those stated for the case group were recruited alongside as controls.

3.2.2.2 Exclusion criteria

Patients who were older than 12 years of age and who did not fit the stated criteria for the case and control group were excluded from the study.

Participation of the respondents was voluntary and informed consent was sought from the parents or guardians who accompanied the patients to the facility after being taken through the study protocol. The study was approved by the Committee on Human Research Publication and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology.

3.3 SAMPLING

3.3.1 Blood sample collection and preparation

Five millilitres (5 ml) of venous blood sample was collected from the antecubital vein of each study participant into BD vacutainer® gel tubes after disinfecting the venepuncture site with 70% ethanol using vacutainer® closed system. The blood samples were then centrifuged at 1500g for 5 minutes and the serum aliquoted and stored at - 80°C until assayed. Serum samples were analysed for urea, electrolytes (sodium, potassium), creatinine,protein and albumin using Selectra Pro S chemistry analyser (Namarka, Germany) and Ellitec reagent from Germany.
Cystatin C was estimated using Diazyme's Cystatin C Assay (Human, Germany).

3.3.2 Anthropometry

Anthropometric parameters measured include height to the nearest centimetre without shoes and weight to the nearest 0.1 kg in light clothing. Study participants were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, and China) and their height measured with a wall-mounted ruler. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2).

3.3.3 Urinalysis

Early morning urine was collected into wide-mouth plastic containers from the respondents and urine protein, urine albumin and urine creatinine levels were estimated using a complete chemistry autoanalyzer (Selectra Pro S) with Ellitec reagents (Namarka, Germany).

3.3.4 Albumin Estimation

3.3.4.1 Principle

At a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of colour at 630 nm is directly proportional to albumin content.

$$BCG + Albumin \xrightarrow{controlled\ pH} Green\ BCG/Albumin\ complex$$

3.3.5 Protein Estimation

3.3.5.1 Principle

The present method is based on the modification of the method developed by (Gornall *et al.*, 1949). Protein forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of protein present when compared to a standard protein concentration.

$$Protein + Cu^{2+} \xrightarrow{alkaline} Coloured\ complex$$

3.3.6 Urea estimation

3.3.6.1 Principle

The present procedure is based on a modification of the method of Talke and Schubert (1965). Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with α-ketoglutarate in the presence of NADH to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction catalyzed by glutamate dehydrogenase (GLDH) resulting in a decrease in absorbance (340 nm) that is directly proportional to the urea nitrogen concentration in the sample.

$$Urea + H_2O \xrightarrow{urease} 2NH_3 + CO_2$$

$$NH_3 + \alpha - ketoglutarate + NADH \xrightarrow{GLDH} L - glutamate + NAD^+$$

3.3.7 Creatinine estimation

3.3.7.1 Principle

Creatinine measurements are used in the assessment of renal dysfunction. Elevated creatinine levels are found in renal diseases and insufficiency with decreased

glomerular filtration (uremia or azotemia if severe); urinary tract obstruction; reduced renal blood flow including congestive heart failure, shock and dehydration.

This method is based on a modification of the kinetic procedure which is fast, simple and avoids interferences (Fabiny and Ertingshausen, 1971), incorporating a surfactant and other ingredients to minimize protein and carbohydrate interferences. Creatinine reacts with picric acid in alkaline conditions to form a colour complex (yellow-orange) which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine in the sample.

$$\boldsymbol{Creatinine + Sodium\ picrate \xrightarrow{alkaline} creatinine - picrate\ complex}$$

3.3.8 Cystatin C estimation

3.3.8.1 Principle

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

3.4 RENAL FUNCTION EQUATIONS

The six renal function equations evaluated are listed below; Four (4) of the equations used serum cystatin C (Cys) while two (2) used serum creatinine (SCr) levels to predict renal function.

3.4.1 Cystatin C-based estimating equation

$$(i)\ \boldsymbol{Le\ Bricon\ (ml/min\ per\ 1.73m^2)} = \left(\frac{\boldsymbol{78}}{\boldsymbol{Cys\ C}}\right) + \boldsymbol{4}$$

$$(ii)\ \boldsymbol{Larsson}\ (\boldsymbol{ml/min}) = \boldsymbol{77.24} \times (\boldsymbol{Cys\ C^{-1.2623}})$$

$$(iii)\boldsymbol{Rule}(\boldsymbol{ml/min\ per\ 1.73m^2} = \boldsymbol{76.6} \times \boldsymbol{Cys\ C^{-1.16}}$$

$$(iv)\ \boldsymbol{Zappitelli}\ (\boldsymbol{ml/min\ per\ 1.73m^2}) = \boldsymbol{75.94} \times \boldsymbol{Cys\ C^{-1.17}}$$

Units: Cystatin C = mg/L

3.4.2 Creatinine based estimating equation

$$(i)\ \boldsymbol{Schwartz}\ (\boldsymbol{ml/min\ per\ 1.73m^2}) = \boldsymbol{36.5} \times \frac{\boldsymbol{height}}{\boldsymbol{Scr}}$$

$$(ii)\ \boldsymbol{Counahan - Baratt} = \boldsymbol{38.4} \times \frac{\boldsymbol{height}}{\boldsymbol{Scr}}$$

Units: Height = cm; Creatinine = [mg/dL (Schwartz); µmol/L (Counahan-Baratt]

3.5 STAGING OF CKD

The GFR results from the various renal function equations were used to stratify the study population into five categories corresponding with the five stages of CKD in the K/DOQI CKD classification (National Kidney, 2002). The staging classified GFR ≥ 90 ml/min/1.73 m² as stage 1; 60-89 ml/min/1.73 m² as stage 2; 30-59 ml/min/1.73 m² as stage 3; 15-29 ml/min/1.73 m² as stage 4; and < 15 ml/min/1.73 m² as stage 5. The Kidney Disease Improving Global Outcome (KDIGO) was also used for classification where appropriate. The staging classified GFR ≥ 90 ml/min/1.73 m² as stage 1; 60-89 ml/min/1.73 m² as stage 2; 45-59

ml/min/1.73 m² as stage 3a; 30-44ml/min/1.73 m² as stage 3b; 15-29 ml/min/1.73 m² as stage 4; and < 15 ml/min/1.73 m² as stage 5.

3.6 STATISTICAL ANALYSIS

All data were entered into Microsoft Excel and double cleaned for multiple entries. Continuous variables were expressed as means ± SD and categorical data were

expressed as absolute numbers and proportions. Continuous variables were compared using the students' *t*-test and categorical variables were compared using Chi-square or Fisher's test as appropriate. Bland-Altman plot was performed to compare creatinine-based equations with cystatin C-based equations for eGFR determination. Sensitivity and specificity values for the predictive equations in detecting subnormal GFR (i.e. GFR < 60 ml/min/1.73 m^2) were also calculated using Receiver Operator Characteristic (ROC) analysis. In all analysis, a p-value <0.05 was considered statistically significant. GraphPad Prism version 5 (GraphPad software, California, USA) for windows and SYSTAT version 12 (SYSTAT software, CA, USA) were used for statistical analysis.

Chapter 4 RESULTS

4.1 GENERAL CHARACTERISTICS OF THE STUDIED POPULATION

The general characteristics of the studied population are as presented in Table 4.1. There was no significant difference in age when the case group (6.1 ± 0.6 years) was compared with the control group (7.5 ± 0.5 years; p=0.077). A comparison of BMI among the case and control groups was not statistically significant (p=0.961) but the respondents within the control group were significantly heavier (24.5 ± 9.8 kg) and taller (1.2 ± 0.2 m) when compared with their counterparts within the case group (19.8 ± 11.3 kg, p=0.041; 1.1 ± 0.3 m, p=0.005). Likewise, the percent body fat (%BF) in the control group (17.2 ± 5.0%) was significantly higher when compared with that in the case group (13.8 ± 4.2%; p=0.001). No significant differences were observed in systolic and diastolic pressures for respondents from the two main groups.

Analysis of the serum analytes measured within the study respondents showed significant increases in urea (5.6 ± 5.4 vs 3.1 ± 1.5 mmol L^{-1}; p=0.004), creatinine (74.2 ± 52.4 vs 49.3 ± 10.5 μmol L^{-1}; p=0.003) and cystatin C (1062 ± 274.3 vs *854.7* ± *109.9* μg L^{-1}; p<0.000) in the case group when compared to the control group. Conversely, serum protein (56.7 ± 14.3 vs 69.5 ± 6.7 g L^{-1}; p<0.000) and albumin (30.7 ± 10.0 vs 39.2 ± 5.0 g L^{-1}; p<0.000) concentrations in the case group were significantly decreased when compared with the control group.

Urinary albumin (3.0 ± 4.5 g L^{-1}) and protein (4.7 ± 5.6 g L^{-1}) concentrations estimated for the case group were significantly higher compared with that estimated in the control group (0.2 ± 0.1 g $L^{-1}$and 1.6 ± 0.9 g L^{-1} respectively). The urinary creatinine concentration was, however, decreased in the case group (3059 ± 2812 mg dL^{-1}) compared with the control group (6971 ± 3764 mg dL^{-1}).

Proteinuria was assessed in the case group and controls by estimating urine protein – creatinine ratio (Upr/cr). Proteinuria was defined as normal (<0.2), significant (0.2 – 2.0) and nephrotic (>2.0). From Table 4.1, a Chi-square for trend analysis showed a significant increase in the numbers of participants in the case and control groups,

respectively, from normal through significant to nephrotic proteinuria.In the control group 4.4% (2/45) of respondents were normal whilst 40.0% (18/45) and 55.6% (25/45) were within the significant and nephrotic proteinuria range respectively. Contrarily, none of the respondents in the case group were normal, however 17.8% (8/45) and 82.2%(37/45) fell within the significant and nephrotic range, respectively.

