PREVALENCE AND CLINICAL FACTORS ASSOCIATED WITH MICROALBUMINURIA IN CHILDREN AGED 2-18YEARS WITH SICKLE CELL ANAEMIA AT KENYATTA NATIONAL HOSPITAL

A dissertation submitted in part fulfilment for the degree of Master of Medicine (Paediatrics and Child Health), University of Nairobi.

Dr Martin Mbiata Muthiga

M B Ch B (U.O.N)
DECLARATION:

I declare this dissertation is my original work and has not been presented for a degree in another university.

Signed: ........................................ Date: ......................................
SUPERVISORS’ APPROVAL:

This thesis has been submitted for examination with our approval as university supervisors.

Professor Musoke
Associate professor; Department of Paediatrics and Child Health
University of Nairobi
Signed: ............................................ Date: ............................................

Dr. Dalton Wamalwa
Senior Lecturer; Department of Paediatrics and Child Health
University of Nairobi
Signed: ........................................ Date: ........................................

Dr. Bashir Admani
Senior Lecturer; Department of Paediatrics and Child Health
University of Nairobi
Signed: ........................................ Date: ........................................
ACKNOWLEDGEMENTS:

My sincerest gratitude to;

- my supervisors for their patience and guidance throughout the process of development and fruition of this work

- the children who participated in this study for their cooperation and bravery, not forgetting their guardians for their consent

- the staff at the Kenyatta National Hospital Haematology clinic as well as the paediatric wards for their help in identifying study participants

- the staff at the University of Nairobi paediatric laboratory for their invaluable contribution

- my family, for their continued support throughout this course
### TABLE OF CONTENTS:

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declaration</td>
<td>2</td>
</tr>
<tr>
<td>Supervisors’ approval</td>
<td>3</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>4</td>
</tr>
<tr>
<td>Table of contents</td>
<td>5</td>
</tr>
<tr>
<td>List of figures</td>
<td>6</td>
</tr>
<tr>
<td>List of tables</td>
<td>7</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>8</td>
</tr>
<tr>
<td>Abstract</td>
<td>9</td>
</tr>
<tr>
<td>1. Background and literature review</td>
<td>11</td>
</tr>
<tr>
<td>2. Study justification</td>
<td>20</td>
</tr>
<tr>
<td>3. Objectives</td>
<td>21</td>
</tr>
<tr>
<td>4. Study methodology</td>
<td>22</td>
</tr>
<tr>
<td>5. Results</td>
<td>33</td>
</tr>
<tr>
<td>6. Discussion</td>
<td>39</td>
</tr>
<tr>
<td>7. Limitations</td>
<td>41</td>
</tr>
<tr>
<td>8. Conclusion and Recommendations</td>
<td>42</td>
</tr>
<tr>
<td>9. References</td>
<td>43</td>
</tr>
<tr>
<td>10. Appendices</td>
<td></td>
</tr>
<tr>
<td>Appendix 1: Questionnaire</td>
<td>46</td>
</tr>
<tr>
<td>Appendix 2: Information and Consent form</td>
<td>48</td>
</tr>
<tr>
<td>Appendix 3: Ethics approval</td>
<td>53</td>
</tr>
<tr>
<td>Appendix 4: BMI charts</td>
<td>55</td>
</tr>
<tr>
<td>Appendix 5: Normal GFR</td>
<td>59</td>
</tr>
</tbody>
</table>
LIST OF FIGURES:

Figure 1: Spectrum and Chronology of Sickle Nephropathy.......................... 14
Figure 2: Study flow chart ........................................................................... 26
Figure 3: Study Profile ................................................................................ 33
Figure 4: Prevalence of Microalbuminuria (MA) ........................................... 34
Figure 5: Microalbuminuria prevalence by age............................................. 34
Figure 6: Association between eGFR and Microalbuminuria ................. 37
LIST OF TABLES

