PREVALENCE AND RISK FACTORS OF CHRONIC KIDNEY DISEASE IN STEADY STATE SICKLE CELL ANAEMIA PATIENTS AGED 5 TO 16 YEARS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

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DECLARATION

I, Nchimunya Machila hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other university.

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ABSTRACT

Background: Improved medical care has led to improved life expectancy of sickle cell anaemia (SCA) patients hence complications associated with SCA such as chronic kidney disease (CKD) are being seen more frequently. Globally, nephropathy of varying severity occurs in 5 to 18 % of the SCA population across all age groups with a third of the adults proceeding to develop CKD while over 30 % of paediatric SCA patients have been documented to have CKD in Africa. The mortality rate in SCA patients CKD is high. This study sought to determine the prevalence and risk factors of CKD in SCA, information which was not available in Zambia prior to this study. This information will guide in targeting and timing of screening for CKD in SCA in children in our population

Objectives: To determine prevalence of haematuria, proteinuria, abnormal estimated glomerular filtration rate (eGFR) and CKD and risk factors of CKD among the steady state SCA patients aged 5 to 16 years at the University Teaching Hospital (UTH), Lusaka.

Methodology: This was a prospective cross - sectional study of 197 children aged 5 to 16 years with SCA at the UTH - Lusaka conducted from August 2014 to July 2015. Demographic and clinical data were collected using a structured questionnaire. Urine and blood samples were used to determine the urine albumin creatinine ratio (ACR) and full blood count /blood biochemistry respectively. CKD was defined and determined using the Kidney Disease Outcome Quality Initiative 2012 guidelines employing urine ACR, dipstick urinalysis and eGFR. In this study, spot urine ACR and dipstick urinalysis were done and repeated three months later if initial tests were abnormal. Data was analysed using SPSS version 21. Chi square and t test were used to compare proportions between groups. Relation between study variables and CKD were examined using logistic regression

Results*:* The median age of the participants was 9 years (range 9 - 12.3 years). Male to female ratio was 1:1. The mean age at diagnosis of SCA was 22 months.

The prevalence of haematuria, proteinuria and CKD among the study participants was 14.2%, 36% and 36 % respectively. Low haemoglobin and elevated mean corpuscular volume (MCV) were associated with CKD-AOR 0.62, 95% CI; 0.46-0.84 and 1.04, 95% CI; 1.01 – 1.08 respectively. Recurrent admissions (due to VOCs, severe anaemia and febrile illness) were also risk factors associated with CKD- AOR 0.52, 95% CI; 0.27-0.98. CKD was not associated with age at enrolment, sex, age at diagnosis of SCA, recurrent Vaso-occlusive crisis (VOCs) or abnormal liver function tests.

Conclusion: The prevalence of CKD among the SCA patients at UTH- Lusaka is high (36%) with lower Haemoglobin, elevated MCV and recurrent admissions being risk factors for developing CKD. SCA patients should be screened for CKD routinely at least once a year. Interventions such as early introduction of hydroxyurea, proactive blood transfusions and ACE inhibitors can reduce the risk of CKD and its progression to end stage renal disease.

Key words: Chronic kidney disease, sickle cell anaemia, Nephropathy.

DEDICATED TO:

-Study participants and their guardians for the opportunity to learn and serve.

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LIST OF ACRONYMS

ACE inhibitors - Angiotensin Converting Enzyme Inhibitors

ACR - Albumin creatinine ratio

ALT - Alanine aminotransferase

AST - Aspartate aminotransferase

CKD - Chronic kidney disease

Cr - Creatinine

DL - Deciliter

GFR - Glomerular Filtration Rate

eGFR - Estimated Glomerular Filtration Rate

ESRD - End Stage Renal Disease

FBC - Full Blood Count

Hb - Haemoglobin

HbS - Haemoglobin S

KIDGO - Kidney Disease Improved Global Outcomes

KDOQI - Kidney Disease Outcome Quality Initiative

MA - Microalbuminuria

MCV - Mean corpuscular volume

SCA - Sickle cell anaemia

VOC - Vaso-occlusive crisis

UTH - University Teaching Hospital

USA- United States of America

DEFINITIONS OF STUDY TERMS

i) **End stage renal disease** - Stage 5 of chronic kidney disease requiring renal replacement therapy.

ii) **Chronic kidney disease** - The presence of structural kidney damage or abnormal kidney function present for three months or more.

iii) **Haematuria** - Presence of blood in urine.

iv) **Hyposthenuria** - Excretion of urine of low specific gravity due to inability of the tubules of the kidneys to produce a concentrated urine or intake of excessive water.

v) **Microalbuminuria** - The excretion in urine of very small amounts of albumin, slightly in excess of 20μg/min or in the range of 30–300 mg/24 hours.

vi) **Proteinuria** - Presence of proteins in urine.

vii) **Recurrent vaso-occlusive crisis** - This is when a patient with sickle cell haemoglobinopathy has 3 or more VOCs in year.

viii) **Sickle cell anaemia** - The inheritance of two abnormal genes coding for haemoglobin of which both are coding for haemoglobin S.

ix) **Steady state in sickle cell anaemia**- A period between crises during which the patient is asymptomatic.

CHAPTER ONE

1.1**BACKGROUND**

Sickle cell anaemia (SCA) is an autosomal recessive disease characterised by the inheritance of two abnormal genes coding for an abnormal haemoglobin, of which both of them code for haemoglobin S (DeBaun et al, 2007). Worldwide, 300,000 babies with sickle cell anaemia are born. Sub-Saharan Africa accounts for 75% of sickle cell anaemia cases (Makani et al, 2013).

Chronic and /or recurrent sickling of red blood cells (RBCs) underlies the mechanism of several complications of SCA. Kidney injury due to recurrent sickling of RBCs predisposes to CKD. The global prevalence of CKD among SCA patients is highly variable. It varies from region to region and is dependent on the age group studied. Worldwide, nephropathy of varying severity occurs in 5 to 18% of the sickle cell population across all age groups with a third of the adults proceeding to develop CKD universally (Makani et al, 2013; Scheinmann et al, 1994; Powars et al, 1991). The prevalence of end stage renal disease (ESRD) in SCA patients regardless of the age group is 11%. The impact of CKD and ESRD increase with advancing age (Powars et al, 2005; Sergeant et al, 2007).

Various studies evaluating the prevalence and risk factors of CKD in sickle cell patients have been done in both resource- limited regions and settings in which health service facilities are relatively good. In countries with better health services such as USA, Saudi Arabia and Brazil revealed the prevalence of CKD in SCA to be 5.1 to 26.5% (Aleem et al, 2008; Bodas et al, 2013; Silva et al, 2012; Yee et al, 2011). This is lower than what has been documented by studies in sub-Saharan Africa in which the prevalence of CKD was found to be as high as 68.4% (Aneke et al, 2014; Madu et al, 2015; Ephraim et al, 2015). All these studies have been done in patients with different profiles. Patient genotype and the settings in which the studies have been done are different leading to variations in the findings. The HbSS genotype is more common in sub-Saharan Africa and this is more aggressive. Therefore, it is expected that SCA patients in our setting would have a high prevalence of complications such as CKD among others.

The true prevalence of CKD in the homozygous HbSS paediatric population is poorly documented in Africa. The few studies that have been done have limitations in terms of the methodology used, age of study participants and set up in which they were done such that their results cannot be generalized hence there is a vacuum for this much needed information.

In Zambia, the prevalence of haematuria and proteinuria was determined to be 32 to 92% and 7 to 43% respectively in both paediatric and adult patients (Musonda et al, 2010; Chansa et al, 2012). However, these studies did not go further to determine the prevalence and risk factors of CKD. Their study population had a bias towards adults and some parameters such as microalbuminuria (the hallmark of CKD) were not included in the analysis of the results. This study sought to determine and document the prevalence and risk factors for CKD in Paediatric steady state SCA patients seen at the University Teaching Hospital in Lusaka, Zambia.

1.2 STATEMENT OF THE PROBLEM.

Zambia has reported a 17.5% carrier rate of the sickle cell gene (Barclay et al, 1970). The UTH haematology out- patient clinic has enrolled over five thousand patients in their care. In 2013 alone, over one thousand SCA patients were seen in the Department of Paediatrics and Child Health in-patient facility with various complications of the disease. With this huge number of SCA patients coupled with their improved survival, it is expected that the burden of kidney diseases in SCA would be significant. Extrapolation from other studies estimates that up to 15 percent (750) of these patients will develop or have CKD (Makani et al, 2013; Powars et al 1991; Scheinman et al, 1994: Abbott KC et al, 2002). All earlier studies carried out at the UTH-Lusaka attempted to describe some indicators of CKD in sickle cell patients yet did not quite determine the prevalence of CKD or its risk factors among SCA patients (Chansa et al, 2010; Musonda et al, 2012). This knowledge gap for clinicians affects the targeting and timing of screening for CKD in children with SCA.

1.3 STUDY JUSTIFICATION

In Zambia, there is a large cohort of sickle cell patients. Currently, over five thousand of them are being followed up in the haematology unit at UTH-Lusaka. Studies in various parts of Africa suggest that the prevalence of CKD among SCA patients is high (Ephraim et al 2015, Madu et al 2015). HbSS is the commonest genotype in Zambia and this is the most severe form of sickle cell haemoglobinopathy. SCA is associated with development of CKD. No definite treatment for CKD is available in our set up. Early screening would enable slowed disease progression with interventions such as use of hydroxyurea and ACE inhibitors. These have been shown to slow down the onset of CKD in SCA patients. This study sought to determine the prevalence and risk factors of CKD in SCA, information that was previously not known in our country prior to this study. Findings from this study will thus form basis for protocol formulation for targeted screening and interventions for diagnosis and slowing down of CKD in SCA patients respectively.

1.4 RESEARCH QUESTIONS

i. What is the prevalence of CKD in steady state SCA patients aged 5 to 16 years seen at UTH in Lusaka, Zambia?

ii. What are risk factors of CKD in steady state SCA patients aged 5 to 16 years seen at UTH in Lusaka, Zambia?

1.5.0 OBJECTIVES

1.5.1 General objective

To determine the prevalence of CKD in steady state SCA patients aged 5 to 16 years seen at UTH in Lusaka, Zambia.

1.5.2 Specific objectives:

i) To evaluate the prevalence of proteinuria in steady state SCA patients aged 5 - 16 years seen at UTH using dipstick urinalysis and urine albumin / creatinine ratio (ACR)

ii) To assess the prevalence of haematuria in steady state SCA patients aged 5 – 16 years seen at UTH using dipstick urinalysis.

iii) To establish the prevalence of abnormal estimated GFR in steady state SCA patients aged 5-16 years at UTH using the updated Schwartz equation.

iv) To estimate the prevalence of CKD in steady state SCA patients aged 5-16 years seen at UTH.

v) To determine the risk factors associated with CKD in steady state SCA patients seen at UTH.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Epidemiology of chronic kidney disease in SCA

In sickle cell haemoglobinopathy, haemoglobin S (HbS) is the result of a single base pair change, thymine for adenine, at the 6th codon of the beta globin. This change encodes valine instead of glutamine in the 6^{th} position in the globin molecule (DeBaun et al, 2007). SCA occurs when both globin genes have the sickle cell mutation. Sickle cell disease is heterozygous inheritance of two genes coding for abnormal haemoglobin of which one must code for HbS (DeBaun et al, 2007). SCA is a big problem in sub-Saharan Africa where 75 percent of the babies born with sickle cell haemoglobinopathy are located (Makani et al 2013). This is a big population potentially at risk of developing SCA associated complications such as CKD. In hyper-osmolar and hypoxic environments, sickle cell haemoglobinopathy is associated with sickling of RBCs. This leads to haemolysis, acute anaemia, vaso-occlusion and increased blood viscosity. All these features are more common in homozygous HbS patients in whom there is high morbidity and mortality when compared to other genotypes of sickle cell haemoglobinopathy (Ephraim et al, 2015). These features of sickle cell haemoglobinopathy have far reaching consequences on several organs of the body leading to complications of which CKD is one of them (DeBaun et al 2007; Phuong-Thu et al, 2000; Alleyne et al, 1975; Statius et al 1997).