Table 4.1General characteristics of the studied population

	Control Group	Case Group	
Parameters	**n=45**	**n=45**	**p-value**
Age (years)	7.5 ± 0.5	6.1 ± 0.6	0.077
Weight (kg)	24.5 ± 9.8	19.8 ± 11.3	0.041
Height (m)	1.2 ± 0.2	1.1 ± 0.3	0.005
BMI (kg m^{-2})	15.9 ± 2.8	15.9 ± 2.1	0.961
Systolic (mmHg)	101.1 ±9.2	101.1 ± 15.2	1.000
Diastolic (mmHg)	67.7 ± 8.8	66.5 ± 13.7	0.637
%BF	17.2 ± 5.0	13.8 ± 4.2	0.001
Serum Analytes			
Sodium ($mmolL^{-1}$)	141.5 ± 1.8	141.5 ±1.9	0.962
Potassium ($mmolL^{-1}$)	4.2 ± 0.3	4.3 ± 0.3	0.708
Urea ($mmolL^{-1}$)	3.1 ± 1.5	5.6 ± 5.4	0.004
Creatinine ($\mu molL^{-1}$)	49.3 ± 10.5	74.2 ± 52.4	0.003
Albumin (gL^{-1})	39.2 ± 5.0	30.7 ± 10.0	< 0.000
Protein (gL^{-1})	69.5 ± 6.7	56.7 ± 14.3	< 0.000
Cystatin C (μgL^{-1})	854.7 ± 109.9	1062 ± 274.3	< 0.000
Urinary Analytes			
Albumin (gL^{-1})	0.2 ± 0.1	3.0 ± 4.5	0.000
Protein (gL^{-1})	1.6 ± 0.9	4.7 ± 5.6	0.000
Creatinine (mg dL^{-1})	6971 ± 3764	3059 ± 2812	< 0.000
Upr/Cr			
Normal (<0.2)	2(4.4)	0(0.0)	
Significant (0.2 – 2.0)	18(40.0)	8(17.8)	
Nephrotic (>2.0)	25(55.6)	37(82.2)	0.004[a]

***Data are presented as mean±SD, median and range; BMI=body mass index; %BF=percent body fat; p-value represents the level of significance when the case group were compared to the control group (paired t-test); UPr/Cr=urine protein-creatinine ratio; [a]Significant when compared (Chi-square for trend*).**

4.2 ESTIMATES OF GFR AS DETERMINED WITH SELECTED EQUATIONS

Estimates of GFR as determined with the selected equations for the studied population are shown in Table 4.2. The mean GFR estimates for the control group were significantly higher ($p=0.006$ and $p<0.0001$) when compared to that estimated for the case group although none of the mean GFR's for the case group dropped below the cut-off of 60 ml min^{-1} 1.73 m^{-2} used for confirmation of the presence of chronic kidney disease. The prevalence of CKD as determined with the Schwartz and Counahan-Baratt equations within the case group was 35.6% (16/45) respectively while the Larsson, Rule and Zappitelli formulae yielded a CKD prevalence of 20.0% (9/45) respectively. Le Bricon, yielded a CKD prevalence of 13.3% (6/45) representing the least recorded prevalence from the same cohorts within the case group.

eGFR Equation	**Control Group (n = 45)**		**Case Group (n = 45)**		
(ml min^{-1} 1.73 m^{-2})	**Mean**	**Range**	**Mean**	**Range**	**p-value**
Schwartz	91.8 ± 16.3	65.5 – 134.5	73.9 ± 39.1	11.5 – 171.6	0.006
< 60	(0.0)		(35.6)		
≥ 60	(100.0)		(64.4)		
Counahan-Baratt	96.5 ± 17.2	68.9 – 141.5	77.8 ± 41.1	12.0 – 180.6	0.006
< 60	(0.0)		(35.6)		
≥ 60	(100.0)		(64.4)		
Larsson	96.5 ± 16.8	73.9 – 132.7	77.6 ± 22.2	36.4 – 132.4	< 0.0001
< 60	(0.0)		(20.0)		
≥ 60	(100.0)		(80.0)		
Rule	93.9 ± 14.9	73.6 – 125.9	76.7 ± 20.2	38.3 – 125.7	< 0.0001
< 60	(0.0)		(20.0)		
≥ 60	(100.0)		(80.0)		
Zappitelli	93.3 ± 14.9	72.9 – 125.4	76.0 ± 20.2	37.8 – 125.2	< 0.0001
< 60	(0.0)		(20.0)		
≥ 60	(100.0)		(80.0)		
Le Bricon	96.8 ± 12.7	79.3 – 123.4	81.7 ± 17.8	46.9 – 123.6	< 0.0001

< 60	(0.0)	(13.3)
≥ 60	(100.0)	(86.7)

Table 4.2 Estimates of GFR as determined with selected equations for the studied population

Continuous data are presented as mean±SD, median and range; categorical data are presented as percentages. P-value defines the level of significance when the case group was compared to the control group (paired t-test).

4.3 STRATIFICATION OF STUDY PARTICIPANTS WITHIN THE CASE AND CONTROL GROUP INTO CKD STAGES USING THE PRESENCE OR ABSENCE OF PROTEINURIA

Table 4.3. presents a distribution of the study participants within the case group into the categories of CKD stages using proteinuria defined by urine protein – creatinine ratio. Thirty-seven (37) out of the 45 participants within the case groups showed proteinuria within the nephrotic range with the remaining 8 showing significant proteinuria. Using the creatinine–based eGFR equations, 14 out of the 37 participants within the nephrotic range had an eGFR ≤60 ml/min/1.73m^2 with the other 23 having an eGFR >60 ml/min/1.73m^2. For the cystatin C–based equations, Larsson, Rule and Zapitelli equations respectively showed 8 of the 37 nephrotic participants with an eGFR ≤60 ml/min/1.73m^{-2} and the remaining 29 having eGFR>60 ml/min/1.73m^{-2}. The Le Bricon equation showed 5 of the 37 within the nephrotic range having an eGFR ≤60 ml/min/1.73m^{-2} with the remaining 32 having eGFR>60 ml/min/1.73m^{-2}.

Table 4.3 Stratification of study participants within the case group into CKD stages using the presence or absence of proteinuria

CKD Staging	Normal n = 0(%)	Significant n = 8(%)	Nephrotic n = 37(%)	Normal n = 0(%)	Significant n = 8(%)	Nephrotic n = 37(%)
	Creatinine-based eGFR equations					
	Schwartz			**Counahan-Baratt**		
Stage 1	0(0.0)	2(25.0)	16(43.2)	0(0.0)	3(37.5)	16(43.2)
Stage 2	0(0.0)	4(50.0)	7(19.0)	0(0.0)	3(37.5)	7(19.0)

Stage 3A	0(0.0)	0(0.0)	2(5.4)	0(0.0)	0(0.0)	4(10.8)
Stage 3B	0(0.0)	0(0.0)	6(16.2)	0(0.0)	0(0.0)	5(13.5)
Stage 4	0(0.0)	2(25.0)	3(8.1)	0(0.0)	2(25.0)	3(8.1)
Stage 5	0(0.0)	0(0.0)	3(8.1)	0(0.0)	0(0.0)	2(5.4)

Cystatin C – based eGFR equations

		Larsson			**Rule**	
Stage 1	0(0.0)	4(50.0)	8(21.6)	0(0.0)	4(50.0)	8(21.6)
Stage 2	0(0.0)	3(37.5)	21(56.8)	0(0.0)	3(37.5)	21(56.8)
Stage 3A	0(0.0)	0(0.0)	5(13.5)	0(0.0)	0(0.0)	6(16.2)
Stage 3B	0(0.0)	1(12.5)	3(8.1)	0(0.0)	1(12.5)	2(5.4)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
		ıpitelli			**Le Bricon**	
Stage 1	0(0.0)	4(50.0)	8(21.6)	0(0.0)	4(50.0)	11(29.7)
Stage 2	0(0.0)	3(37.5)	21(56.8)	0(0.0)	3(37.5)	21(56.8)
Stage 3A	0(0.0)	0(0.0)	5(13.5)	0(0.0)	1(12.5)	5(13.5)
Stage 3B	0(0.0)	1(12.5)	3(8.1)	0(0.0)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Proteinuria was classified using urine protein to creatinine (Upr/cr) ratio cut-offs as follows: normal (Upr/cr <0.2); significant (Upr/cr 0.2 – 2.0) and nephrotic (Upr/cr >2.0). eGFR = estimated GFR

Table 4.4 presents a distribution of the study participants within the control group into the categories of CKD stages using proteinuria defined by urine protein – creatinine ratio. Generally across the distribution, none of the respondents within the control group had a GFR value ≤60 ml/min/1.73m². Two (2) out of the 45 respondents had proteinuria within the normal range whilst 18 (40.0%) and 25 (55.6%) fell within the significant and nephrotic ranges, respectively. For the creatinine-based equations, the 18 respondents with significant proteinuria had eGFR >60 ml/min/1.73m^{-2} likewise the 25 respondents who fell within the nephrotic range. The percentage distribution outcome for the cystatin c-based

equations was similar to that of the creatinine-based equations with variations in the point percentages estimated for stages 1 and 2.