Table 1: Albuminuria cut-off values .............................................................. 16
Table 2: Prevalence of Microalbuminuria in Sickle Cell Disease .................. 17
Table 3: Interventional Studies on Microalbuminuria .................................. 20
Table 4: Values of K for paediatric patients .................................................. 30
Table 5: Population Characteristics .............................................................. 36
Table 6: Factors associated with Microalbuminuria .................................... 38
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>Albumin/Creatinine ratio</td>
</tr>
<tr>
<td>AER</td>
<td>Albumin Excretion Rate</td>
</tr>
<tr>
<td>CAPE</td>
<td>Cellulose Acetate Paper Electrophoresis</td>
</tr>
<tr>
<td>eGFR</td>
<td>estimated Glomerular Filtration Rate</td>
</tr>
<tr>
<td>ESRD</td>
<td>End Stage Renal Disease</td>
</tr>
<tr>
<td>FSGS</td>
<td>Focal Segmental Glomerulosclerosis</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>Hb F</td>
<td>Fetal haemoglobin</td>
</tr>
<tr>
<td>Hb S</td>
<td>Sickle Haemoglobin</td>
</tr>
<tr>
<td>HbSC</td>
<td>Sickle cell Haemoglobin C disease</td>
</tr>
<tr>
<td>HbSS</td>
<td>Homozygous sickle cell anaemia</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
<tr>
<td>MA</td>
<td>Microalbuminuria</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non steroidal Anti Inflammatory Drug</td>
</tr>
<tr>
<td>SCA</td>
<td>Sickle Cell Anaemia</td>
</tr>
<tr>
<td>SCD</td>
<td>Sickle Cell Disease</td>
</tr>
<tr>
<td>SCN</td>
<td>Sickle Cell Nephropathy</td>
</tr>
<tr>
<td>SCT</td>
<td>Sickle Cell Trait</td>
</tr>
<tr>
<td>UTI</td>
<td>Urinary Tract Infection</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
ABSTRACT

Background: Sub-Saharan Africa has a high burden of sickle cell disease. Better care has led to increased life expectancy, switching focus from early mortality to morbidity caused by organ damage. One of the organs involved is the kidneys, with damage heralded by subclinical changes such as microalbuminuria (MA) that can be diagnosed early allowing for timely intervention. This can reduce progression to and incidence of end stage renal disease (ESRD). The prevalence of microalbuminuria among children with SCD in our setting, and need for routine screening is unknown.

Objectives: To determine prevalence and clinical factors associated with microalbuminuria in children aged 2-18 years with sickle cell anaemia at Kenyatta National Hospital (KNH)

Methods: This was a cross sectional descriptive study carried out at KNH. We recruited 110 eligible subjects whose records were reviewed to confirm sickle cell genotype then interviewed to establish number of past admissions in the preceding year, hydroxyurea use and history of severe vaso-occlusive phenomena. Physical examination was carried out and anthropometric measures recorded. A spot clean catch urine sample and venous blood samples were then collected and analysed for quantification of protein levels in urine, serum creatinine and haemoglobin. Estimated glomerular filtration rate (eGFR) was calculated for each subject and classified by K/DOQI guidelines.

Results: The study population (n=110) comprised 62(56%) male and 58(44%) female subjects with a mean age of 7.53(+3.7) years.

The mean haemoglobin concentration for the study population was 7.9(1.2) g/dl.
Over half the study group were on hydroxyurea at time of recruitment (61%) and only nine subjects had history of severe vaso-occlusive phenomena. Fifty nine subjects (54%) had a raised eGFR and only 2% had a low eGFR.

The prevalence of microalbuminuria (MA) was 39.1% with our youngest child with MA being 2 years old.

There was significant association with hyperfiltration ($p=0.006$, OR 3.06 95% CI 1.36 to 6.92) but no significant association with age ($p=0.805$), gender ($p=0.926$), BMI ($p=0.538$), number of hospitalisations ($p=0.679$) or haemoglobin concentration ($p=0.58$).

**Conclusion:** The prevalence of MA in our population is high and warrants regular screening of all SCD patients. The onset was also noted to be at an early age thus screening should start as soon as the diagnosis is made. eGFR should also be monitored as there was significant association between MA and hyperfiltration.
1. BACKGROUND AND LITERATURE REVIEW

Introduction

Sickle cell disease is a chronic haemolytic disease characterised by the presence of the mutated β-globin gene, βS-globin. Sickle cell disease (SCD) is a generic term for a group of disorders that includes homozygous sickle cell anemia (HbSS), sickle cell hemoglobin C disease (HbSC), sickle cell thalassemia disease (S/thal) and other compound heterozygous conditions. Homozygous HbSS and compound heterozygosity for β0-thalassemia have the most severe phenotype\(^3\)\(^,\)\(^4\). It is estimated that >70% of SCD is in Africa distributed mainly in western, central and eastern Africa with trait prevalence of between 5 and 40%\(^4\). In our local setting we mainly encounter genotypes Hb SS and Hb AS (sickle cell trait), the later having a less severe phenotype.