In North America, the prevalence of CKD is 8.3 to 26.5% as demonstrated by two studies which enrolled predominantly paediatric study participants with mean age of 13.5 ±4.4 years (Bodas et al, 2013; Yee et al, 2011). In both studies, there was a predominance of patients with a genotype of HbSS. A study done in Saudi Arabia on prevalence of CKD among sickle cell patients had results comparable with the findings in the USA. The study in Saudi Arabia also included adults as well and found a prevalence of CKD to be 20.3% (Aleem et al, 2008). In sharp contrast, a much lower prevalence of CKD of 5.1% was found in a research done in Brazil despite having a study population that was exclusively composed of adults in whom the sickle cell

related complications including CKD are expected to be high (Silva et al, 2012). This difference in the research results between the studies done in the USA and Saudi Arabia on one hand and studies done in Brazil on the other hand demonstrate that the profile of patients and setting in which a study has been done can affect research findings.

In Africa, three studies done in both paediatric and adult populations in West African countries i.e. two in Nigeria and one in Ghana showed the prevalence of CKD in sickle cell patients to be high as 68.4% (Aneke et al, 2014; Madu et al, 2015; Ephraim et al 2015). These studies demonstrated that CKD is common in sickle cell patients. SCA associated complications such as CKD are more prevalent in adults than in the paediatric SCA populations. This has been reflected in a study done in Nigeria which revealed a higher prevalence of CKD in adults in which all the 100 study participants in that cohort had some form of CKD though none had ESRD (Aneke et al, 2014). Another study in Nigeria followed up a cohort of 220 SCA patients (median age of the study participants was 24 years) over a period of 7 years and found prevalence of CKD to be 20.3% with 1.7% of them requiring renal replacement therapy (Madu et al 2015). These two studies done in Nigeria also demonstrate that even within the same country study results may not be replicated because of variations in the study methodology, profile of patients and the setting of the area in which the study is being conducted. The prevalence of CKD in sickle cell patients in Ghana is 39.2% in both adults and paediatric sickle cell populations though further stratifying of available data shows the prevalence of CKD in the paediatric population and adults to be 31.6% and 68.4 % respectively. Proteinuria and CKD in this population was far more common in HbSS genotype than in HbSC genotype. The prevalence and intensity of CKD increased with older age, a finding consistent with what has been reported in literature (Silva et al, 2012; Ephraim et al, 2015).

Proteinuria, Haematuria and abnormal estimated glomerular filtration rate (eGFR) are among the clinical features of CKD. Collectively, their prevalence influences the overall prevalence of CKD. Proteinuria, especially microalbuminuria (MA) is a hall mark of glomerular injury. The prevalence of proteinuria among SCA patients ranges between 15 to 30%. Studies done in countries such as Brazil, the USA, Saudi Arabia

and India have shown a prevalence of proteinuria on average. These studies have been done in environments different from ours. The genotype of their sickle cell population is not mainly the severe form (HbSS), their patients are diagnosed early and measures to prevent complications are initiated in good time. This results in low prevalence of SCA associated complications such as CKD. The methodology used for studies done outside African setting may not be applicable in our setting by virtue of our setting being a low resource setting.

2.2. Pathogenesis and pathophysiology of SCA associated chronic kidney disease

CKD is the presence of structural kidney damage or abnormal kidney function for three months or more as defined by presence of proteinuria, haematuria or a eGFR of less than 60 ml/min/1.73m^2 (Kopple et al 2001, KDOQI 2012). The CKD associated with sickle cell haemoglobinopathy can involve damage to multiple parts of the kidney. The hemodynamic changes that occur with chronic anaemia and renal hypoxia due to recurrent vaso-occlusion and hemolysis related endothelial dysfunction can lead to CKD (Phuong-Thu et al, 2000). The findings in CKD are haematuria, proteinuria, abnormal estimated GFR, hyposthenuria, electrolyte imbalance, abnormal renal function tests, and kidney structural abnormalities. Biological markers of CKD such as creatinine can be normal even in the face of advanced CKD because of hyperfiltration and lean mass in sickle cell patients hence they are not reliable markers for CKD in SCA patients. Microalbuminuria is an early marker of CKD and it can be present even before dipstick urinalysis reveals proteinuria and / or haematuria (Scheinmann et al, 2009).

Renal tubular defects: Hypoxia, acidotic and hyper-osmolar environment of the inner medulla promote sickling of the RBCs resulting in impaired renal medulla blood flow, ischaemia, micro-infarct and papillary necrosis cause renal tubular defects. SCA patients also have reduced vasa recta, abnormal dilatation or obliteration of the remaining capillaries hence negatively affecting the counter current multiplication and

exchange system of the inner medulla resulting in the inability to concentrate urine well (DeBaun et al, 2007; Ataga et al, 2000; Allon et al, 1990). This is worsened by repeated sludging causing thrombosis, progressive infarction and necrosis of the papillae and the inner medulla. The ability to acidify urine and excrete potassium in SCA patients can be impaired due to impaired tubular function (Statius et al, 1992). Failure to acidify urine can manifest as an incomplete renal tubular acidosis and can be seen in the setting of renal insufficiency even in the mildest form renal impairment.

Abnormalities in the renal haemodynamics: Increased eGFR and renal plasma flow have been documented in sickle cell patients and these are due to compensatory hyper secretion of vasodilator prostaglandins in response to sickling. The resultant hyper filtration causes glomerular capillaries sclerosis (glomerular damage) leading to progressive renal insufficiency which is more common in older patients especially adults (Phuong-Thu et al, 2000; Aloni et al, 2014).

Haematuria: Haematuria is a common occurrence in SCA patients. The cause of haematuria is related to the pathological events in the inner medulla and papillae of sickle cell patients. Sickling of RBCs in the vasa recta causes increased blood viscosity, micro-thrombi formation and ischaemic necrosis causing structural changes leading to haematuria. Haematuria may originate from either kidney. In 80% of the patients, bleeding is from the left the kidney (Ataga et al, 2000; Allon et al, 1990)

Proteinuria: Proteinuria is encountered more frequently in SCA than in other haemoglobinopathies with an incidence of 20 to 30 % in SCA patients (Scheinmann et al, 2009; Falk et al, 1994). The mechanisms of glomerular damage are varied and due to but not limited to mesangial phagocytosis of sickled red cells, immune complex mediated glomerulonephritis and glomerular hyperfiltration leading to glomerular injury and hypertrophy. These effects are primarily due to the basic pathological process-sickling of the red blood cells with subsequent episodic ischaemia of the end organ (DeBaun et al, 2007). Ineffective erythropoiesis, severe anaemia, hypertension, proteinuria, nephrotic syndrome and haematuria are pre-azotaemic predictors of chronic renal failure (Powars et al, 1991). The kidneys of SCA patients have pathologic findings which include prominent glomeruli distended with

blood along with necrosis and pigmentation of the tubular cells (Sydenstricker et al, 2003).

Glomerular enlargement and congestion are more common in children older than 2 years of age and more marked in the juxtamedullary glomeruli (Buckalew et al, 1974). Other prominent lesions in the renal medulla include focal scarring, interstitial fibrosis, tubular atrophy and infiltration with lymphoid cells. Iron deposits primarily in the proximal tubule are common though pigment casts may also be seen (Phuong-Thu, 2000). These abnormalities of the renal medulla and papillae in SCA associated CKD are due to decreased blood flow in the vasa recta which can lead to medullary and papillary necrosis and fibrosis. Structural glomerular abnormalities seen in SCA associated CKD are focal segmental glomerulosclerosis (FSGS) and membranoproliferative glomerulonephritis – like (MPGN- like) disease. Definite MPGN type (immune complex type) have been reported in literature (Phuong-Thu et al, 2000).

2.3 **Manifestations *of SCA associated nephropathy in Children***

Clinical features of CKD in SCA include the following:

Haematuria: This is a common finding in SCA patients. The prevalence of haematuria depends on the region and the group of patients studied. In West and East Africa, the prevalence of haematuria in SCA is between 3.3% and 13.3% in the paediatric age group (Anigilage et al,2013; Osei-Yeboah et al ,2011) and this is similar to the 3% and 8.5% that has been reported in both adults and children in Brazil and Saudi Arabia respectively (Silva et al, 2012; Aleem et al, 2008).In Zambia, the prevalence of haematuria in the sickle cell population across all age groups at the UTH is between 32 and 92% (Chansa et al, 2012; Musonda et al, 2010). These differences could be due to variations in patient profile e.g. sickle cell genotype and how well patients are managed e.g. early initiation of drugs such as hydroxyurea which reduces risk of complications such as CKD.

Proteinuria: Proteinuria in SCA is one of the markers of CKD. In North America, the prevalence of proteinuria is between 15.9 and 38.5% in the age group 2 to 21 years with

proteinuria being noted in children as young as three years of age, earlier than anticipated with higher haemoglobin being protective against proteinuria and CKD (Yee et al, 2011; Aygun et al, 2011; Olivera et al, 2008).

Renal complications in SCA are more prevalent in older patients and this is evidenced by a study in Saudi Arabia in which prevalence of proteinuria was 41% in both adults and children but more common in the older patients among the study participants (Aleem et al, 2008). In an Asian paediatric population, in India, the prevalence of proteinuria was 19.2%. Advancing age again was a risk factor for microalbuminuria as it was more common in older children especially those above the age of 9 years (Datta et al, 2003). This is in contrast with what was found in a study in Brazil where the prevalence of proteinuria was 3% despite the study population being exclusively composed of adults. This seem to suggest that older age has no effect on the prevalence of proteinuria SCA. However, it is documented in various studies and literature that older age come with increased risk of complications in sickle cell patients (Yee et al, 2011; Aleem et al, 2008; Silva et al, 2012; Aygun et al, 2011; Olivera et al, 2008). This difference could be due to differences in patient profile e.g. sickle cell genotype and how well patients are managed e.g. early initiation of drugs such as hydroxyurea which reduces risk of complications such as CKD.