Table 4.4 Stratification of study participants within the control group into CKD stages using the presence or absence of proteinuria

CKD Staging	Normal n = 2(%)	Significant n = 25(%)	Nephrotic n = 2(%)	Normal n = 18(%)	Significant n = 25(%)	Nephrotic n = 18(%)
			Creatinine-based eGFR equations			
	Schwartz			**Counahan-Baratt**		
Stage 1	1(50.0)	10(55.6)	11(44.0)	2(100.0)	14(77.8)	13(52.0)
Stage 2	1(50.0)	8(44.4)	14(56.0)	0(0.0)	4(22.2)	12(48.0)
Stage 3A	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 3B	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
			Cystatin C – based eGFR equations			
	Larsson			**Rule**		
Stage 1	0(0.0)	10(55.6)	17(68.0)	0(0.0)	10(55.6)	16(64.0)
Stage 2	2(100.0)	8(44.4)	8(32.0)	2(100.0)	8(44.4)	9(36.0)
Stage 3A	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 3B	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Zapitelli			**Le Bricon**		
Stage 1	0(0.0)	10(55.6)	13(52.0)	0(0.0)	10(55.6)	18(72.0)
Stage 2	2(100.0)	8(44.4)	12(48.0)	2(100.0)	8(44.4)	7(28.0)
Stage 3A	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 3B	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Proteinuria was classified using urine protein to creatinine (Upr/cr) ratio cut-offs as follows: normal (Upr/cr <0.2); significant (Upr/cr 0.2 – 2.0) and nephrotic (Upr/cr >2.0). eGFR = estimated GFR

4.4 REGRESSION LINE GRAPH OF URINE PROTEIN:CREATININE RATIO AGAINST GFR ESTIMATED WITH CREATININE-BASED AND CYSTATIN C-BASED EQUATIONS RESPECTIVELY

Figure 4.1 shows a regression line graph of urine protein – creatinine ratio (Upr/cr) against GFR as estimated using each of the renal function estimating equations.

Overall, there was an inverse relationship between Upr/cr and GFR as estimated with the renal function estimating equations with the line of best fit being delineated with the equation $y = \beta x + \alpha$ where β, represents the gradient and α represents the y-intercept.

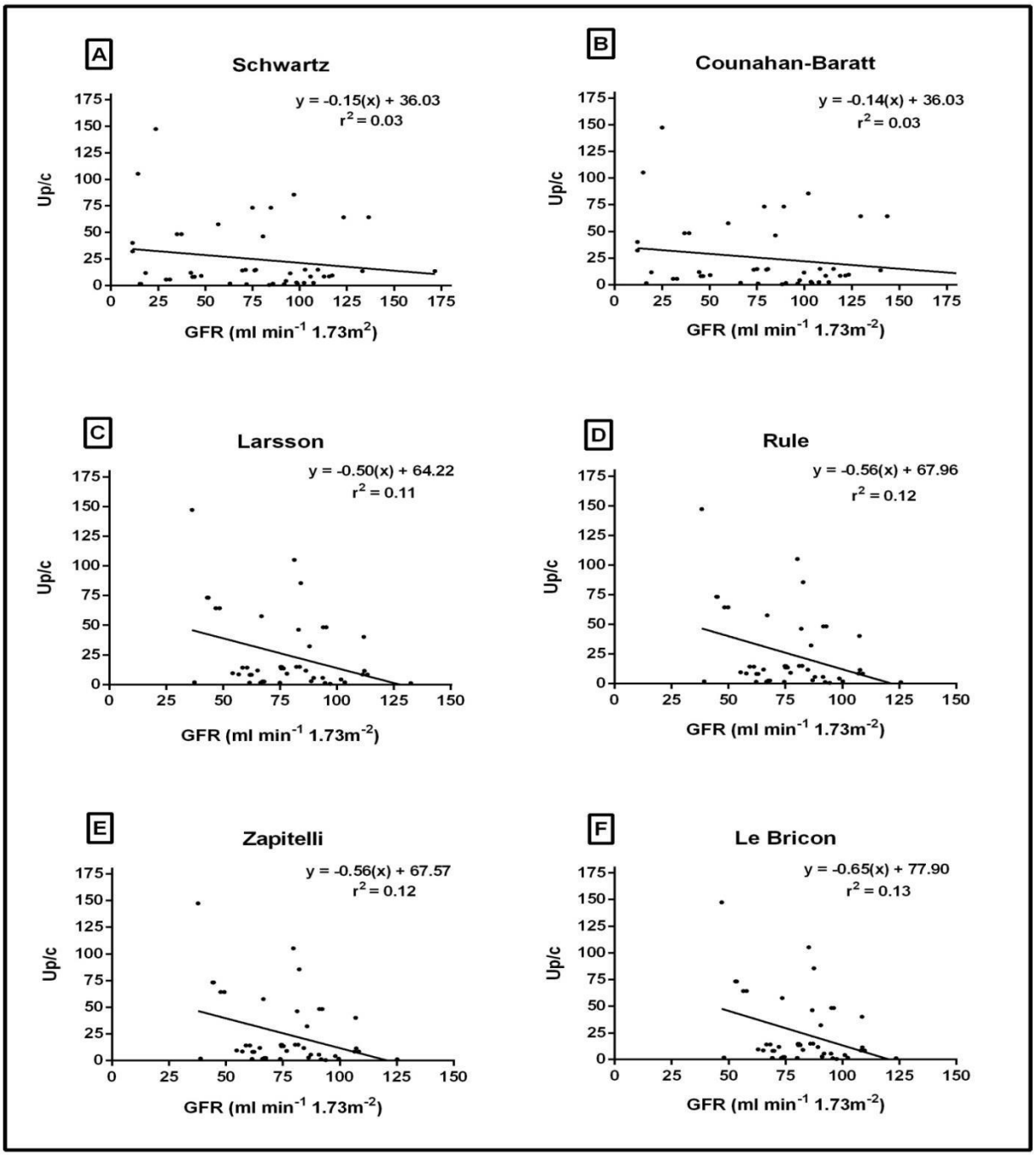

Figure 4.1 Regression line graph of urine protein:creatinine ratio against GFR estimated with creatinine-based and cystatin c-based equations respectively.

4.5 STRATIFICATION OF STUDY PARTICIPANTS WITHIN THE CASE AND CONTROL GROUP INTO CKD STAGES USING THE PRESENCE OR ABSENCE OF ALBUMINURIA

Table 4.5 presents a distribution of the study participants within the case group into the categories of CKD stages using albuminuria defined by urine albumin – creatinine ratio (UACR mg/g). Thirty-seven (37) out of the 45 participants within

the case group showed macroalbuminuria with the remaining 8 showing microalbuminuria. Using the creatinine – based eGFR equations, 16 out of the 37 participants within the macroalbuminuria range had an eGFR ≤60 ml/min/1.73m^2 with the other 21 having an eGFR >60 ml/min/1.73m^2. For the cystatin C – based equations, Larsson, Rule and Zapitelli equations respectively showed 9 of the 37

macroalbuminuric participants with an eGFR ≤60 ml/min/1.73m^2 and the remaining 28 having eGFR>60 ml/min/1.73m^2. The Le Bricon equation showed 6 of the 37 within the macoalbuminuria range having an eGFR ≤60 ml/min/1.73m^2 with the remaining 31 having eGFR>60 ml/min/1.73m^2.

Table 4.6 presents a distribution of the study participants within the control group into the categories of CKD stages using albuminuria defined by urine albumin – creatinine ratio (UACR mg/g). Seventeen (17) out of the 45 participants within the control group showed macroalbuminuria with the remaining 28 showing microalbuminuria. Generally across the distribution, none of the respondents within the control group had a GFR value ≤60 ml/min/1.73m^2 using the cystatin C based equations. For the creatinine-based equations, the 28 respondents with microalbuminuria had eGFR >60 ml/min/1.73m^{-2} likewise the 17 respondents who fell within the macroalbuminuric range. The overall percentage distribution outcome for the cystatin c-based equations was similar to that of the creatininebased equations with variations in the point percentages estimated for stages 1 and
2.

Table 4.5 Stratification of study participants within the case group into CKD stages using the presence or absence of albuminuria

CKD Staging	MicroAlb n = 8(%)	MacroAlb n = 37(%)	MicroAlb n = 8(%)	MacroAlb n = 37(%)
		Creatinine-based Equations		

	Schwartz		Counahan-Baratt	
Stage 1	5(62.5)	13(35.1)	6(75.0)	13(35.1)
Stage 2	3(37.5)	8(21.6)	2(25.0)	8(21.6)
Stage 3A	0(0.0)	2(5.4)	0(0.0)	4(10.8)
Stage 3B	0(0.0)	6(16.2)	0(0.0)	5(13.5)
Stage 4	0(0.0)	5(13.5)	0(0.0)	5(13.5)
Stage 5	0(0.0)	3(8.1)	0(0.0)	2(5.4)
	Cystatin C- based Equations			
	Larsson		**Rule**	
Stage 1	4(50.0)	8(21.6)	4(50.0)	8(21.6)
Stage 2	4(50.0)	20(54.1)	4(50.0)	20(54.1)
Stage 3A	0(0.0)	5(13.5)	0(0.0)	6(16.2)
Stage 3B	0(0.0)	4(10.8)	0(0.0)	3(8.1)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Zapitelli		**Le Bricon**	
Stage 1	4(50.0)	8(21.6)	5(62.5)	10(27.0)
Stage 2	4(50.0)	20(54.1)	3(37.5)	21(56.8)
Stage 3A	0(0.0)	5(13.5)	0(0.0)	6(16.2)
Stage 3B	0(0.0)	4(10.8)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Albuminuria was classified using urine albumin to creatinine (UACR mg/g) ratio cutoffs as follows: normal (UACR <30.0); Microalbuminuria(UACR 30 - 300) and Macroalbuminuria (UACR >300). eGFR = estimated GFR