Pathology of sickle cell disease (SCD) is attributed to the propensity of the mutant haemoglobin (Hb) to polymerise when deoxygenated. This polymerisation depends on intracellular sickle haemoglobin (HbS) and fetal haemoglobin (HbF) concentrations, degree of cell deoxygenation, acidosis, change in temperature, infection, fever and exercise.\(^3\)\(^,\)\(^4\)

This process of haemoglobin polymerisation causing red blood cell sickling takes place in various vascular beds resulting in obstruction of blood flow with resultant ischaemia and necrosis. Repeated episodes cause damage to several organ systems such as the spleen, musculoskeletal system, the lungs and kidneys.

Pathogenesis of Sickle Cell Nephropathy

Chronic sickling underlies several mechanisms of kidney injury.\(^5\)\(^,\)\(^6\)\(^,\)\(^7\) The arterial side of the renal microvasculature has a low oxygen tension and hyper tonicity plus low pH of the renal medulla promote the formation of haemoglobin polymers in the red cells with deformation.
of the cells resulting in an increase in the blood viscosity, functional venous engorgement, and interstitial edema. These predispose the renal microcirculation to ischemia and infarction.\textsuperscript{4, 5, 8}

This compromise in circulation causes gradual obliteration of the medullary vasculature causing segmental scarring and interstitial fibrosis. The development of collateral vessels and their abnormal orientation in the medulla interferes with the counter current exchange mechanism, culminating through the years in irreversible loss of medullarytonicity.\textsuperscript{5}

This early renal damage is manifested clinically as loss in the ability to concentrate urine, termed hyposthenuria, and results in polyuria in affected patients. Initially reversible by repeated blood transfusion this state becomes permanent after the age of 15yrs due to medullary fibrosis causing damage to the collecting ducts. This process may contribute to further episodes of sickling by causing dehydration thus fuelling the disease process.\textsuperscript{5}

Tubular dysfunction is also seen at the proximal tubules with relative ‘hyperfunction’ characterized by increased creatinine secretion and sodium and phosphate reabsorption, possibly to compensate for poor distal tubular function that is more affected by medullary fibrosis. The clinical relevance of this is an overestimation of glomerular filtration rate (GFR) using standard formulae thus overestimation of renal function. It has been shown in experimental work that the functional reserve in clearing creatinine is reduced, with a blunted increase in clearing an intravenous creatinine load in these patients, reflecting a subclinical nephron mass reduction.\textsuperscript{5, 7, 9}

The glomerular changes begin as early as the first decade of life in otherwise asymptomatic SCD patients.\textsuperscript{5, 10, 11, 12, 13} The earliest glomerular changes are seen as high renal blood flow causing hyperfiltration and glomerular hypertrophy. Initially thought to be secondary to immune complex deposition, evidence was found lacking and the pathogenesis is now
known to be a combination of processes.\textsuperscript{5, 14, 15} Increased prostaglandin secretion from the ischaemic medulla causes afferent vasodilatation thus increased intraglomerular pressure and glomerular hypertrophy. Efferent obstruction also contributes to this increased pressure leading to hyperfiltration with gradual loss of glomerular permselectivity to both size and charge. Larger molecules, such as albumin, abnormally permeate the restrictive pores of the glomerular wall. This albumin excretion can be detected and has been shown to be an early sensitive clinical marker of glomerulopathy.\textsuperscript{5, 10, 11, 14, 15}

Glomerular lesions seen in sickle nephropathy are typically focal segmental glomerulosclerosis (FSGS), beginning in the perihilar region.\textsuperscript{5, 15} Development of these lesions is caused directly by the proteinuria as well as endothelial damage induced by the occluding sickled cells, growth hormones and cytokines whose production is induced by proteins in the capsular space.\textsuperscript{5, 14, 15}

With loss of nephrons, pressure is transmitted to the remaining units, thus secondary damage due to compensatory hyperfiltration even in initially unaffected units. This process is progressive leading to chronic renal insufficiency and end stage renal disease (ESRD).\textsuperscript{14, 15}

Hematuria is another feature of renal involvement. It may be dramatic though transient, a presentation more common with sickle cell trait (SCT). Aetiology includes papillary necrosis, (left) renal vein thrombosis, hypertension and medullary cell carcinoma.\textsuperscript{3}

The chronology of these events is represented in figure.1 below.
Microalbuminuria

Normal individuals usually excrete very small amounts of protein in the urine. Persistently increased protein excretion is usually a marker of kidney damage. The excretion of specific types of protein, such as albumin, depends on the type of kidney disease that is present. Increased excretion of albumin is a sensitive marker for glomerular disease.