The prevalence of proteinuria among SCA patients in West and East Africa is between 9.4 and 28.2%. This is based on four studies in Ghana, Nigeria, Uganda and Tanzania which were done in the paediatric age group. These studies also demonstrated that higher haemoglobin (Hb) is a protective factor against the development of proteinuria and CKD, while advance in age is a risk factor for proteinuria and CKD eventually (Abhulimen et al, 2011; Mawanda et al, 2011; Richard et al, 2012). In Zambia, prevalence of proteinuria is 7 to 43% among the sickle cell patients at UTH- Lusaka in the age group 10 to 43 years (Chansa et al, 2012; Musonda et al, 2010). This is much higher than the average 15 to 30% reported in West and East African countries.

Abnormal eGFR: Estimated GFR has been subject to various studies in SCA patients as it is one of the parameters that get deranged in CKD in SCA). The abnormality in eGFR can be either an abnormally low eGFR or high eGFR – hyperfiltration (Aloni et al, 2014). Estimated GFR is initially higher than normal and then begin to decline with age advancement. Hyperfiltration is associated with glomerular damage. The prevalence of hyper filtration is variable, reported to be high in the USA (76%) and Brazil (53%) and relatively low in Nigeria (30.6%). The prevalence of eGFR abnormalities vary from region to region. It is also dependent on the age group studied showing a decline with advance in age (Silva et al, 2012; Bodas et al, 2013; Aygun et al 2011).

Hyposthenuria: Excretion of urine of low specific gravity, due to inability of the tubules of the kidneys to produce concentrated urine. This can be evidenced by low urine osmolality or abnormally low specific gravity (Scheinman et al, 2009).

Other renal abnormalities in SCA nephropathy: Some of the renal abnormalities associated with SCA include gross haematuria, papillary necrosis, nephrotic syndrome, renal infarction, pyelonephrosis, end stage renal insufficiency and renal carcinoma (Scheinmann et al, 2009; Luciana et al, 2002).

2.4 Risk factors of chronic kidney disease in SCA

There are various risk factors associated with CKD in SCA and these include advanced age, severe anaemia, HbSS genotype, recurrent VOCs, delay in the diagnosis of SCA, nephrotoxic drugs and poor health seeking behavior. Deranged liver function tests, i.e. alanine aminotransferase (ALT) and aspartate aminotransferase(AST), have also been found to be risk factors for SCA associated CKD (Aleem et al, 2008; Guash et al 1997 Silva et al, 2012; Ephraim et al, 2015; Datta et al, 2003; Osei et al, 2011; Bodas et al 2013, Aygun et 2011).

2.5. Diagnosis of chronic kidney disease and Challenges of estimation of glomerular filtration rate/renal function in SCA patients

Various studies have been carried out to assess renal functional status in SCA patients with variations in outcomes depending on the indicators used and age group studied. Relative increase of the eGFR in SCA children and tubular secretion of serum creatinine (Cr) results in low serum Creatinine. Biochemical indicators like creatinine, urea, and potassium may remain within normal range in SCA patients with renal complications and imminent renal failure. Proteinuria has been identified to be persistent and increase progressively with the increase in severity of renal damage (Scheinmann et al, 2009). Thus, urinalysis is a valuable tool for screening urinary abnormalities as it detects proteinuria, haematuria and abnormal urinary specific gravity though it cannot detect microalbuminuria (MA) that heralds early renal involvement (Aok et al, 1990; Ide et al, 2011). MA is excretion of albumin in small amounts (30-300mg/dL) in urine in 24 hours. Proteinuria especially MA is a very important marker of glomerular injury in patients with renal disease thus it is useful in early detection of CKD in SCA patients (Ide et al, 2011; Levey et al 2005). CKD in SCA encompasses all structural and functional abnormalities of the kidneys seen in SCA patients.

The diagnosis of CKD requires presence of either:
evidence of structural or functional abnormalities (abnormal urinalysis, MA, haematuria, or histology) that persists for at least three months with or without a decreased GFR as defined by an estimated glomerular filtration rate (eGFR) less than 60mL/min/1.73m^2

or

eGFR less than 60mL/min/1.73m^2 for at least three months with or without any evidence of kidney damage (KDOQI guideline, 2012; Levey et al, 2005).

2.6. **Management and outcomes of SCA associated nephropathy**

In SCA patients, the pre-azotaemic phase of CKD manifested by hypertension, proteinuria and severe anaemia predict end stage renal disease (ESRD). The survival time for SCA patients with CKD is about four years after diagnosis despite dialysis and the treatment outcome of the azotaemic phase is dismal (Powars et al, 1991). The mortality rate following ESRD diagnosis in SCA patients is three times higher than in patients with ESRD without sickle cell anaemia. Control of the indicators of the of CKD with interventions such as use of hydroxyurea, angiotensin converting enzyme (ACE) inhibitors and the proactive blood transfusion have proven to be helpful as they can slow down the progression of CKD to ESRD (Stall et al, 2011; Becker et al, 2011; Paydas et al, 1996; Guash et al 1997).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

This was a prospective cross sectional study in known SCA anaemia patients at the University Teaching Hospital (UTH) in Lusaka, Zambia.

3.2 Study Site

The study site was the emergency room and the haematology clinic, in the Department of Paediatrics and Child Health at UTH. UTH is a tertiary referral hospital in Lusaka, Zambia and it is home to several specialties of which hematology is one of them-a specialty under which this research was conducted. The haematology clinic runs on an outpatient basis on Fridays every week except on public holidays while the emergency room operates on a daily basis. The haematology clinic was started in 1973 and to date more than 5000 SCA patients are registered with this clinic.

3.3 Study duration

The study was conducted over a 12-month period beginning August 2014 and ending July 2015.

3.4 Target Population

Known SCA patients in a steady state aged 5 -16 years of age attending haematology clinic and those who were being reviewed in the emergency room in the department of Paediatrics and Child Health at the UTH.

3.5 Inclusion and exclusion criteria

a) Inclusion criteria:

i. Age 5-16 years
ii. Asymptomatic for at least 4 weeks
iii. Sickle cell anaemia patients with Hb SS electrophoresis results
iv. Parental/Guardian consent (with child assent where applicable)

b) Exclusion criteria:

i. Confirmed or suspected urinary tract infection
ii. Pregnant patient or those patients having menses
iii. Refusal to consent/assent

3.6 Sample size

Despite a huge variation in the prevalence of CKD in SCA from region to region, the average worldwide prevalence of nephropathy in SCA patients across all age groups is 5 to 18 percent. Based on the estimated prevalence of 15% chronic kidney disease in sickle cell anaemia patients, 196 participants were enrolled in order to identify the true prevalence of chronic kidney disease with a precision of +/-5% and the level of significance at 95%. The sample size was calculated as illustrated below using the formula for prevalence studies shown below:

$N = [Z^2 X P (1-P)]/E^2$

where N=sample required,

Z =Z statistic =1.96 (95% significance level),

P= expected prevalence 0.15 (assuming a 15% prevalence of CKD in SCA patients)

E= margin error of 5%

Therefore, $N = [(1.96)^2 X 0.15(1-0.15)]/ (0.05)^2 =196$.

Sample **size was 196.**

3.7 Sampling method

Random sampling method was used to select patients that participated in this study. Files for eligible patients attending sickle cell clinic were identified and given numbers and then computer generated numbers matching with the numbers given to files for eligible patients were put on a table and were picked at random. For each computer generated number picked at random, the patient whose file had that corresponding study number was called in together with the guardian and invited to take part in the study. The patients were enrolled in the study after parental/guardian consent (and assent from the participant where applicable) was obtained.

3.8 Data collection

i) A structured questionnaire was used to collect socio-demographic details, medical history of study interest such as frequency of VOCs, admissions and blood transfusions in the preceding one year, drug history and age at diagnosis of SCA

ii) Laboratory forms were used to collect data for the laboratory in investigations

3.9 Study procedure:

After obtaining consent (and child assent where applicable), each of the enrolled participants had an interviewer administered data collection questionnaire filled in. This questionnaire collected demographic data, past and current medical history and this was followed by physical examination and collection of specimens. It must be mentioned that the questionnaire was piloted prior to being used for this study. The data collected from twenty study participants who were involved in the piloting of the study questionnaire formed part of the data for this study because no changes were made to the variables under study.

The specimens that were collected from study participants are:

i. Urine for dipstick urinalysis and urine ACR.

ii. Blood-a total of 4 milliliters was drawn from each study participant and the blood was subjected to haematological test (FBC) and biochemistry analysis i.e. urea, Cr, AST and ALT.

The study participants with proteinuria, haematuria and / or abnormal eGFR were reviewed at three months from the date of enrollment to repeat the urinalysis, urine ACR and / calculation of eGFR to assess for presence of CKD (As per study definition of CKD-refer to study definitions). The participants who became symptomatic i.e. those who developed VOCs, febrile illnesses, urinary tract infections while waiting for the three months to come for review for purposes of this study had to be re-enrolled into the study after being asymptomatic for four weeks.

Once results showed haematuria and/ or features suggestive of a urinary tract infection on dipstick urinalysis, the urine was subjected to urine microscopy, culture and sensitivity. All the laboratory results from this study were forwarded to the attending physician with copies being retained for the study.

3.10.0 VARIABLES

3.10.1 Primary outcomes

i. **Proteinuria**: Proteinuria was assessed by use of dipstick urinalysis and urine ACR. The proteinuria of 1+ or more on dipstick urinalysis and/ or microalbuminuria on urine ACR constituted an outcome of proteinuria. MA is excretion of albumin in small amounts in excess of 20µg/min or 30-300mg/dL in urine in 24 hours. Proteinuria especially MA is a very important marker of glomerular injury in patients with renal disease thus it is useful in early detection of CKD in SCA patients even before the symptoms appear.

ii. **Haematuria**: Haematuria was determined by dipstick urinalysis. Haematuria of 1+ or more constituted an outcome for haematuria.

iii. **Abnormal eGFR**: The eGFR was calculated using an updated Schwartz equation for eGFR (eGFR = 41.3 (height / serum creatinine). A eGFR less than 60mL/min/1.73m^2 constituted an outcome of abnormal eGFR. The updated Schwartz formula was chosen because it has been validated in the paediatric population. Its limitation is over estimating the eGFR while the alternative formula – chronic kidney disease epidemiology equation is not validated in the

paediatric population. CKD has various grades based on the estimated GFR as shown in table 1 below based on the KIDGO guidelines.

iv. **Chronic kidney disease**: The presence of one or more of the above mentioned primary outcomes for a minimum period of three months constituted an outcome of CKD. To be able to determine the presence of CKD, all participants with proteinuria, haematuria and / or abnormal eGFR were reassessed for persistence of proteinuria, haematuria and / or abnormal eGFR at three months after initial assessment.

Table 1: Categories of CKD.

GFR category	GFR (ml/min/1.73 m^2)
Grade 1	≥90
Grade 2	60 -89
Grade 3	30 – 59
Grade 4	15 – 29
Grade 5	< 15

3.10.2 Independent variables:

i. Age at enrollment in years
ii. Sex
iii. Number of admissions in the past one year
iv. Frequency of vaso-occlusive crises (VOCs) in the preceding one year
v. Age at diagnosis of SCA in months
vi. Haemoglobin
vii. Liver function tests (ALT and AST)
viii. Frequency of blood transfusions in the past one year
ix. Platelet count

3.11.0 Data management

3.11.1. Data collection:

A standardized data questionnaire for each participant was used for data collection and participants were identified by codes to ensure anonymity.