Table 4.6 Stratification of study participants within the control group into CKD stages using the presence or absence of albuminuria

CKD Staging	MicroAlb n = 28(%)	MacroAlb n = 17(%)	MicroAlb n = 28(%)	MacroAlb n = 17(%)
	Creatinine-based equations			
	Schwartz		**Counahan-Baratt**	
Stage 1	16(57.1)	6(35.3)	21(75.0)	8(47.1)
Stage 2	12(42.9)	11(64.7)	7(25.0)	9(52.9)
Stage 3A	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Stage 3B	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Cystatin C-based equations

	Larsson		**Rule**	
Stage 1	15(53.6)	12(70.6)	14(50.0)	12(70.6)
Stage 2	13(46.4)	5(29.4)	14(50.0)	5(29.4)
Stage 3A	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 3B	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)

	Zapitelli		**Le Bricon**	
			12(42.9)	12(70.6)
Stage 1	12(42.9)	11(64.7)		
Stage 2	16(57.1)	6(35.3)	16(57.1)	5(29.4)
Stage 3A	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 3B	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stage 5	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Albuminuria was classified using urine albumin to creatinine (UACR mg/g) ratio cutoffs as follows: normal (UACR <30.0); Microalbuminuria(UACR 30 - 300) and Macroalbuminuria (UACR >300). eGFR = estimated GFR

4.6 REGRESSION LINE GRAPH OF URINE ALBUMIN:CREATININE RATIO AGAINST GFR ESTIMATED WITH CREATININE-BASED AND CYSTATIN C-BASED EQUATIONS RESPECTIVELY.

Figure 4.2 shows a regression line graph of urine albumin – creatinine ratio (UACR) against GFR as estimated using each of the renal function estimating equations. Overall, there was an inverse relationship between UACR and GFR as estimated with the renal function estimating equations with the line of best fit being delineated with the equation $y = \beta x + \alpha$ where β, represents the gradient and α represents the y-intercept.

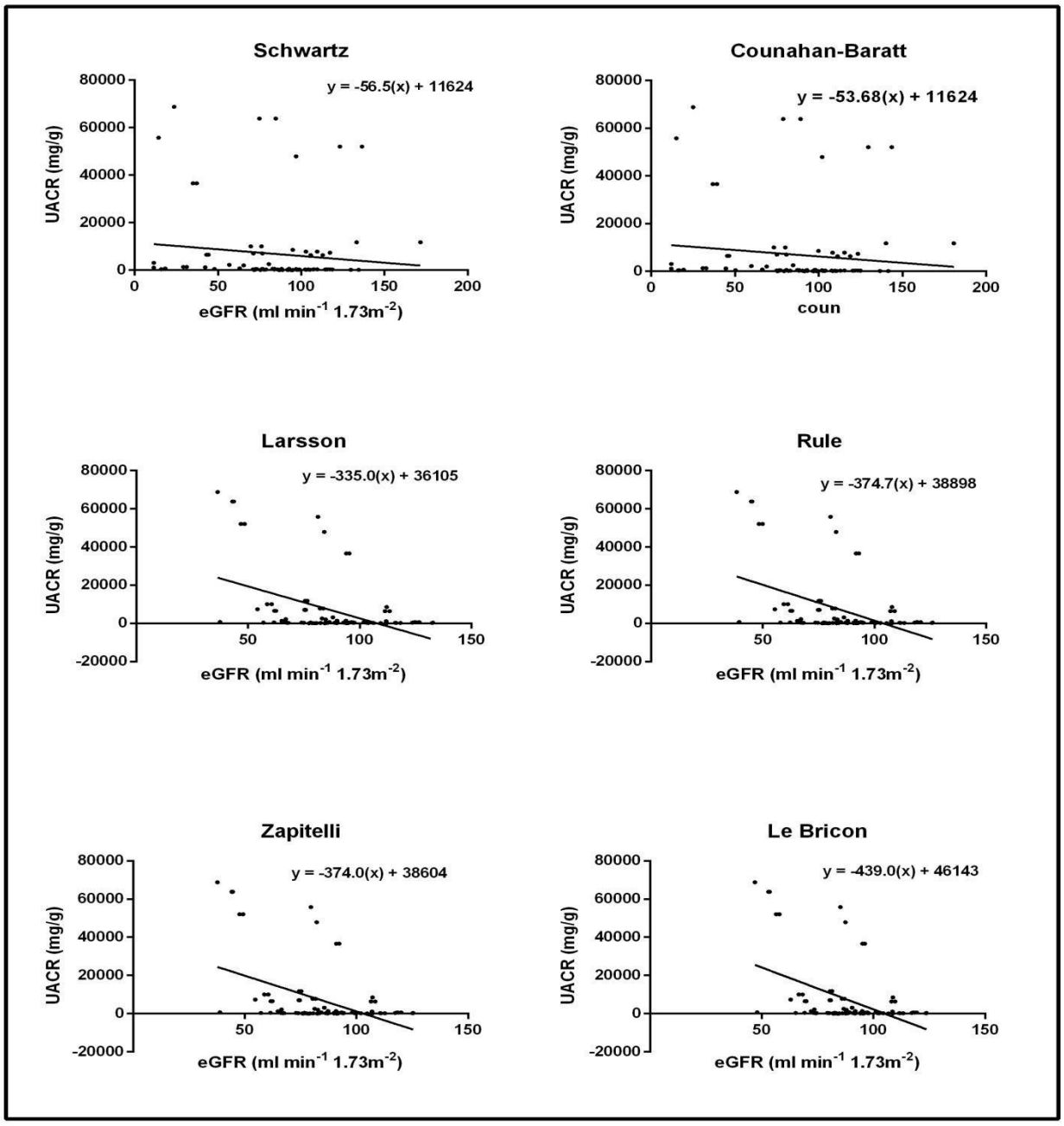

Figure 4.2 Regression line graph of urine albumin:creatinine ratio against GFR estimated with creatinine-based and cystatin c-based equations respectively.

4.7 COMPARABILITY OF THE EGFR EQUATIONS USING THE BLANDALTMAN PLOT

Table 4.7 examines the comparability of the estimated GFR (eGFR) equations within the case group using the Bland-Altman Plot. The Bland-Altman plot is a graphical method to compare two measurement techniques by plotting the differences between the two techniques against the averages of the two techniques. The plot is useful to reveal a relationship between the differences and the

magnitude of measurements, to look for any systematic bias and to identify possible outliers. Bias is defined as the mean difference between the two estimating equations being compared and precision is defined as the standard deviation (SD) of the difference between the two estimating equations being compared. Out of the six (6) eGFR equations utilized for the study, two (2) were creatinine-based (Schwartz &Counahan-Baratt) with the other four (4) being Cystatin C-based (Larsson, Rule, Zappitelli and Le Bricon). For all comparisons, a positive bias indicates overestimation from the first equation in relation to the second equation while a negative bias indicates underestimation from the first equation in relation to the second equation. The comparisons were conducted as creatinine-based vs creatinine-based, creatinine-based vs Cystatin C-based and then Cystatin C-based vs Cystatin C based. Overall, Rule vs Zappitelli yielded the least bias (0.6) and precision (0.03) followed by Larsson vs Rule (bias = 0.9; precision = 2.0), then Larsson vs Zappitelli (bias = 1.5; precision = 2.0), then Counahan-Baratt vs Schwartz (bias = 3.9; precision = 2.0), then Le Bricon vs Larsson (bias 4.2; precision = 4.4), then Le Bricon vs Rule (bias = 5.1; precision = 2.5) and then finally Le Bricon vs Zappitelli (bias = 5.7; precision = 2.5). The ranking of Rule, Larsson and Zappitelli equations among the first three of the comparisons is further buttressed by the exact CKD prevalence rates of 20.0% each of those equations yielded. Comparison between creatinine-based vs Cystatin C-based equations yielded extremely high precision values as indicated in Table 4.7.

Table 4.7 Comparability of eGFR equations among the case group using BlandAltman Plot

eGFR Equations	Bias	Precision	Limits of Agreement
Creatinine-based equation Counahan-Baratt vs Schwartz	3.9	2.0	-0.1 – 7.8
Creatinine-based vs Cystatin C			
Schwartz *vs* Le Bricon	-7.7	43.4	-92.8 – 77.3
Schwartz *vs* Larsson	-3.6	45.4	-92.6 – 85.5
Schwartz *vs* Rule	-2.7	44.5	-89.9 – 84.5

Schwartz *vs* Zappitelli	-2.0	44.5	-89.2 – 85.2
Counahan-Baratt *vs* Le Bricon	-3.9	45.3	-92.6 – 84.8
Counahan-Baratt *vs* Larsson	0.3	47.2	-92.3 – 92.8
Counahan-Baratt *vs* Rule	1.2	46.3	-89.6 – 91.9
Counahan-Baratt *vs* Zappitelli	1.8	46.3	-88.9 – 92.6
Cystatin C based equations			
Le Bricon *vs* Larsson	4.2	4.4	-4.5 – 12.8
Le Bricon *vs* Rule	5.1	2.5	0.3 – 9.9
Le Bricon *vs* Zappitelli	5.7	2.5	0.9 – 10.5
Larsson *vs* Rule	0.9	2.0	-3.0 – 4.8
Larsson *vs* Zappitelli	1.5	2.0	-2.3 – 5.4
Rule *vs* Zappitelli	0.6	0.03	0.6 – 0.7

eGFR-estimated glomerular filtration rate; bias-was defined as the mean difference between the two estimating equations; precision-was defined as the SD of the difference between the two estimating equations being compared

4.8 CATEGORIZATION OF CKD STAGING USING EGFR EQUATIONS AS DEFINED BY KDOQI AND KDIGO

Figure 4.3 presents a line plot of CKD staging as defined by the KDOQI against the absolute number of respondents within the case group who fell within each defining criterion. Rule, Larsson and Zappitelli equations showed 12 (26.7%) respondents in Stage 1 (GFR ≥90), 24 (53.3%) in Stage 2 (GFR: 60 - 89), 9 (20.0%) in Stage 3 (GFR: 30 - 59) and 0 (0.0%) respectively for Stages 4 (GFR: 15 - 29) and Stage 5 (≤15). Counahan-Baratt and Schwartz equations saw a gradual decline of case group numbers from Stage 1 through to Stage 5 with alternating patient numbers with the exception of Stage 4 where both estimated 5 (11.1%) persons each. From an initial data-point value of 15 (33.3%) yielded by the Le Bricon formulae, there was a sharp rise in the number of cases for Stage 2 followed by a gradual decline in numbers to a least value of (0.0%) at Stages 4 and 5 respectively. It is worthy to note that with the exception of the creatinine-based equations (Schwartz and Counahan-Baratt) which were able to pick respondents in Stages 4 and 5 respectively, none of the Cystatin C-based equations did same.