Microalbuminuria was described more than three decades ago as a predictor of nephropathy and is recognized as a sign of abnormal vascular function and increased vascular permeability. Measurement of albumin in urine thus has an important role in secondary prevention, to decide treatment and monitor response to treatment.

Various methods for urine collection are used in clinical practice to measure albumin in urine. The amount of albumin excreted in urine during a 24-hour period has been considered the gold standard. However 24-hour urine collections may be associated with
significant collection errors, largely due to improper timing and missed samples, leading to over-collections and under-collections.

Timed overnight collections or shorter timed daytime collections may reduce the inconvenience of a 24-hour collection, but are still associated with collection errors. In addition, errors due to incomplete bladder emptying are relatively more important in shorter collection intervals.

More practical and easier alternatives are collection of a first morning void or a spot (random) urine sample. It has been suggested that a first morning void is to be preferred over a spot urine sample, because the former is less influenced by factors such as hydration status and physical activity, reducing the variability that is caused by these factors. From a practical point of view, however, spot urine samples are preferred because they can be collected during consultation at the doctor’s office and therefore pose the least inconvenience for patients. According to the National Kidney Foundation (NKF), clinical practice guidelines, under most circumstances, untimed spot urine samples should be used to detect and monitor proteinuria in children and adults. It is usually not necessary to obtain a timed urine collection (overnight or 24-hour) for these evaluations in either children or adults. First morning specimens are preferred, but random specimens are acceptable if first morning specimens are not available.

In terms of translation of results the following cut offs are used (table 1):
Table 1: Albuminuria cut off values

<table>
<thead>
<tr>
<th>Terms</th>
<th>24-hour urine sample UAE (mg/24hours)</th>
<th>Timed overnight urine sample AER (μg/min)</th>
<th>Spot (random) urine sample UAC (mg/l)</th>
<th>ACR (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoalbuminuria</td>
<td>&lt;30</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30 to 300</td>
<td>20 to 200</td>
<td>20 to 200</td>
<td>30 to 300</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>&gt;300</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

Key: UAE - a urinary albumin excretion, AER - albumin excretion rate, UAC – urinary albumin concentration, ACR – albumin: creatinine ratio, *values gender independent

It is also important to note vigorous exercise, infection, fever, congestive heart failure, marked hyperglycemia, marked hypertension, pyuria, and hematuria may elevate urinary albumin excretion over baseline values.

**Prevalence of Microalbuminuria**

Microalbuminuria screening has been proposed in detecting pre-clinical renal involvement in SCD based on the pathologic process described earlier.\(^{10,11,12}\)

Several studies in various regions have described the prevalence of microalbuminuria in this population of patients and this has been found to range from 5% to 26% with association found to age, haemoglobin (Hb) level, body mass index (BMI) and GFR.\(^{5,6,9,10-12,15-20}\)

As shown in table 2, clinical variables associated with presence of microalbuminuria are varied. Generally most studies have shown significant association between microalbuminuria and age as well as baseline Hb.

In Jamaica King et al\(^{10}\) in a cross sectional study on SCD patients diagnosed and followed up from birth found a prevalence of MA of 18.4% with their youngest patient testing positive at 2.8yrs old. Previous studies\(^ {6,9,21}\) had reported no MA in patients under 5 years. Most
reports show hyperfiltration in this patient population with significance of this association is only shown by King et al\textsuperscript{10} and Mc Pherson et al \textsuperscript{7}. Contrary to reports associating MA to early kidney disease a cross – sectional study by Aokoundou-N’Guessan et al\textsuperscript{17} in Ivory Coast suggests microalbuminuria as a marker of advanced renal disease based on association with lower GFR. This study however included 75 patients of all ages, mean age 16.58±9.08yrs as well as various SCD phenotypes including Hb SS and Hb SC as well as sickle cell trait (Hb AS). Prevalence of microalbuminuria was 17.3% and a significant association with lower GFR and presence of anaemia. This association suggested significant renal impairment. No relation to age, disease duration or BMI was found. Mc Pherson et al\textsuperscript{7} however showed more chronic kidney disease in Hb SC than SS populations which could account for this result.