3.11.2. Data security:

Data was stored in a lockable cabinet with the principal investigator having sole access to the lockable cabinet.

3.11.3. Data entry

The data was entered on the Epidata software with double entry being performed to reduce on human errors.

3.11.4 Data analysis:

Data were analysed using the statistical software package SPSS version 21. All statistical tests were at 5 percent significance level. The Independent Samples T-test and the Pearson's chi-squared were used to compare mean values and proportions between groups with and without CKD respectively. The relationship between study variables and outcome variable of interest (CKD) was examined using logistic regression. Selection for logistic regression model was considered at level $P < 0.20$ or known clinical significance. Backward selection method was used to obtain the final logistic regression model. The backward selection method removes terms one at a time beginning with the largest p-value and continuing until all remaining effects are significant at a specified level or removing more terms results in poorer fit.

3. 12 Ethical considerations:

a) **Ethical approval**: Before the study was carried out, ethical approval and permission were obtained from ERES converge IRB and the study site respectively.

b) **Informed consent**: The purpose of the study was explained to the parents / guardians and participants. It was emphasized that participation was purely voluntary with no financial rewards or gains for taking part. Patients and / or parents/guardians were free to withdraw from the research at any time with no repercussions for refusing to participate or for withdrawing. Consent was sought from parents/guardians (and child assent from participants where applicable).

c) **Privacy and Confidentiality**: Data collected was kept under lock and key with only the principal investigator having access to the data. The data and specimen collected during this research was used for this research purposes only.

d) **Benefits**: The benefits for the participants in this study are that those who were found with renal abnormalities were referred for appropriate care as per standard procedure at UTH. Those whose results were normal now know what their current functional status is and were counseled on the need to have their renal functional status assessed regularly.

e) **Gain in scientific knowledge**: Benefits of this study to SCA population is that baseline data concerning CKD and its risk factors in SCA were determined at UTH. This research has the potential to guide protocols at UTH with regards to targeted and timing of screening for renal abnormalities in SCA patients. This in turn can lead to identifying those with renal dysfunction early hence interventions to slow down the progression to ESRD can be instituted in good time.

f) **Description of risks:** The disadvantages of being participants in this study were the fact that participants experienced some pain when samples of blood were

being drawn. Research participants spent more time in the SCA clinic/ emergency room longer than necessary during their regular reviews.

g) **Emergency care for research related injury:** For research related injuries, the principal investigator under took to take full responsibility to arrange treatment at UTH as per UTH procedure for all those who would have sustained research related injuries while taking part in this study. It must be mentioned that no study participant sustained any research related injuries while taking part in this study.

CHAPTER FOUR

4.0 RESULTS

During the study period, 216 SCA patients eligible for this study were invited to take part of which Seven (7) of the parents refused to allow their children to participate. Eleven (11) of the participants did not meet the inclusion criteria and thus were excluded. A total of 198 patients were enrolled. All but one completed the follow up. Consequently, 197 study participants were considered in the final analysis. The response rate was about 92%. Refer to figure 1 for participant flow chart.

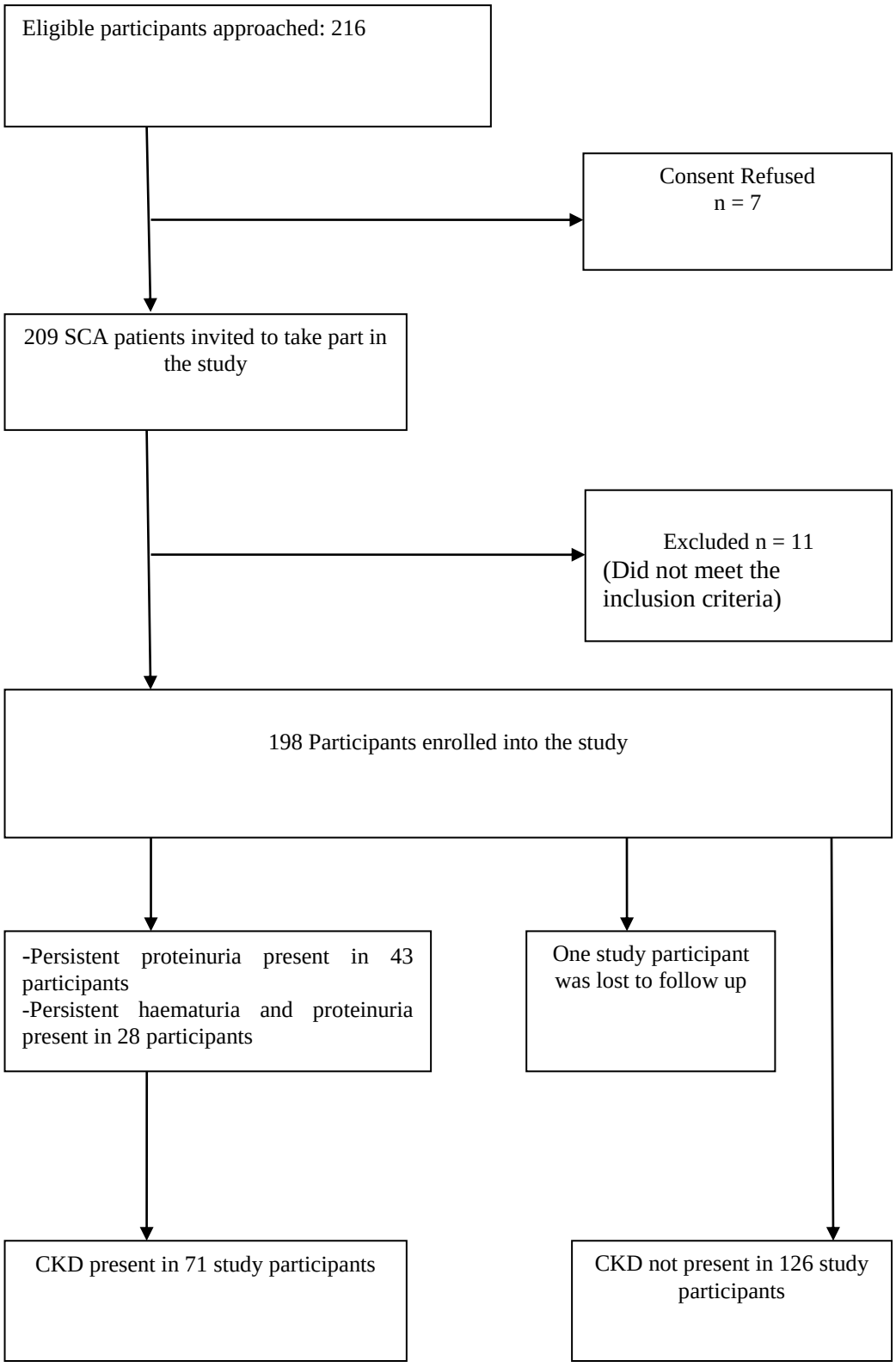

Figure 1: Study flow chart.

4.1 Descriptive Demographic and Clinical Characteristics of study participants

The median age of the participants was 9 years (interquartile range being 9 - 12.3 years). Figure 2 shows the age distribution histogram. The male to female ratio was 1:1 as shown in table 2. There was no statistically significant difference in mean age between males and females (P-value = 0.42). All the study participants were of African origin. A large proportion of the study participants (92.4%) were underweight with body mass index (BMI) < 18.5 Kg /m^2 while the remainder had normal BMI.

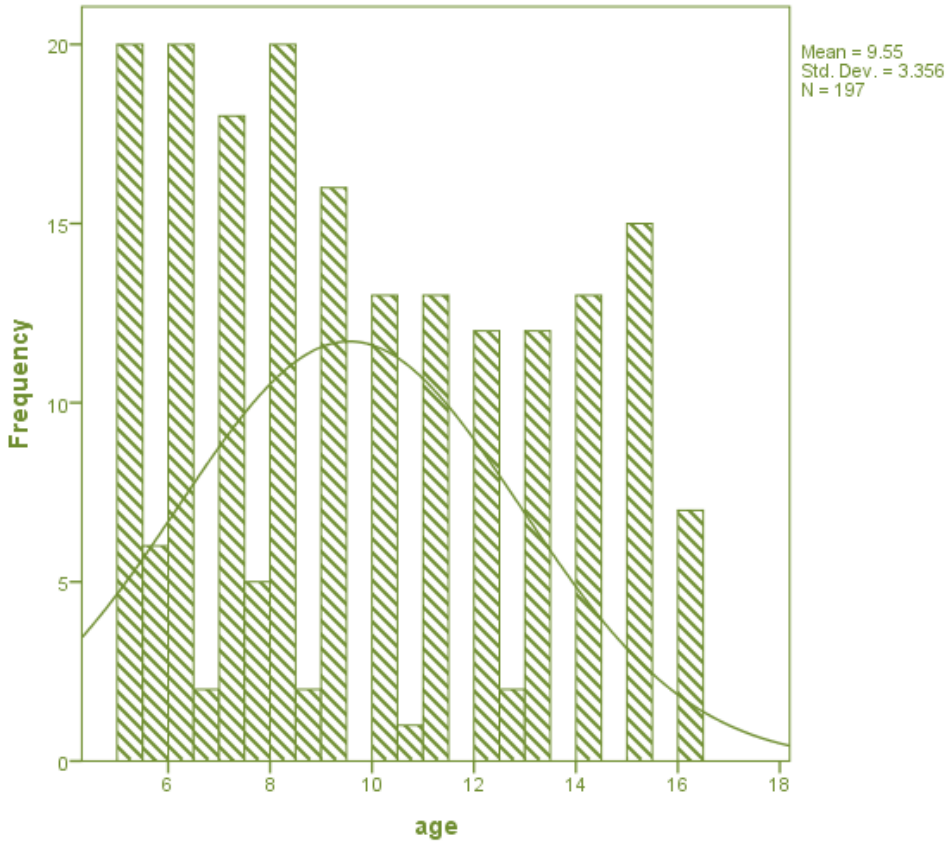

Figure 2: Age distribution histogram.

The median age at diagnosis of SCA was 22 months (range being 3 to 168 months). The median age at diagnosis of SCA for females versus males was 18 months and 24 months respectively see table 2. The difference was not statistically significantly (P value – 0.59).

Table 2: Age in months at diagnosis of SCA

Sex	Participants	Mean Age (months)	Standard deviation	Median Age (months)	Minimum Age (months)	Maximum Age (months)
Male	99	32.99	± 27.54	24	3	120
Female	98	33.35	± 32.64	18	3	168
Average for All participants	197	33.17	± 30.11	22	3	168

4.2. Prevalence of proteinuria and haematuria

On first dipstick urinalysis 41 (20.8%) of participants had proteinuria while only 32 (16.2%) of the participants had proteinuria on second dipstick urinalysis. Urine ACR was positive in 87 (44.2%) of the participants during first visit but this reduced to 36% after repeat ACR on second visit. Employing a combination of dipstick urinalysis and urine ACR showed a prevalence of persistent proteinuria in this study to be 36%. This study also found that 28 (14.2%) of the participants had persistent haematuria.