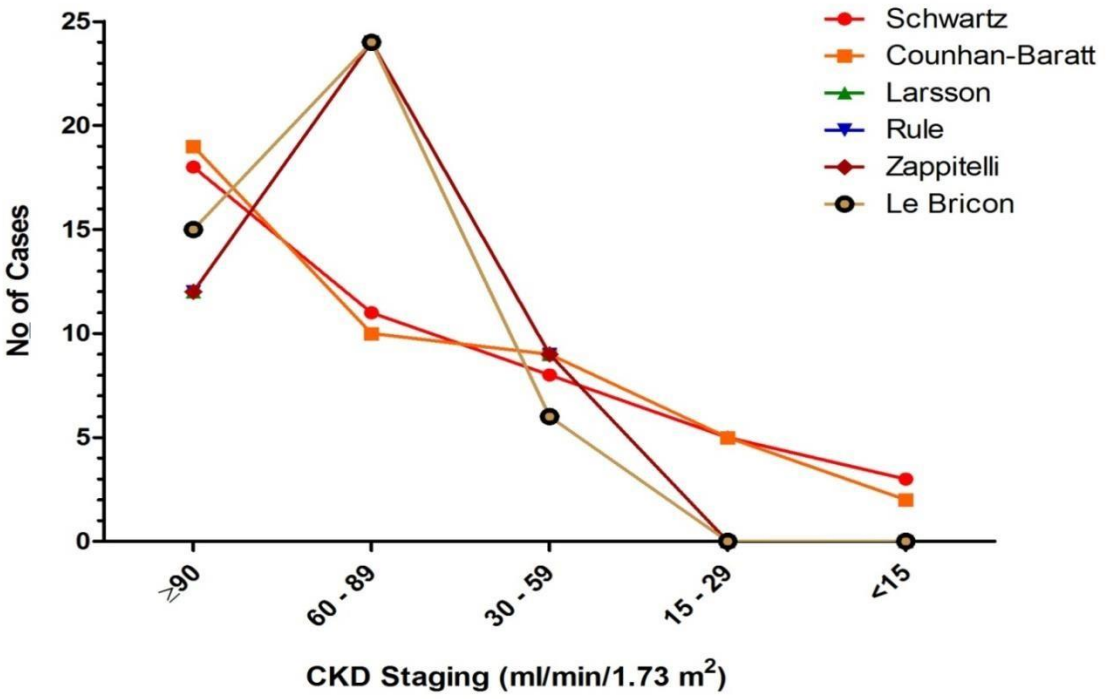

Figure 4.3 Line graph of number of study participants within the case group categorized by CKD staging using the eGFR equations

Figure 4.4 depicts the same line graph as detailed in Figure 4.3 but with stratification of Stage 3 (30 - 59) into the new classification criteria of Stage 3A (45 - 59) and Stage 3B (30 - 44). Out of the 9 respondents who were hitherto categorized as Stage 3 by the Larsson and Zappitelli formulae respectively, 5 (11.1%) were in Stage 3A and 4 (8.9%) were in Stage 3B. Out of the 9 respondents categorized as Stage 3 by the Rule equation, 6 (13.3%) were in Stage 3A and 3 (6.7%) were in Stage 3B. The 6 respondents categorized as Stage 3 by the Le Bricon equation were all in Stage 3A. Out of the 8 respondents who were categorized in Stage 3 by the Schwartz equation, 2 (4.4%) were in Stage 3A and the remaining 6 (13.3%) were in Stage 3B thus signifying the numbers which are actually approaching end stage renal failure. Out of the 9 respondents who were categorized as Stage 3 by the Counahan-Baratt equation, 4 (8.9%) were in Stage 3A and the remaining 5 were in Stage 3B also signifying the numbers approaching end stage renal failure.

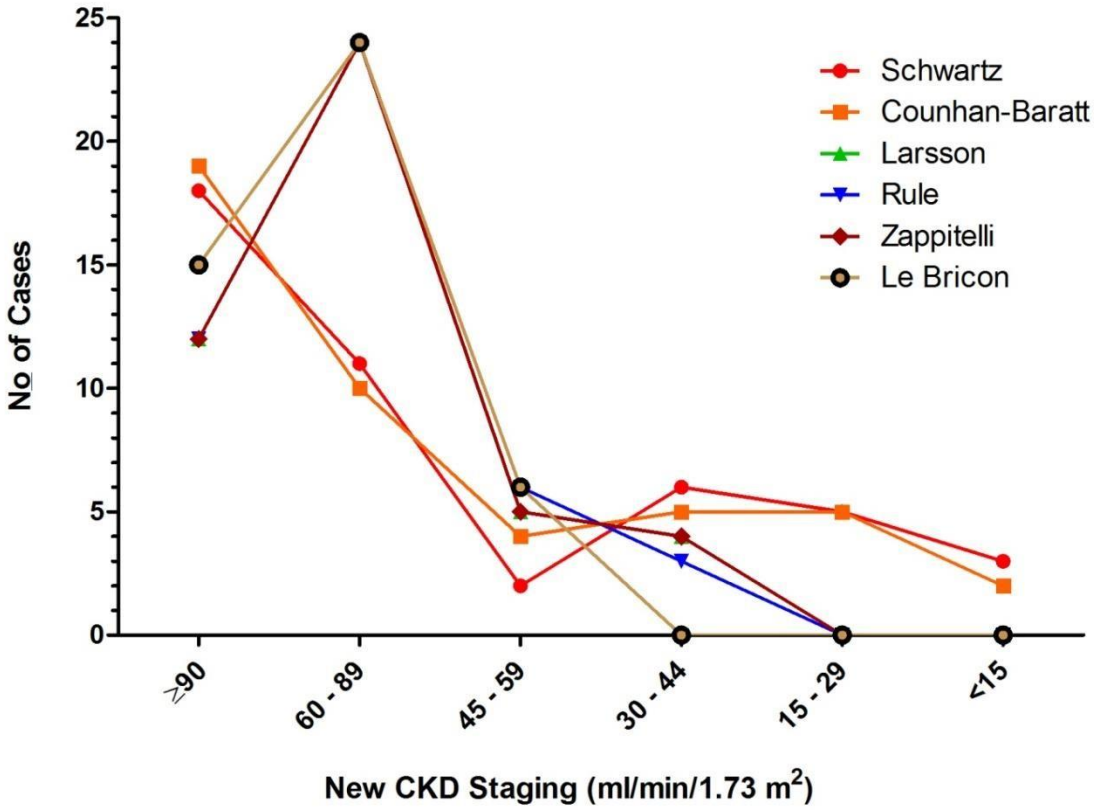

Figure 4.4 Line graph of number of study participants within the case group categorized by the new CKD staging using the eGFR equations

4.9 LOGISTIC REGRESSION OF STUDY PARAMETERS AGAINST EGFR EQUATIONS

Tables 4.8 and 4.9 shows univariate logistic regression analysis of the study parameters against CKD as defined by either a creatinine-based equation or cystatin C-based equation. Outcomes from the Schwartz equation showed that age (OR = 0.80, p = 0.044), weight (OR = 0.93, p = 0.027) and height (OR = 0.04, p = 0.013) of the respondents were less likely to be associated with CKD as defined by the Schwartz equation. A rise in the concentration of urea (OR = 1.98, p = 0.001) was associated with a 2 times risk for CKD as defined by the Schwartz formulae. A rise in creatinine (OR = 1.11, p = 0.002) concentration was linked with a one on one outcome for the presence of CKD. The same trend was observed for the Counahan-Baratt equation (Table 4.8a). Upr/cr had an urge over UACR in that, Upr/cr stood out as a significant predictor of CKD either with the use of creatinine-based or Cystatin C-

based equation. UACR showed statistical significance with the Cystatin C-based eGFR equations but not with the creatininebased equations (Tables 4.8 and 4.9)