**Table 2: Prevalence of Microalbuminuria in Sickle Cell Disease**

<table>
<thead>
<tr>
<th>Author</th>
<th>Country/Study site</th>
<th>Subjects</th>
<th>Prevalence and correlates of microalbuminuria</th>
</tr>
</thead>
</table>
| King et al\textsuperscript{10} | Jamaica/Sickle Cell Unit, University of West Indies | N=244                   | -prevalence 18.4%  
- youngest 2.8yrs  
- significant association with dactilytis, glomerular hyperfiltration  
- increasing age, lower Hb and HbF  
- predictors of positive MA  
- no relation with gender, LDH\textsuperscript{ii}, reticulocyte count, ACS\textsuperscript{iii}, CVA\textsuperscript{iv}, WBC\textsuperscript{v} counts |
| Aokoundou-N’Guessan et al\textsuperscript{17} | Ivory Coast/Yopougon Teaching Hospital | N=75                    | -prevalence 17.3%  
- significant association with lower GFR, anaemia  
- no relation to age, disease duration, BMI |
<p>|                         |                             | SCD                     |                                                                                                             |
|                         |                             | all ages                |                                                                                                             |
|                         |                             | (ave16.58±9yrs)         |                                                                                                             |</p>
<table>
<thead>
<tr>
<th>Author</th>
<th>Country/Study site</th>
<th>Subjects</th>
<th>Prevalence and correlates of microalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Datta et al</td>
<td>India/Mahatma Ghandi Institute of Medical Sciences</td>
<td>N=64</td>
<td>-prevalence 18.8% (Hb SS prevalence 19.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCD</td>
<td>-higher prevalence in &gt;9yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;14yrs</td>
<td>-no significant relation to Hb level, number of hospitalizations, crisis state</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-no difference in mean creatinine</td>
</tr>
<tr>
<td>Abhulimen-Iyoha</td>
<td>Nigeria/University of Benin Teaching Hospital</td>
<td>N=69</td>
<td>-prevalence 20.3%</td>
</tr>
<tr>
<td>Imuetinyan et al</td>
<td></td>
<td>Hb SS</td>
<td>-significant association with increase in age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-16yrs</td>
<td>-normal blood pressure in all, no association to Hb</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-2 patients with MA &lt;5yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-MA at average age of 10.9±4.9yrs</td>
</tr>
<tr>
<td>McPherson et al</td>
<td>USA/Georgia Comprehensive Sickle Cell Clinic, Grady</td>
<td>N=410</td>
<td>-prevalence 20.7%(23% in Hb SS)</td>
</tr>
<tr>
<td></td>
<td>Memorial Hospital</td>
<td>SCD</td>
<td>-significant association with age, lower Hb and high GFR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-21yrs</td>
<td>-26.5% had chronic kidney disease by KDIGO(^\text{vii}) study definition</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14.8% stage 1, 11.6% stage 2</td>
</tr>
</tbody>
</table>

\(^{\text{v}}\)Hb SS homozygous sickle cell anaemia, \(^{\text{vi}}\)LDH lactate dehydrogenase, \(^{\text{vii}}\)ACS acute chest syndrome, \(^{\text{viii}}\)CVA cerebral vascular accident, \(^{\text{vii}}\)WBC white blood cell, \(^{\text{vii}}\)Kidney Disease: Improving Global Outcomes study

**Management Strategies for Microalbuminuria**

Interventional studies have shown significant benefit of hydroxyurea (HU) and angiotensin converting enzyme inhibitors (ACEI) use as shown in table 3.