Table 3: frequency of proteinuria and haematuria among the participants

Parameter	Visit one	Visit two
Proteinuria on dipstick Urinalysis	20.8%	16.2%
Proteinuria on urine ACR	44.2%	36%
Haematuria on urinalysis	16.8%	14.2%

4.3 Prevalence of abnormal eGFR

No study participant was found to have an eGFR below 60 mL/min/1.73m^2. Actually 160 (81%) of the study participants had eGFR levels which were higher than normal with the average eGFR being 181.6 mL/min/1.73m^2 (SD= ±34.36).

4.4 Prevalence of chronic kidney disease

Participants who had an abnormal eGFR, proteinuria and / or haematuria for at least three months fulfilled the study definition of CKD and were deemed to have CKD. The prevalence of CKD among the study participants was found to be 36% i.e. 71 study participants fulfilled the study definition of CKD and these had persistent proteinuria and / or haematuria as shown in figure 3.

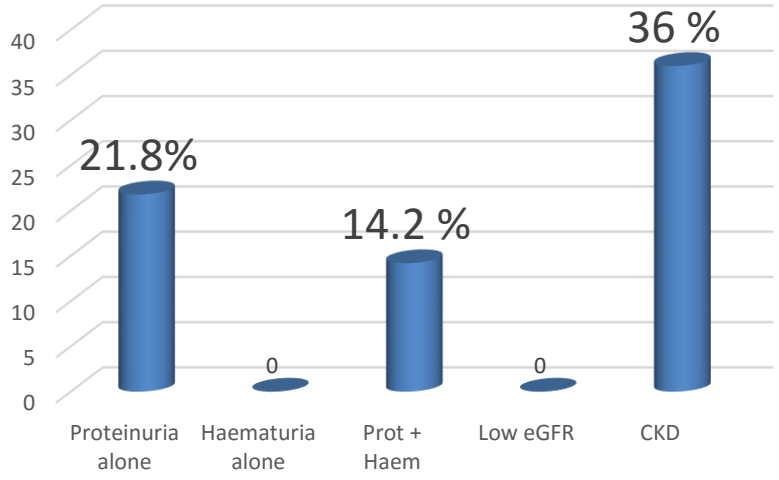

Figure 3. Frequency of Primary outcomes (%)

No study participant had eGFR less than 60 mL/1.73m^2/min or haematuria alone.

4.5 Risk factors for chronic kidney disease in SCA

4.5.1 **Haematological and serum biochemistry findings**

The mean haemoglobin (Hb) was 7.4g/dL (SD=1.22g/dL). The median platelets count was 410 $x10^9$ platelets /L (range: 43 x 10^9/L to 901 x 10^9 $/L$). The mean WBC was 14.1 (SD= ±4.34) x 10^9 /L while the mean MCV was 80.6 (SD = ± 10.05) fL. The reference range for MCV being 76 to 96 fL.

Mean Hb was 7.43 g/dL with a standard deviation of ±1.222 as shown below.

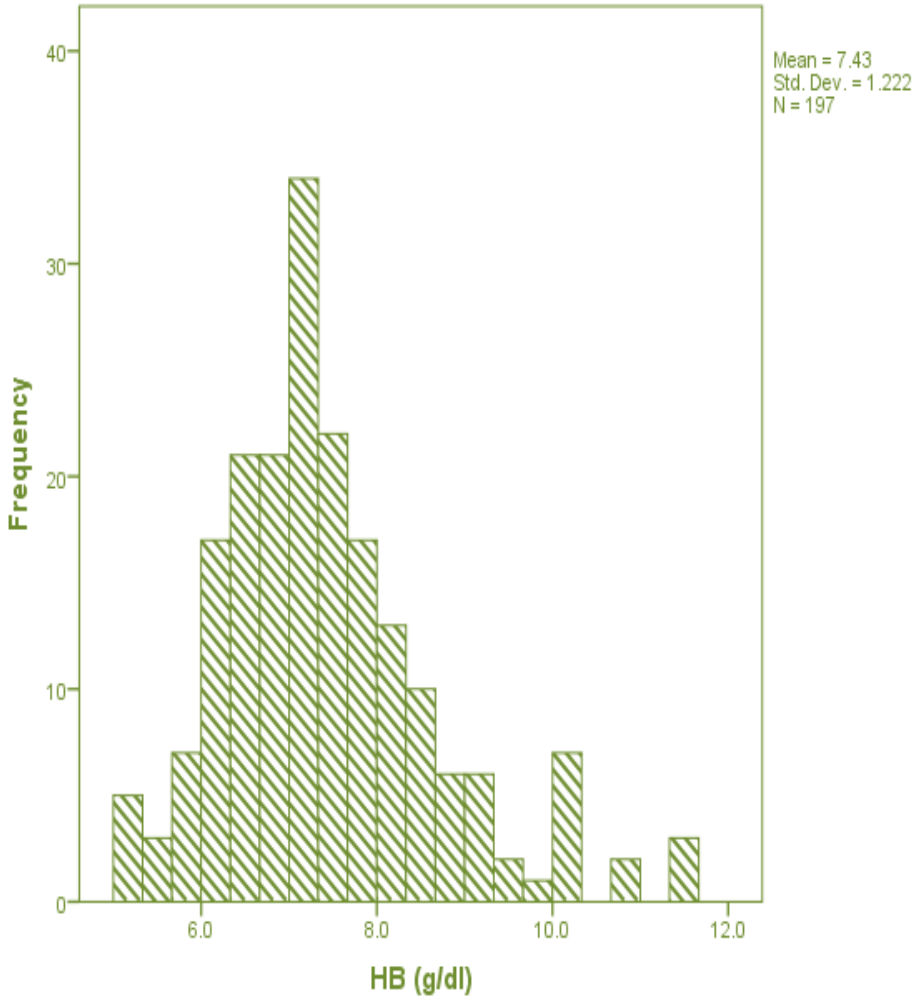

Figure 4: Distribution of Hb among the study participants.

The mean urea level was 2.9 (SD = ±1.18) mmol/L. The median creatinine level was 41.7 micromoles/L (range: 12.2 - 120.3 micromoles/L). None of the study participants had a creatinine level that was higher than normal for age.

Table 4-Summary statistics for Platelets, WBC, MCV, Urea and Creatinine:

	Platelets count/Litre	White cell count/Litre	MCV (fL)	Urea (mmol/L)	Creatinine (umol/L)
Number participants	197	197	197	197	197
Mean	420.7	14.1	80.6	2.9	47.2
Std. Deviation	± 140.07	± 4.34	± 10.05	± 1.18	± 19.63
Median	410	13.61	80	2.67	41.7
Minimum	43	3.08	57	1.08	12.2
Maximum	901	28.6	99.9	6.87	120.3

The mean platelet count was 420.7 (SD ± 140.7) x 10^9 / L while the mean for MCV and creatinine were 80.6 FL (SD ± 10.05) and 47.2 umol/L (SD ± 19.63) respectively.

The mean serum albumin level was 41.8 g/L (SD ±5.31). The median ALT 22.0 IU/L (range: 7.3 IU/L to 72 IU/L). The median AST was 39.5 IU/L (with the range being 12.8 iu/L to 139.0 IU/L). Urine specific gravity was normally distributed and PH was also normally distributed with the mean PH being 6.

Table 5: Summary statistics of ALT and AST

	ALT	AST
Number of participants	197	197
Mean	24.4 iu	44.3 iu
Standard deviation	± 11.7	± 24.6
Median	22 iu	39.5 iu
Minimum	7.3 iu	12.8 iu
Maximum	72 iu	139 iu

4.5.2 Frequency of blood transfusions

Eighty-one (41%) of the study participants had at least one blood transfusion in the preceding one year prior to enrolment into this study. Figure 3 shows the frequency of blood transfusions among the study participants who received blood transfusion(s) in the preceding one year prior to the study.

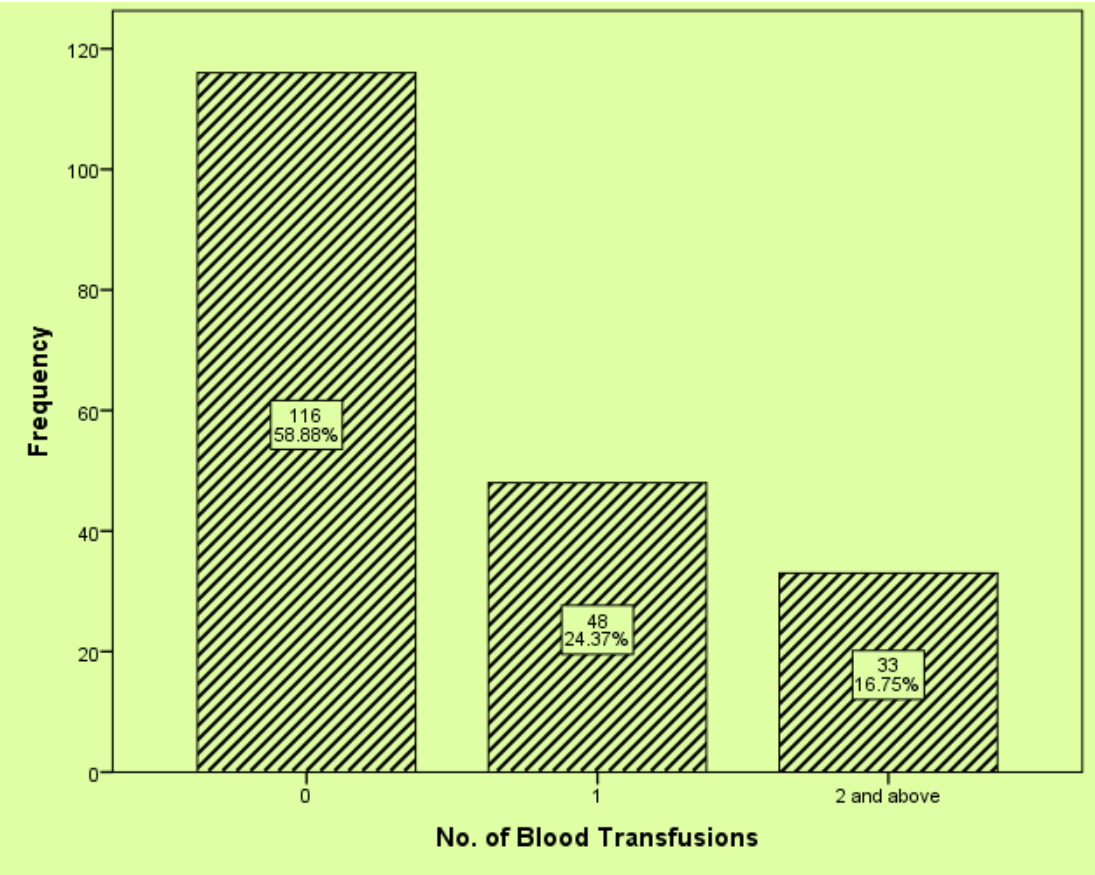

Figure 5: Frequency of blood transfusions among study participants.

4.5.3 Frequency of VOCs

One hundred and Fourty seven (75%) of the study participants had at least one VOC in the preceding one year prior to the study while 45 (22.8%) had three or more VOCs during the period under consideration. Close to 26% of the study participants reported no VOCs in the twelve months prior to enrolment into this study as shown in the table below.