Table 4.8 Logistic regression of study parameters against eGFR equations

	Schwartz		Counahan-Baratt		Larsson	
Parameters	**OR**	**P-value**	**OR**	**P-value**	**OR**	**P-value**
Age (years)	0.80	0.044	0.83	0.044	0.78	0.038
Weight (kg)	0.93	0.027	0.93	0.027	0.96	0.216
Height (m)	0.04	0.013	0.04	0.013	0.37	0.427
BMI (kg m^{-2})	0.85	0.276	0.85	0.276	0.94	0.732
Systolic (mmHg)	0.99	0.960	0.99	0.960	0.97	0.272
Diastolic (mmHg)	1.03	0.207	1.03	0.207	1.02	0.542
%BF	0.99	0.870	0.99	0.870	1.10	0.274
Gender						
Female	1		1		1	
Male	2.67	0.256	2.67	0.256	0.48	0.376
Serum Analytes						
Na (mmol L^{-1})	1.13	0.469	1.13	0.469	0.66	0.080
K (mmol L^{-1})	0.91	0.934	0.91	0.934	6.86	0.171
Urea (mmol L^{-1})	1.98	0.001	1.98	0.001	0.92	0.384
Creatinine (µmol L^{-1})	1.11	0.002	1.11	0.002	0.98	0.131
Albumin (g L^{-1})	1.02	0.541	1.02	0.541	1.04	0.324
Protein (g L^{-1})	1.04	0.141	1.04	0.141	1.04	0.217
Cystatin C (µgL^{-1})	1.00	0.772	1.00	0.772	15.06	1.000
Urinary Analytes						
Albumin (mg dL^{-1})	1.00	0.629	1.00	0.629	1.00	0.065
Protein (mg dL^{-1})	1.00	0.375	1.00	0.375	1.00	0.102
Creatinine (mg dL^{-1})	0.99	0.217	0.99	0.217	1.00	0.928
UACR	1.00	0.927	1.00	0.927	1.00	0.003
Upr/cr	1.03	0.005	1.03	0.005	1.04	0.001

OR = odds ratio; 95%CI = 95% confidence interval;Upr/cr =Urine protein: creatinine ratio

Table 4.9 Logistic regression of study parameters against eGFR equations

	Rule		Zappitelli		Le Bricon	

Parameters	OR	P-value	OR	P-value	OR	P-value
Age (years)	0.86	0.163	0.86	0.163	0.79	0.127
Weight (kg)	0.96	0.216	0.96	0.216	0.93	0.138
Height (m)	0.37	0.427	0.37	0.427	0.14	0.195
BMI (kg m^{-2})	0.94	0.732	0.94	0.732	0.96	0.863
Systolic (mmHg)	0.97	0.272	0.97	0.272	0.88	0.024
Diastolic (mmHg)	1.02	0.542	1.02	0.542	0.98	0.563
%BF	1.10	0.274	1.10	0.274	1.09	0.357
Gender						
Female		1		1		1
Male	0.48	0.376	0.48	0.376	0.52	0.487
Serum Analytes						
Na (mmol L^{-1})	0.66	0.080	0.66	0.080	0.64	0.121
K (mmol L^{-1})	6.86	0.171	6.86	0.171	30.94	0.062
Urea (mmol L^{-1})	0.92	0.384	0.92	0.384	0.97	0.737
Creatinine (µmol L^{-1})	0.98	0.131	0.98	0.131	0.98	0.257
Albumin (g L^{-1})	1.04	0.324	1.04	0.324	1.05	0.299
Protein (g L^{-1})	1.04	0.217	1.04	0.217	1.03	0.307
Cystatin C (µgL^{-1})	15.06	1.000	15.06	1.000	2.07	1.000
Urinary Analytes						
Albumin (mg dL^{-1})	1.00	0.065	1.00	0.065	1.00	0.250
Protein (mg dL^{-1})	1.00	0.102	1.00	0.102	1.00	0.445
Creatinine (mg dL^{-1})	1.00	0.928	1.00	0.928	0.92	0.112
UACR	1.00	0.003	1.00	0.003	1.00	0.001
Upr/cr	1.04	0.001	1.04	0.001	1.06	0.001

OR = odds ratio; 95%CI = 95% confidence interval; Upr/cr =Urine protein: creatinine ratio

4.10 RECEIVER OPERATOR CHARACTERISTICS OF THE STUDY VARIABLES

The receiver operator characteristics (ROC) curve is a fundamental tool for diagnostic test evaluation which creates an ROC curve and a complete sensitivity/specificity report. The diagnostic performance of the renal function test methods evaluated in this study or their accuracy to discriminate diseased cases from normal cases was assessed using ROC and the data presented in Tables 4.10, 4.11 and 4.12. With the Schwartz and Counahan-Baratt equations, age ≤5 years (AUC=0.750, p=0.002), weight ≤15.0 kg (AUC=0.751, p=0.001) and height ≤1.1 m (AUC=0.758, p=0.001) were significantly associated with the presence of CKD respectively as defined by both equations. For the serum analytes measured, a urea concentration >4.9 mmol L^{-1} (p=0.000) and a creatinine concentration >65.5 µmol L^{1} (p=0.000) were significantly associated with the presence of CKD as defined by both equations respectively. For the Larsson, Rule and Zappitelli equations, sodium concentration ≤142.2 mmol L^{-1}, creatinine concentration ≤46.3 µmol L^{-1} and cystatin C concentration >1213 were the cut-offs which were significantly associated with the presence of CKD. For the urinary analytes, albumin concentration >520 mg dL^{-1}, protein concentration >360.0 mg dL^{-1} and urinealbumin-creatinine ratio (UACR) >47901.5 were the cut-offs which were significantly associated with the presence of CKD. For the cystatin C-based equations, the Le Bricon equation showed a slight variation with age ≤4 years, weight ≤17.0 kg and height ≤1.0 m as the cut-offs which were significantly associated with the presence of CKD. Contrary to the findings of other cysatin Cbased equations, systolic pressure ≤90 mmHg was also significantly associated with the presence of CKD in the Le Bricon equation. Furthermore, for the Le Bricon equation, serum concentration of creatinine did not show any significant association with the presence of CKD but sodium (≤142.2 mmol L^{-1}) and cystatin C (>1323.3) were significantly associated with the presence of CKD. For the urinary analytes, albumin concentration (>520 mg dL^{-1}), protein concentration (>360 mg

dL^{-1}), creatinine concentration (≤13.8 µmol L^{-1}) and UACR >47901.5 were significantly associated with the presence of CKD.

Table 4.10 Receiver operator characteristics of the study variables and the cut-offs for predictability of the presence of CKD

Variables	Schwartz					Counahan-Baratt				
	Criterion	Sensitivity	Specificity	AUC	p-value	Criterion	Sensitivity	Specificity	AUC	p-value
Age	≤5.0 (years)	81.3	66.2	0.750	0.002	≤5.0	81.3	66.2	0.750	0.002
Weight (kg)	≤15.0	68.8	77.0	0.751	0.001	≤15.0	68.8	77.0	0.751	0.001
Height (m)	≤ 1.1	81.3	68.9	0.758	0.001	≤ 1.1	81.3	68.9	0.758	0.001
BMI (kg m^{-2})	≤14.8	50.0	79.3	0.556	0.592	≤14.8	50.0	79.3	0.556	0.592
Systolic (mmHg)	≤80.0	25.0	100.0	0.490	0.926	≤80.0	25.0	100.0	0.490	0.926
Diastolic	>59 (mmHg)	100.0	34.5	0.654	0.061	>59	100.0	34.5	0.654	0.061
%BF	≤10.6	50.0	72.4	0.519	0.844	≤10.7	50.0	72.4	0.519	0.844
Gender *Male*		87.5	27.6	0.575	0.209	>142.6	87.5	27.6	0.575	0.209
Serum Analytes						>4.6				
Na (mmol L^{-1})	>142.6	37.5	82.8	0.554	0.590	>4.9	37.5	82.8	0.554	0.590
K (mmol L^{-1})	<4.6	100.0	0.0	0.912	0.914	>65.5	100.0	0.0	0.912	0.914
Urea (mmol L^{-1})	>4.9	81.3	94.6	0.863	0.000	>26.4	81.3	94.6	0.863	0.000
Creatinine (µmol L^{-1})	>65.5	93.8	96.6	0.942	0.000	>60.4	93.8	96.6	0.942	0.000
Albumin (g L^{-1})	>26.4	75.0	51.7	0.534	0.727	≤1196.2	75.0	51.7	0.534	0.727
Protein (g L^{-1})	>60.4	56.3	72.4	0.629	0.169		56.3	72.4	0.629	0.169

Table 4.11 Receiver operator characteristics of the study variables and the cut-offs for predictability of the presence of CKD

Cystatin C (μgL^{-1})	≤1196.2	93.8	31.0	0.511	0.905		93.8	31.0	0.511	0.905
Urinary Analytes										
Albumin (mg dL^{-1})	≤30.0	62.5	58.6	0.555	0.560	≤30.0	62.5	58.6	0.555	0.560
Protein (mg dL^{-1})	>1580.0	12.5	100.0	0.529	0.754	>1580.0	12.5	100.0	0.529	0.754
Creatinine (mg dL^{-1})	≤20.8	62.5	62.1	0.606	0.246	≤20.8	62.5	62.1	0.606	0.246
UACR	>343.3	100.0	31.0	0.509	0.925	>343.3	100.0	31.0	0.509	0.925
Upr:cr	>4.28	87.5	70.3	0.764	0.0001	>4.28	87.5	70.3	0.764	0.0001

AUC – area under the curve; UACR – urine-albumin-creatinine ratio;Upr:cr – urine protein-creatinine ratio

Larsson Variables	Rule					Rule				
	Criterion	Sensitivity	Specificity	AUC	p-value	Criterion	Sensitivity	Specificity	AUC	p-value
Age (years)	≤7.0	100.0	44.4	0.603	0.210	≤7.0	100.0	44.4	0.603	0.210
Weight (kg)	≤25.0	100.0	0.0	0.606	0.204	≤25.0	100.0	0.0	0.606	0.204
Height (m)	≤1.3	100.0	38.9	0.600	0.239	≤1.3	100.0	38.9	0.600	0.239
BMI (kg m^{-2})	≤16.5	88.9	50.0	0.576	0.380	≤16.5	88.9	50.0	0.576	0.380