Table 6: Frequency of VOCs among study participants

Frequency of VOCs in the past one year	Number of participants	percent of participants
None	51	25.9 %
One	58	29.4 %
Two	43	21.8
Three or more	45	22.8 %

4.5.4 Frequency and causes of admissions

About 125 (63.5%) of study participants reported at least one hospital admission in the previous twelve months prior to enrolment into this study while 72 (36.5%) of them reported no admissions during the same period under consideration- see figure 6.

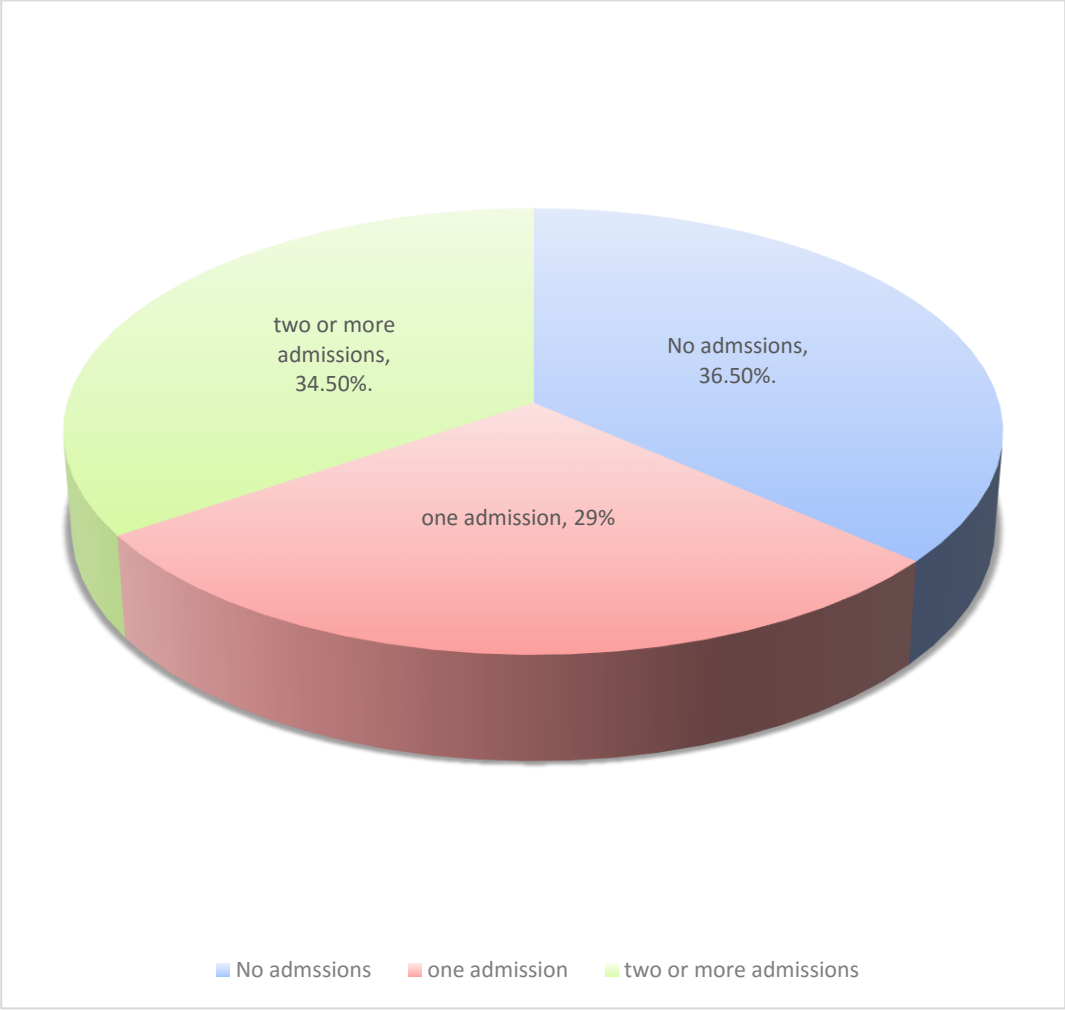

Figure 6: Frequency of admissions among study participants.

The most common cause of admissions was VOC which accounted for the 46 (37%) of admissions, with severe anaemia accounting for 34 (27%) of the admissions while a combination of severe anaemia, VOC and febrile illness accounted for the remaining causes of admissions as shown in figure 6.

Table 7: Causes and frequency of admission

Frequency	Number of participants	Percentage of participants
VOCs	**46**	**37 %**
Severe anaemia	**34**	**27 %**
Severe anaemia and VOC	**29**	**23 %**
VOCs & febrile	**11**	**9 %**
VOCs, Severe anaemia & febrile illness	**3**	**2 %**
Febrile illness	**2**	**2%**

4.5.5 Analysis of risk factors associated with chronic kidney disease in SCA

a) Bivariate analysis for association of independent variables with CKD:

Bivariate analysis was conducted to examine association of the study variables with CKD. At 5% significance level the following variables were associated with CKD; admission status (P = 0.06), Hb (P< 0.01), MCV (P = 0.01), Urea (P = 0.03) and PH (P = 0.02). Table 6 and 7 show the bivariate analysis results.

Table 8: Bivariate analysis for categorical variables using chi square

VARIABLE	CKD ABSENT (n = 126)		CKD PRESENT (n = 71)		P-VALUE
	N	%	N	%	
Age group:					
5 - 9 years	72	57.10%	37	52.10%	0.50
10 - 16 years	54	42.90%	34	47.90%	
Sex:					
Male	65	51.60%	34	47.90%	0.62
Female	61	48.40%	37	52.10%	
Blood Transfusion:					
No	71	56.30%	45	63.40%	0.34
Yes	55	43.70%	26	36.60%	
VOC:					
No	29	23.00%	22	31.00%	0.22
Yes	97	77.00%	49	69.00%	
Admission in the past one year:					
No	40	31.70%	32	45.10%	0.06
Yes	86	68.30%	39	54.90%	
BMI:					
Underweight (<18.5)	115	91.30%	67	94.40%	0.43
Normal weight (18.5 - 24.9)	11	8.70%	4	5.60%	

Table 9: Bivariate analysis for continuous variables using t test

Variable	CKD ABSENT (n = 126)	CKD PRESENT (n = 71)	P-value
	Mean (SD)	**Mean (SD)**	
Age at enrolment (years)	9.4 (3.33)	9.8 (3.42)	0.54
Age at diagnosis (months)	35.2 (31.85)	29.5 (26.58)	0.20
Weight	25.8 (9.84)	25.7 (8.04)	0.96
Height	125.2 (15.14)	126.3 (16.65)	0.65
BMI	15.7 (1.80)	15.7 (1.56)	0.97
Hb	7.6 (1.25)	7.1 (1.07)	<0.01
Platelets	432.1 (137.67)	400.4 (143.0)	0.13
WBC	13.9 (4.61)	14.3 (3.83)	0.59
MCV	79.3 (9.91)	83.0 (9.94)	0.01
ALT	24.6 (11.65)	24.1 (11.85)	0.78
AST	44.3 (26.46)	44.4 (21.22)	0.98
ALB	41.9 (5.57)	41.6 (4.85)	0.72

Severe anaemia and higher MCV were associated with CKD (P value < 0.01 and 0.01 respectively).

b) Logistic regression analysis

Logistic regression analysis was conducted to identify independent factors associated with CKD. Children with no admission record in the past one year had on average 48% reduced odds for CKD [Odds Ratio (OR) = 0.52, 95% Confidence Interval (CI) = 0.27 – 0.98, P-value = 0.04]. For every 1 unit increase in Hb, the odds for CKD on average reduced by 38% (OR = 0.62, CI = 0.46 – 0.84, P-value < 0.01). For every increase of 1 unit in MCV the odds for CKD increased on average by about 4% (OR = 1.04, CI = 1.01 – 1.08, P-value = 0.01), see table 8 below.

Table 10: Logistic regression analysis predicting CKD

Variable	Crude Odds ratio (95% CI)	Adjusted Odds ratio (95%CI)	P-value
Admission in the past one year:			
Yes	1	1	
No	0.57 (0.31 - 1.03)	0.52 (0.27 - 0.98)	0.04
Low Hb	0.64 (0.48 - 0.85)	0.62 (0.46 - 0.84)	< 0.01
High MCV	1.04 (1.01 - 1.07)	1.04 (1.01 - 1.08)	0.01

4.6 Other results

One hundred and eighty-two (92%) of the study participants were on folate and deltaprim, whereas only 15 (8%) were on a combination of folate, deltaprim and hydroxyurea. None of the study participants were on nephrotoxic drugs for a prolonged period.

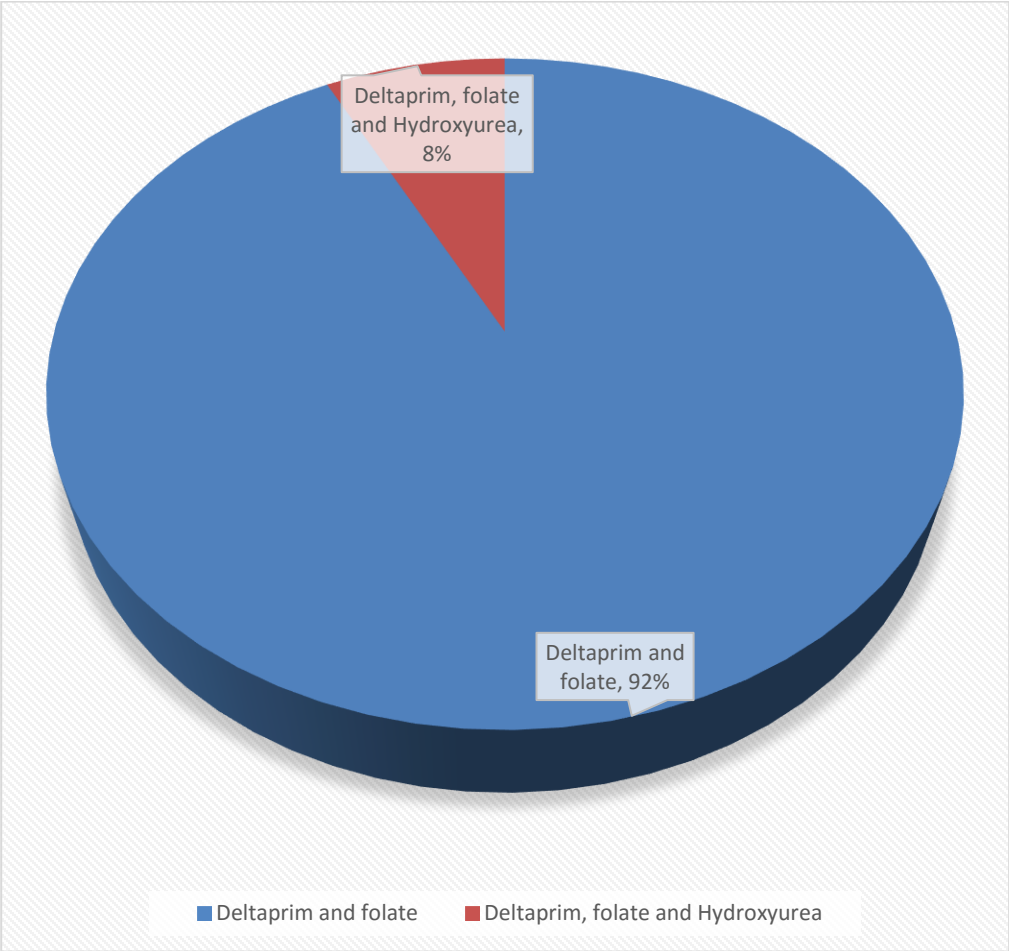

Figure 7: Drug history for study participants

All the study participants were noted to be pale on clinical examination. None of the participants had oedema and only one had lymphadenopathy (generalised). All the study participants had normal CVS examination, respiratory examination, and genito-urinary examination. There were 35 (17.8%) study participants with abnormal GIT findings. Summary of the abnormal GIT findings are shown in tables 11, 12 and 13. One study participant had abnormal musculo-skeletal system examination- this abnormality was scoliosis. In terms of CNS findings clinically, one study participant had abnormal central nervous system examination - left side hemiparesis secondary to an old stroke.

Table 11: Abnormal gastrointestinal tract findings.

Abnormal GIT findings	Frequency	Percentage
Mild splenomegaly (spleen< 4 cm below costal margin)	14	40
Moderate Splenomegaly (spleen 4-8 cm below costal margin)	10	28.6
Moderate Hepatosplenomegaly (liver & spleen 4-8 cm below costal margin)	5	14.3
Mild hepatosplenomegaly (liver and spleen below 4cm below costal margin)	3	8.6
Hepatomegaly	3	8.6
Total	35	100.0

Splenomegaly either on its own or in combination with hepatomegaly was present in 32 (16.2%) of the study participants.

Table 12: Frequency of splenomegaly according to age groups

Age group	Frequency	Percentage
5 – 10	22	68.8%
11 – 15	10	31.2%
Total	32	100.0%

The age group 5 to 10 years had the highest frequency of splenomegaly.

Table 13: Frequency of CKD in participants with splenomegaly

CKD Status	Frequency	Percent
No CKD	27	84.4%
CKD	5	15.6%
Total	32	100.0%

Approximately 16% of participants with splenomegaly had CKD. This was not statistically significant ($P > 0.05$)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of chronic kidney disease:

A prevalence of CKD of 36 percent was found among the SCA paediatric population at UTH. This high prevalence of CKD falls within the range of 5.1 to 68.4% found in various studies in paediatric and adult populations in low income countries and is much similar to what was found in the Ghanaian study which found a prevalence of CKD among sickle cell patients to be 31.6% in the paediatric age group (Ephraim et al, 2015). However, there is sharp contrast with what was found in countries with relatively better health facilities such as Brazil which found the prevalence of CKD among adult study participants with sickle cell to be 5.1% (Silva et al, 2012). In Zambia, we have a high disease burden of sickle cell and the commonest genotype is HbSS. The latter is the most severe form of sickle cell haemoglobinopathy. This coupled with the high disease burden, poor health services, late diagnosis and poor health seeking behaviour in our setting may explain the high prevalence of CKD among the study participants. Interventions such as hydroxyurea and proactive blood transfusions to prevent SCA associated complications are not adequately utilized in our health facilities. In resource rich countries like the USA, availability of screening programs that diagnose sickle cell early, good health seeking behaviour, dedicated units that offer interventions such as hydroxyurea and proactive blood transfusions and probably the sickle cell genotype prevalent is a less aggressive form hence their low prevalence of CKD (Alvarez et al, 2012; Yee et al 2011).

5.2 Prevalence of proteinuria:

Persistent Proteinuria in this study was found be 36% among the study participants. Dipstick urinalysis only identified 16.2 percent of the participants with persistent proteinuria while the urine ACR identified all the study participants with persistent proteinuria hence demonstrating its superiority over dipstick urinalysis. The results of this study concur with the findings of two earlier studies done at UTH which reported a prevalence of proteinuria in sickle cell patients of up to 41%. These studies predominantly enrolled participants outside the paediatric age group (Chansa et al, 2012;

Musonda et al 2010). The Zambian studies results are also comparable with various studies in several parts of the world which ranged from 15.9 to 41 percent in areas such as the USA, West and Eastern African countries that recruited adults as well (Yee et al, 2011; Aleem et al, 2008; Alleyne et al, 1975; Aygun et al, 2011; Bodas et al, 2013; Guash et al, 1997). It is known that the risk of proteinuria like any other complication in SCA patients is high with increase in age (Powars et al, 1991; Powars et al, 2005), thus some studies have reported increase in age as an associated risk factor to development of proteinuria. This study however shows that renal dysfunction appears earlier, a fact that has been documented in literature hence screening for renal abnormalities in our SCA population should begin early. This will allow patients with renal abnormalities to be identified early so that measures to slow down worsening of renal dysfunction such as use of hydroxyurea and proactive blood transfusions are instituted early.

5.3 Prevalence of Haematuria:

Persistent haematuria was present in 14.2% of the study participants and this prevalence is comparable with 13% reported in three studies in Saudi Arabia, Ghana and Tanzania done in the paediatric population age group respectively. However, the prevalence of haematuria in this study was significantly lower than that in two earlier studies done at UTH which demonstrated a prevalence of haematuria ranging from 32 to 92% among sickle cell patients (Chansa et al, 2012, Musonda et al 2010). It is expected and known that the prevalence of haematuria in adult SCA patients is higher than that found in children as frequency of complications is higher with increase in age (Powars et al, 1991; Powars et al 2005). This difference could be due to the fact the two earlier studies recruited both adults and children with a bias towards adults and their sample sizes were small hence reflecting findings mainly in adults as most study participants were above the paediatric age group. Ours is a resource limited setting in which SCA patients are diagnosed late as evidenced by the median age of diagnosis among the study participants was 22 months, this coupled with poor health seeking behavior and poor medical facilities the risk of SCA associated complications is high.

5.4 Prevalence of abnormal eGFR

No study participant was found to have a below normal eGFR, however 160 (81%) of the study participant had hyperfiltration. The hyperfiltration is due to the tubular secretion of creatinine and over estimation of the eGFR by available formulae e.g. the Schwartz and the CKD epidemiology equation. Hyperfiltration has been linked to kidney injury, thus is not a good sign. Studies in Brazil, USA and Saudi Arabia show hyperfiltration of up to 76% findings similar to what was found in this study (Aleem et al, 2008; Silva et al, 2012). The hyperfiltration which occurs in SCA patients actually may be the reason why none of the study participants was found to have a below normal eGFR.

5.5 Risk factors of CKD in SCA

The possible risk factors for CKD in SCA were evaluated but only three were identified to be associated with CKD in SCA in this study. Low haemglobin and elevated MCV were risk factors associated with CKD (adjusted odds ratio 0.62, CI 95%; 0.46-0.84 and 1.04, 95% CI; 1.01-1.08 respectively). High MCV in SCA is because of folate deficiency and this in turn causes megaloblastic anaemia. For every increase of 1 unit in MCV, the odds for CKD increased on average by about 4 (P-value = 0.01). The finding that increased MCV above the normal is a risk factor for CKD can be explained by the fact that folate deficiency causes anaemia which in turn predisposes to CKD as anaemia is a risk factor for CKD. Thus, poor adherence to daily folate prophylaxis can results in megaloblastic anaemia that predisposes to CKD.

In this study, it was noted there was no association between recurrent VOCs and CKD (P value > 0.05) which is contrary to what is expected and known about recurrent VOCs. Recurrent VOCs over long periods of time predispose to end organ damage including CKD (Michael et al 2007). The possible reason for lack of association between recurrent VOCs and CKD in this study could be due to possible poor recall by patients or caregivers of VOC events because caregiver could have changed or VOCs were mild and did not require hospital admission.

No significant statistical association between CKD and age at diagnosis of SCA was found. It would be expected that those who were diagnosed early with SCA and managed properly to reduce complications would have reduced risks for complications including CKD and vice versa would be true. Severe genotype, late diagnosis, no/delayed institution of hydroxyurea/proactive blood transfusions and poor compliance to medications in our settings can lead to early manifestations of SCA associated complications.

Statistically, no association was found between age at enrollment into this study and CKD and this can be explained by the fact that some markers of and / or CKD appear earlier than the age of five years which was the lower age limit of the participants. Abnormalities of renal function in SCA patients have been reported to occur as early as two years (Yee et al, 2011; Aygun et al, 2011; Oliver et al, 2008). Older age increases the degree of renal dysfunction as evidenced by reduced eGFR in a study done in Brazil which showed a significant decline in eGFR in some of their study participants with advanced age (Silva et al, 2012).

Blood transfusions did not confer any protection against CKD in this study as was shown in the results section ($P > 0.05$), a finding similar to what was found in study done in the USA in which chronic blood transfusion did not confer protection against CKD among the sickle cell study participants (Yee et al, 2011). It is possible that those who received blood may have already developed CKD prior to the transfusions or they did not receive adequate number of blood transfusions for the later to be protective against CKD. It would be expected that regular blood transfusions would reduce the concentration of RBCs with HbSS hence reducing the risk for SCA associated complications such as recurrent VOCs, severe anaemia and spleen sequestration of RBCs which in turn cause recurrent hypoxia leading to end organ damage causing problems such as CKD. There is need to research this area more so as to determine the timing and number of proactive blood transfusions needed to offer protection against CKD in SCA.

Poorly controlled SCA has regular complications such as recurrent VOCs and severe anaemia which would lead to chronic hypoxia which in turn lead to significant growth failure (evidenced by significantly low BMI) and end organ damage such as CKD among other complications. There was no association between low BMI and CKD. This can be explained partly by the possibility of reduced creatinine production due to reduced muscle bulk coupled with tubular secretion of creatinine and over estimation of the eGFR by the available formulae. Sex of the participants was not associated with CKD. This is expected as literature shows no sex bias as regards the development of CKD in SCA population. Literature has documented that deranged liver function is associated with deteriorating eGFR though this study found no association between derangement of LFTs and CKD (Guash et al, 1997). This finding is reflecting a weakness in the nature of this study design as an association cannot be identified easily in a cross section study. Long term follow up may be necessary to arrive at logical conclusion as regards the association between eGFR and deranged LFTs. Thrombocytosis is expected to increase the risk of CKD as it promotes occlusion of blood vessels but in this study, no association was found between thrombocytosis and CKD despite significant number of SCA patients having thrombocytosis. History of recurrent admissions is a risk factor for CKD and this is due to the fact that the causes of admissions are anaemia, VOCs and febrile illness. These are interrelated and all eventually lead to hypoxia which in turn lead to SCA associated complications including CKD.

CHAPTER SIX

6.1. Conclusion

The prevalence of CKD, proteinuria and haematuria among the SCA patients at UTH- Lusaka is high. Low haemoglobin, elevated MCV and history of recurrent admissions (due to VOCs, severe anaemia and febrile illness) are risk factors for developing CKD. Blood transfusions do not seem to offer protection against CKD development in SCA. Age at diagnosis of SCA, recurrent VOCs, abnormal LFTs, sex, BMI, thrombocytosis are not associated with CKD in SCA.