Table 4.12 Receiver operator characteristics of the study variables and the cut-offs for predictability of the presence of CKD

Systolic (mmHg)	≤99.0	88.9	66.7	0.707	0.051	≤99.0	88.9	66.7	0.707	0.051
Diastolic (mmHg)	≤ 62.0	77.8	52.8	0.591	0.444	≤62.0	77.8	52.8	0.591	0.444
%BF	>11.8	88.9	50.0	0.640	0.149	>11.8	88.9	50.0	0.640	0.149
Gender	*Female*	33.3	80.6	0.569	0.439	*Female*	33.3	80.6	0.569	0.439
Serum Analytes										
Na (mmol L^{-1})	≤142.2	100.0	47.2	0.705	0.010	≤142.2	100.0	47.2	0.705	0.010
K (mmol L^{-1})	>4.4	66.7	80.6	0.645	0.280	>4.4	66.7	80.6	0.645	0.280
Urea (mmol L^{-1})	>3	77.8	52.8	0.500	1.000	>3.0	77.8	52.8	0.500	1.000
Creatinine (µmol L^{-1})	≤46.3	77.8	63.9	0.707	0.022	≤46.3	77.8	63.9	0.707	0.022
Albumin (g L^{-1})	>25.3	100.0	41.7	0.574	0.416	>25.3	100.0	41.7	0.574	0.416
Protein (g L^{-1})	>49.7	100.0	47.2	0.659	0.075	>49.7	100.0	47.2	0.659	0.075
Cystatin C (µgL^{-1})	>1213.0	100.0	100.0	1.000	<0.000	>1213.0	100.0	100.0	1.000	<0.000
Urinary Analytes										
Albumin (mg dL^{-1})	>520.0	66.7	91.7	0.750	0.021	>520.0	66.7	91.7	0.750	0.021
Protein (mg dL^{-1})	>360.0	77.8	77.8	0.758	0.016	>360.0	77.8	77.8	0.758	0.016
Creatinine (mg dL^{-1})	≤13.8	55.6	72.2	0.563	0.610	≤13.8	55.6	72.2	0.563	0.610

Table 4.13 Receiver operator characteristics of the study variables and the cut-offs for predictability of the presence of CKD

UACR	>47901.5	55.6	97.2	0.785	0.006	>47901.5	55.6	97.2	0.785	0.006
Upr:cr	>8.5	88.9	74.1	0.833	<0.0001	>8.5	88.9	74.1	0.833	<0.0001

AUC – area under the curve; UACR – urine-albumin-creatinine ratio;Upr:cr – urine protein-creatinine ratio

	Zappitelli					Le Bricon				
Variables	**Criterion**	**Sensitivity**	**Specificity**	**AUC**	**p-value**	**Criterion**	**Sensitivity**	**Specificity**	**AUC**	**p-value**
Age (years)	≤7.0	100.0	44.4	0.603	0.210	≤4.0	100.0	0.0	0.665	0.046
Weight (kg)	≤25.0	100.0	0.0	0.606	0.207	≤17.0	100.0	53.9	0.688	0.023
Height (m)	≤1.3	100.0	38.9	0.600	0.239	≤1.0	100.0	56.4	0.684	0.027
BMI (kg m^{-2})	≤16.5	88.9	50.0	0.576	0.380	≤15.7	83.3	58.9	0.556	0.562
Systolic (mmHg)	≤99.0	88.9	66.7	0.707	0.051	≤90.0	100.0	74.4	0.848	<0.000
Diastolic (mmHg)	≤62.0	77.8	52.8	0.591	0.444	≤60.0	83.3	53.9	0.641	0.242
%BF	>11.8	88.9	50.0	0.640	0.149	>11.8	100.0	0.0	0.637	0.173

Table 4.14 Receiver operator characteristics of the study variables and the cut-offs for predictability of the presence of CKD

Gender	Female	33.3	80.6	0.569	0.439	Female	33.3	79.5	0.564	0.561
Serum Analytes										
Na (mmol L^{-1})	≤142.2	100.0	47.2	0.705	0.014	≤142.2	100.0	43.6	0.718	0.033
K (mmol L^{-1})	>4.4	66.7	80.6	0.645	0.280	>4.4	83.3	79.5	0.748	0.070
Urea (mmol L^{-1})	>3.0	77.8	52.8	0.500	1.000	>3.0	100.0	53.9	0.622	0.159
Creatinine (µmol L^{-1})	≤46.3	77.8	63.9	0.707	0.022	≤46.3	83.3	61.5	0.690	0.114
Albumin (g L^{-1})	>25.3	100.0	41.7	0.574	0.416	>25.3	100.0	38.5	0.607	0.333
Protein (g L^{-1})	>49.7	100.0	47.2	0.659	0.075	>49.7	100.0	43.6	0.650	0.152
Cystatin C (µgL^{-1})	>1213.0	100.0	100.0	1.000	<0.000	>1323.3	100.0	100.0	1.000	<0.000
Urinary Analytes										
Albumin (mg dL^{-1})	>520.0	66.7	91.7	0.750	0.021	>520	66.7	87.2	0.759	0.008
Protein (mg dL^{-1})	>360.0	77.8	77.8	0.758	0.016	>360	83.3	74.4	0.692	0.018
Creatinine (mg dL^{-1})	≤13.8	55.6	72.2	0.563	0.610	≤13.8	83.3	48.7	0.778	0.001
UACR	>47901.5	55.6	97.2	0.785	0.006	>47901.5	83.3	97.4	0.885	0.000
Upr:cr	>8.5	88.9	74.1	0.833	<0.0001	>57.6	83.3	26.19	0.861	0.003

AUC – area under the curve; UACR – urine-albumin-creatinine ratio; Upr:cr – urine protein-creatinine ratio

Table 4.57 Receiver operator characteristics of the study variables and the cut-offs for predictability of the presence of CKD

Chapter 5 DISCUSSION

5.1 DISCUSSION

Glomerular filtration rate (GFR) is a summation of the filtration rate of each functional nephron and thus provides an estimate of functional renal mass (Mattman *et al.*, 2006) and as such, accurate knowledge of GFR is vital for provision of appropriate medical care, primarily, dosing of drugs. Chronic kidney disease (CKD) is defined by the presence of kidney damage (e.g, any structural or functional abnormality involving pathological, laboratory or imaging findings) for >3 months or a GFR <60 ml/min/1.73m^2 for >3 months. CKD is often asymptomatic in its early states and as a result it is both underdiagnosed and under-reported. It is, therefore, imperative that modalities that could lead to early identification of a diagnosis of reduced GFR, i.e. CKD should be encouraged for early interventions and minimization of morbidity and mortality. The K/DOQI report recommends clinical laboratories to report an estimate of GFR using a predictive equation in addition to reporting the serum creatinine (Hogg *et al.*, 2003). In children and adolescents with early chronic kidney disease and well-maintained fluid and electrolyte balance, the urinalysis may be entirely normal. Therefore, a reduced GFR may serve as the only clinical sign of kidney damage in such individuals.

On the backdrop of such information, this study was conducted in a cohort of children with varied medical conditions ranging from polycystic kidney disease (PKD), posterior urethral valve, hydronephrosis, steroid resistant nephrotic syndrome (SRNS), proven recurrent UTI to those on antihypertensive drugs. Some patients also had co-morbid conditions comprising PUV and UTI, hydronephrosis and UTI and Hydronephrosis and PUV as the case group and a control group who were diagnosed with medical conditions other than that stated for the case group to explore the utilization of the renal function estimating equations and other parameters in predicting the presence of CKD among the study cohorts.

5.2 PREVALENCE AND PREDICTORS OF CKD

This study explored two creatinine-based equations (Schwartz and CounahanBaratt equations) and four cystatin C-based equations (Larsson, Rule, Zapitelli and Le Bricon equations) as estimates of GFR for the case group and their stratification using the K/DOQI guidelines. The prevalence of CKD ranged from as low as 13.3% (Le Bricon) through 20.0% (Larsson, Rule and Zapitelli respectively) and 35.6% (Schwartz and Counahan-Baratt respectively). None of the study participants from the control group had an eGFR <60 ml/min/1.73m^2. In exploring agreements between the equations using the Bland-Altman plot for method comparison, the Rule vs. Zapitelli equations yielded the least precision (0.03) and bias (0.6) followed by the Larsson vs. Rule (precision = 2.0, bias = 0.9) then the Larsson vs Zapitelli (precision = 2.0, bias = 1.5). The Schwartz vs Counahan-Baratt equations yielded a precision of 2.0 and bias of 3.9. The varied prevalence rates estimated is as a result of inherent variations in the composition of the formulae which combines either serum creatinine or cystatin C with demographic variables which in the case of creatinine are related to production rates. The four cystatin C-based equations examined did not classify any of the study participants in the case group in Stages 4 and 5 but rather classified 9 (20.0%; Rule, Larsson and Zapitelli equations) and 6 (13.3%; Le Bricon) in Stage 3, respectively. The Schwartz equation showed 8 (17.7%) in Stages 4 and 5 respectively, whilst the CounahanBaratt equation showed 7 (15.5%) in Stages 4 and 5. Inter-comparability assessment showed the ability of the cystatin C-based equations to overestimate GFR when compared to the creatinine-based equations which also underestimated GFR. Studies have noted a number of inherent pitfalls in the development and use of estimating equations, with the first being the intrinsic biological variability in the GFR itself, which has been noted to have within-day (Rehling *et al.*, 1986) and between-day variability (Van Acker *et al.*, 1995). The physiological variability in the production and in the extra-glomerular elimination of creatinine will also contribute to variability in the creatininebased eGFR measurements; then factors in inter-laboratory variation

in terms of measurement of creatinine (Coresh *et al.*, 2002). Given the outcomes of prevalence rates observed, it is most plausible to suggest the clinical utilization of the creatinine-based eGFR equations ahead of the cystatin C-based equations with the reason clearly being that attention could be given to patients classified in Stages 4 and 5 for prompt intervention and further minimization of morbidity and mortality. Hogg *et al.* (2003) clearly outlined the importance of early identification of a child with a persistent moderate to severe decrease in GFR, i.e. <60 ml/min/1.73 m² (NKF-DOQI Stage 3) for > 3 months, since at this level of dysfunction, children are more likely to present with the associated complications and hence benefit from early identification (National Kidney, 2002). The other unique challenge the eGFR formulae presents is the use of height which is not generally measured at the time of blood collection and as such could present difficulties with ease of reporting paediatric eGFR. The other problem is the use of constants that are specific to a particular method for creatinine measurement.