6.2. Study limitations

There were a number of limitations encountered in this study and these are as follows

i. The data that was obtained from this research may not reflect the true picture in sickle cell anemia patients seen at local clinics within Lusaka due to the fact that patients with the most severe complications are all seen at the UTH
ii. The Schwartz formula used to calculate the eGFR though validated for use in children is known to overestimate the eGFR by as much as 45 mL/min/1.73m^2 hence the values of eGFR may not be a true reflection of the actual GFR. Of the available formulae. Alternative formulae will still overestimate the eGFR and have not been validated in children as of now hence the limitations.
iii. Though structural abnormalities leading to CKD are accompanied by functional abnormalities, structural abnormalities were not identified as no abdominal ultrasounds were done due lack of certified manpower to carry out this test therefore this vital information was not obtained.

6.3. Recommendations

Following this study, the recommendations are as follows:

i. UTH- haematology unit should add routine screening for renal dysfunction in SCA patients to the protocols for managing SCA anaemia patients to ensure that patients with renal problems are identified early. Measures to include in the screening of CKD in SCA are dipstick urinalysis, Urine ACR, urea and creatinine, calculation of eGFR and abdominal ultrasound with emphasis on the renal system at least once a year in all SCA patients aged two years and above as it has been documented renal complications are already evident even at the age of two years. Patients should have BP measurement routinely at each review as hypertension is one of the manifestations of CKD.

ii. UTH- haematology unit should formulate protocols to initiate interventions which slow down the progression of CKD in SCA early. These measures include early introduction of hydroxyurea, ACE inhibitors and proactive blood transfusions for SCA patients who have recurrent Severe anaemia among others complications to reduce the risk of developing CKD and / or progression of CKD to ESRD. Although blood transfusions did not seem protective against CKD in SCA in this study, proactive blood transfusions need to be advocated for as a tool for preventing sickle cell related complications such like CKD as further research is being awaited on this subject.

iii. A follow up study to determine how many patients with SCA develop ESRD and its associated factors in our environment especially that none of the study participants had evident symptoms of CKD prior to screening.

iv. A similar study should be done in the adult SCA so that the disease burden in adults with SCA is determined as the prevalence of CKD with SCA is known to be high in adult SCA population.

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APPENDICES

APPENDIX A: INFORMATION SHEET

A STUDY ON THE PREVALENCE AND RISK FACTORS OF CHRONIC KIDNEY DISEASE IN SICKLE CELL ANAEMIA PATEINTS AGED 5 TO 16 YEARSIN A STEADY STATE SEEN AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

Why are we giving you this form?

We are giving you this form so as to give you information about the above named study and also to give you a chance to ask questions about this study and then you can decide if you would like your child to take part in this study. This study wants to find out how often proteins and blood are found in urine and to assess how well the kidneys are working in asymptomatic sickle cell anaemia patients (aged 5 years to 16years) seen in the at the University Teaching Hospital, Lusaka, Zambia.

2. Who is carrying out this study?

Dr Nchimunya Machila is doing the study as part the requirements of specialist training at the University of Zambia School of Medicine.

3. Background Information

You are being asked to take part in the above mentioned study, where we would like to find out how often proteins and blood are found in urine (as these can point to damaged kidneys) and how well kidneys are functioning in Sickle cell anaemia patients seen at UTH. By participating in this study we will be able to get the information that may help in order to make relevant guidelines and interventions regarding sickle cell anaemia associated kidney problems. We believe this is very vital information to all of us and you would help by participating in this study.

4. What happens in this research study?

Once you consent, you will be interviewed now and then you child will be examined, and some blood and urine taken for tests. A total of 4mLs of blood will be collected and 10 mL of urine. The information collected will be kept confidential.

5. Possible Problems

We believe that the processes being carried will not be harmful to you/your child while participating in this study although needle prick will cause pain to your child while collecting a blood sample. However, if we notice anything dangerous/harmful to your child as a result of participating in this study, we will let you know. The principal Investigator will take responsibility of all research related injuries and will arrange for appropriate medical treatment at the UTH as per UTH standard care for your child if your child sustains research related injuries while taking part in this study.

6. Benefits of this study

It is hoped that the study will help produce information on what proportion of sickle cell anaemia patients have chronic kidney disease and it will also assess how well kidneys are functioning in these patients. At individual level, you will know how well the kidneys of your child are functioning.

If the kidneys of your child are found to be functioning abnormally, your child will be referred for relevant care within UTH as per UTH standard procedure**.**

7. Confidentiality

Your name/the name of your child will never be made public by the investigators. The information generated will be treated as confidential-the same as all medical records at the health facilities. A code that makes it very difficult for anyone to identify your child will be used to identify the research information gathered during this study from your child. All information will be stored in a secure place. Information from this study will be used for research purposes and may be published; however, your name/the name of child will not be made public by the investigators**.** It is possible that, after the study is

over, we may want to look again at the laboratory results and review data collected during this study to help us answer another question(s). If this happens, still your name/the name of your child will not be made public by the investigators.

8. Research Related Injury

In the event that a problem results from a study-related procedure, the principal investigator Dr Nchimunya Machila in LUSAKA should be notified (0n +260 977 648587) or contact the ethics committee of the university of Zambia (see contact details section), and your child will be facilitated to seek and receive appropriate medical care at the University Teaching Hospital. It must be mentioned that the principal Investigator will take full responsibility of all research related injuries that may occur during this study.

9. Contact Details

Should you want further information about this study or your child's rights as a participant/your rights as parent/guardian to a participant please use the details provided below:

Dr Nchimunya Machila **Principle Investigator.** **University Teaching Hospital,** **Department of Paediatrics and Child Health.** **Cell Phone number: +260-977 648587** **Email: nmachila@gmail.com**	**The Secretary,** **ERES CONVERGE IRB,** **33 Joseph Mwilwa Road,** **Lusaka, Zambia.** **Cell phone: +260955 155633.**

APPENDIX B: CONSENT FORM

A STUDY ONTHE PREVALENCE ANDRISK FACTORSOF CHRONIC KIDNEY DISEASE IN SICKLE CELL ANAEMIA PATEINTS SEEN AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.)

I_____ (*parent/guardian's name) has been informed about the study. I volunteer to have my child participate in the above named study. A copy of this form signed by me and the *principal investigator /research assistants has been given to me.

Signature/Thumb: _____

Date : _____

Interviewer:

I have explained this study to the participant's *parent/guardian and participant. I am available to answer any questions now or in the future regarding the study and the participant's rights.

Principal investigator/research assistant: _____

Signature: _____

Date: _____

* Delete appropriately

APPENDIX C: ASSENT INFORMATION SHEET

A STUDY ON THE PREVALENCEAND RISK FACTORSOF CHRONIC KIDNEY DISEASE IN SICKLE CELL ANAEMIA PATEINTS SEEN AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.)

1. Why are we giving you this form?

Kidney disease(s) are becoming common in children of your age with your condition (sickle cell anaemia) and the extent and risk factors for this problem needs to be evaluated. We are giving you this form because we ASKING you to take part in the above mentioned study. This form will give you the information about the above named study. After reading this form you will be given a chance to ask questions about the said study after which you will decide whether to take part or not to take part in this study that is trying to find out the magnitude and risk factors of chronic kidney disease in sickle cell anaemia patients and how well the kidneys are functioning in this group of patients seen in the Department of Paediatrics and Child Health at the University Teaching Hospital, Lusaka, Zambia.

2. Who is carrying out this study?

Dr Nchimunya Machila who is currently in training to become a specialised doctor for children is the principal investigator of this study.

3. Background Information: In this study we are trying to see how often proteins and blood are found in urine (proteins and blood in urine can be indicators of a kidney disease) and to evaluate how well the kidneys are working in patients with sickle cell anaemia (age 5 to 16 years). Kidney disease(s) occur in children like you at the University Teaching Hospital.

Once you accept to take part in this study, the doctor will talk to you and your guardian then he will examine you. The doctor will then collect a few mL of blood and urine for purposes of this study. You will experience some pain during blood collection as a needle will be used in the process of collecting blood however this should not last very long.

The importance of you taking part in the study is that you will assist the doctor to try and come up with information that will be useful in helping us to detect kidney disease early in children with sickle cell disease so at to initiate appropriate treatment promptly. This study will also help you to know how well your kidneys are functioning with the condition you have.

APPENDIX D: ASSENT FORM

A STUDY ON PREVALENCE AND RISK FACTORS OF CHRONIC KIDNEY DISEASE IN PATIENTS WITH SICKLE CELL ANAEMIA SEEN AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

Participant:

I_____ **(**participant's name) has been informed about the above named study. I volunteer to participate in the study. A copy of this form signed by me and the principal investigator/research assistant has been given to me.

Signature/Thumb : _____

Date : _____

***Principal investigator/research assistant:**

I have explained this study to the above named study participant and I am available to answer any questions now or in the future regarding this study and the participant's rights.

Principal Investigator/research assistant: _____

Signature: _____

Date : _____

APPENDIX E: DATA COLLECTION SHEET.

A STUDY ON THE PREVALENCE AND RISK FACTORSOF CHRONIC KIDNEY DISEASE IN SICKLE CELL ANAEMIA PATEINTS AGED 5 TO 16 YEARS IN A STEADY STATE SEEN AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

Participant's study number:

Initials of participant :

I) Demographics:

a) Age: .....

b) Sex: 1) Male 2) Female

c) Race: 1) Asian 2) Arabs 3) blacks 4) European

II) Presenting Complaints:

III: Review of systems:

a) Cardio-Respiratory system:

1) Normal 2) if abnormal, specify …….....

b) Gastrointestinal system:

1) Normal 2) if abnormal, specify …….....

c) Genital-urinary system:

1) Normal 2) if abnormal, specify …….....…….

d) Neurology system:

1) Normal 2) if abnormal, specify …….....…….

e) Other systems:

1) Normal 2) if any abnormality specifies system affected and abnormality found:

IV) Past medical history:

1) Age at diagnosis of SCA: ----------

2) Number of blood transfusions in a period of one year.....

3) Previous admissions and co morbidities:

a) Any recent admissions (In the last 4 weeks): 1) No. 2) Yes,

If yes

State Diagnosis: ----------

b) Frequency of VOCs per year: ----------

c) 1). Renal disease 2) HTN 3) DM 4)TB 5) HIV 6) others …….

*** circle appropriately**

V) Drug history:

VI) Physical examination:

a) Vitals:

i. BP:
ii. Temperature:
iii. Respiratory rate
iv. Pulse:

b) Anthropometry

i. Weight:
ii. Height:
iii. Body mass index (BMI):
iv. Weight for height standard deviation:

c) General examination

i. Pallor : a) mild b) moderate b) severe

ii. Oedema: a) yes b) No

iii. Lymphadenopathy : a) No b) yes, if yes specify region(s) affected:

d) Systemic examination

System	**Normal**	**If abnormal, Specify findings**
Respiratory system		
Cardiovascular system		
Gastrointestinal system		
Musculo-skeletal system		
Central nervous system		

VIII) Laboratory results:

Biochemistry, FBC and microalbuminuria results

Test	M/albuminuria	eGFR	FBC	Haematuria	Urea, Cr and LFTs
Results					

Urinalysis by dipstick:

Principal investigator/ Research assistant: …….……

Signature: .....

Date: …….....

*Delete appropriately.