5.3 ANTHROPOMETRIC VARIABLES

On analyzing the variables to explore those which will serve as predictors in addition to eGFR to enhance the clinical utilization of the creatinine-based equations in determining the presence of CKD, a receiver operator characteristic analysis was performed with the presence or absence of CKD as the outcome variable. Variables comprising age (≤5 years; sensitivity = 81.3%, specificity = 66.2%), weight (≤15.0 kg; sensitivity = 68.8%, specificity = 77.0%) and height (≤1.1 m; sensitivity = 81.3%, specificity = 68.9%) were significantly associated with CKD as defined by the Schwartz and Counahan-Baratt equations respectively. A logistic regression analysis of these variables (age, weight, height), however,/*+ showed a significantly less likelihood of such variables being independent risk factors for CKD. In the light of these findings, the argument of an early onset of CKD within the case group could be advanced with suggestion of dire effects on growth which subsequently affects body weight and height as the children grow. Foster *et al.* (2011) and Schärer (1990)

in their respective studies reported that children with CKD are at increased risk for growth retardation and muscle wasting which supports the arguments sustained from this study. This is exactly so because the case group was drawn from a population with known underlying medical conditions linked with the kidney. Harambat *et al.* (2012) in their study on the epidemiology of CKD in children iterated that the progression of CKD is variable and depends on the underlying disease, the severity of the initial injury and the presence of additional risk factors.

Although percentage body fat (%BF) showed a significant decrease in the case group compared to the control group, it did not stand out as an independent risk factor for CKD in the further analysis carried out and likewise body mass index. The lack of association between body mass index and CKD from this study could be as a result of the direct mathematical association between BMI, weight and height. With a sustained argument of a direct effect of progression of CKD on growth retardation, there is a direct consequence on weight and height which will hitherto impact negatively on BMI and as such reduce a direct strength and association between CKD and BMI. The possibility of distortions of measuring BMI in children with CKD can therefore be inferred and as such interpretation of BMI measurements in children with CKD should be made with caution given the line of argument expressed thus far.

5.4 PROTEINS AND HYPERTENSION

Analysis of paediatric retrospective data suggests that many factors such as anaemia, hypoalbuminaemia, hyperphosphataemia and hypocalcaemia might be associated with the rate of CKD progression (Furth *et al.*, 2007). From this study, serum protein and albumin concentrations was significantly lower in the case group when compared to the control group with a urinary protein and albumin concentration which was significantly higher when compared with the control group. This significant finding is in consonance with the assertions of Furth *et al.* (2007). Studies have reported hypertension and proteinuria as the

most important risk factors for renal disease progression in both adult and paediatric CKD (Ardissino *et al.*, 2003; Jafar *et al.*, 2003). Flynn *et al.* (2008) and Mitsnefes *et al.* (2010) reported that hypertension is present in approximately 50% of children with CKD and masked hypertension (i.e. normal casual but elevated ambulatory blood pressure) is often present. From the analysis of data in this study, hypertension (systolic or diastolic) did not come out as a significant predictor and an independent risk factor for renal disease progression in this cohort of study participants. A plausible explanation could be due to the fact that a section of the children who fell within the case group were on anti-hypertensive drugs at the time of recruitment into the study.

5.5 TRADITIONAL KIDNEY FUNCTION MARKERS

Attending difficulties with the clinical utilization of either serum urea and serum creatinine to estimate GFR is well documented (National Kidney, 2002). Serum creatinine is affected by factors other than GFR, principally creatinine production is related to body size, especially muscle mass. This leads to considerable variability between children of different ages and a relatively wide range for serum creatinine in normal individuals. Serum creatinine, urea and cystatin C analytes were significantly increased in the case group when compared to the control group. Subsequent analysis showed a better agreement between serum creatinine and serum urea with CKD as defined with the Schwartz and Counahan-Baratt equations. Cystatin C did not stand out as an independent marker in the logistic regression analysis but correlated well with the cystatin C-based equations in the receiver operator characteristics analysis as expected. Indeed, it is difficult to accurately estimate the level of kidney function or to detect earlier stages of CKD using serum creatinine alone as the measure of kidney function given the fact that in many patients, the GFR must decline to approximately half of the normal level before the serum creatinine rises above the upper limit of normal (National Kidney, 2002).

5.6 URINARY ANALYTES

Proteinuria is an important biomarker strongly associated with CKD and is an indicator of underlying glomerular disease or renal tubular dysfunction. The National Kidney Foundation recommends the evaluation of individuals for CKD by either blood testing to estimate GFR or by urinary screening for the detection of proteinuria. Tests for total urine protein are preferred in children, except for those with diabetes who should be screened for albuminuria (Vassalotti *et al.*, 2007). Not only is proteinuria a marker of kidney damage, but it also responsible for progressive kidney injury. The study therefore ascertained the clinical value and possible utilization of urine albumin:creatinine ratio (UACR) and urine protein:creatinine (Upr/cr). Increases in urinary protein either by damage to the glomerular capillary wall or by decreased tubular reabsorption of protein causes injury to renal tubular cells (D'amico and Bazzi, 2003). Exposure of tubules to urinary proteins causes interstitial inflammation and subsequent fibrosis (Eddy, 2005) as well as apoptosis in proximal tubular cells (Erkan *et al.*, 2007). A number of paediatric studies have demonstrated that elevated urine total protein is an independent risk factor for a progressive decline in kidney function (Peterson *et al.*, 1995; Ardissino *et al.*, 2003; Wühl *et al.*, 2004). The study observed an inverse relationship between both UACR and Upr/cr with eGFR thus implying higher albuminuria or proteinuria as the level of eGFR decreased. The most striking finding is the similarity in numbers and distribution of the participants in the case group when stratified by albuminuria (microalbuminuria = 8; macroalbuminuria = 37) and proteinuria (significant = 8; nephrotic = 37) respectively. This significant finding gives credence to the ability of either UACR or Upr/cr to help identify and stage CKD in a cohort of children with one or more renal disease as the same trend could not be said for stratification within the control group using UACR and Upr/cr. It is therefore worthy to note from this study that proteinuria and albuminuria are significant markers of CKD progression as related in the study of Wingen *et al.* (1997). Further analysis showed that Upr/cr had an urge over UACR in that, Upr/cr stood out as a

significant predictor of CKD either with the use of creatinine-based or cystatin C-based equation. UACR showed statistical significance with the Cystatin C-based eGFR equations and not with the creatinine-based equations (Tables 4.8 and 4.9). Furthermore, UACR did not classify any of the participants with microalbuminuria as having CKD whilst Upr/cr classified two out of the eight who had significant proteinuria as having CKD (Tables 4.3 and 4.5). It is thus evident that high levels of proteinuria increases the rate of renal progression in this cohort of children studied with Upr/cr showing a better performance compared to UACR and as such a better predictor of CKD progression in this studied population.

Chapter 6 CONCLUSION

6.1 CONCLUSION

This study assessed the clinical utilization of the two creatinine-based equations (Schwartz and Counahan-Baratt) and four Cystatin C-based equations (Rule, Zappitteli, Larsson and Le Bricon) to predict the presence of chronic kidney disease (eGFR <60 ml/min/1.73 m^2). The prevalence of CKD ranged from 13.3% through 20.0% to 35.6% depending on the type of renal function equation adapted. Weight, age, height and proteinuria (defined by urine protein-creatinine ratio) were identified as significant predictors of progression to CKD stage. The Schwartz and Counahan-Baratt equations showed the best agreement with the predictors and showed a natural spread of the respondents within the case group across the five stages of CKD as defined by KDOQI guidelines.

6.2 RECOMMENDATION

1. Given that this study is the first of its kind to explore the presence of CKD in children using the estimating equations, it is timely for paediatric nephrologists in-country to discuss these issues and if possible, develop a consensus regarding the need for screening studies (primarily urine protein-creatinine ratio) to help in the early identification of children with CKD.

2. More studies should be conducted in the area to shore-up the database on CKD prevalence among children in the country so that conclusive outcomes could be set for better management of children with CKD.

3. The outcome of this study has provided preliminary evidence which supports the fact that the estimating equations provide simple alternatives for the early identification of CKD in children. Integration of the KDOQI guidelines into routine clinical practice will provide a means

of early identification and therefore potentially successful intervention, for many individuals with CKD who would otherwise be destined to present later with serious sequelae of untreated kidney failure.

4. This study should be replicated with a large sample size and should be expanded to involve other paediatric units.

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