Falk et al\(^{15}\) aimed to determine prevalence, pathologic features of sickle cell nephropathy (SCN) and response to ACEI. The study population included adults and children seen over a six year period at 2 centres. General prevalence of microalbuminuria was 26% with biopsies
of 10 adult participants showing FSGS and glomerular enlargement as the typical pathologic lesions. None of the children had increased creatinine levels and proteinuria was more frequent in Hb SS population. ACE inhibitor enalapril was then administered for 2 weeks in these 10 subjects with results showing an average 57% decline in protein excretion that was maintained at 25% of baseline 2 weeks after stopping the medication. In one patient the decline in proteinuria continued even after stopping treatment.

McKie et al\textsuperscript{21} in Georgia also studied the prevalence, prevention and treatment of microalbuminuria among HbSS patients aged 3-20yrs old. This study found a prevalence of 19.4\%, with a positive test associated with higher mean age and lower Hb. The average age of patients testing positive for microalbuminuria in the study was 14.95yrs and average age of onset of microalbuminuria to be 11.8 $\pm$ 3.93yrs on follow up of those who had initially tested negative. Further hydroxyurea was started in those with frequent vaso-occlusive episodes, acute chest syndrome and those positive for microalbuminuria/proteinuria. ACEI were instituted for persistent proteinuria and those who developed proteinuria while on hydroxyurea that had been started based on vaso-occlusive crises or acute chest syndrome. Results showed decline in microalbuminuria in 44\% of those started on hydroxyurea and only 1 out of the 19 patients on hydroxyurea for one of the other indications developed microalbuminuria. Eighty eight percent of those on both hydroxyurea and ACEI had reduction in microalbuminuria though there was concern about development of hyperkalemia.

Other studies have since shown benefit of this combination treatment.\textsuperscript{33, 34}

Locally unpublished work on Renal Manifestations in Children with Sickle Cell Anaemia done in 1981 by Owino A.\textsuperscript{38} showed urine concentration impairment, a tendency to a lower GFR
than controls and reduced urine flow rate. He also found 3 out of his 21 patients with proteinuria with none being of nephrotic range, 2 of the 21 had macroscopic hematuria and 3 had significant pyuria. Of note is that all his subjects were inpatients admitted in various crises.

Table 3: Interventional Studies on Microalbuminuria

<table>
<thead>
<tr>
<th>Author Country</th>
<th>Subjects</th>
<th>Results – response to management strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falk et al\textsuperscript{15} USA/Universities of Duke and North Carolina</td>
<td>N=381 310 adults 71 children SCD</td>
<td>-23-79% reduction in proteinuria with 2 weeks of enalapril unrelated to reduction in BP - proteinuria at 75% of baseline on stopping ACE inhibitor</td>
</tr>
<tr>
<td>Kathlene McKie et al\textsuperscript{21} USA/Medical College of Georgia</td>
<td>N=191 Hb SS 3-20yrs</td>
<td>-normalisation of MA 44% with hydroxyurea 56% with ACE Inhibitor 88% with combination treatment - risk of developing hyperkalemia necessitating cessation of ACE Inhibitor</td>
</tr>
</tbody>
</table>

2. STUDY JUSTIFICATION

Health care measures in management of SCD have led to improved survival from acute causes such as sequestration and infections. This means that the population of SCD patients surviving to adulthood is increasing thus need to focus on causes of morbidity that can be prevented. It has been shown that up to 20% of adults with SCD end up with end stage renal disease (ESRD) requiring dialysis or transplant the response to which is guarded.
ESRD develops secondary to the progressive glomerular changes described earlier. These glomerular changes are largely asymptomatic but are detectable due to increased loss of albumin in the urine. This measure can be used for screening, thus allow for early intervention by use of hydroxyurea and ACE inhibitors that retard progression of renal injury.

The prevalence of microalbuminuria among children with SCD is not known in local settings thus this study aimed to provide baseline data to inform on the need to institute regular screening and investigate clinical factors that may be associated with microalbuminuria.

3. STUDY OBJECTIVES

**Primary Objective:**

To determine the prevalence of microalbuminuria in children with sickle cell anaemia between ages two and eighteen years at KNH.

**Secondary Objective:**

To determine clinical factors associated with microalbuminuria (MA) in children with sickle cell anaemia aged between two and eighteen years. The clinical factors to be considered included age, gender, body mass index, estimated glomerular filtration rate, number of admissions in the preceding year, use of hydroxyurea and haemoglobin level.
4. STUDY METHODOLOGY

Study design:
Cross sectional descriptive study

Study site:
The study was conducted in Kenyatta National Hospital (KNH) which is a national referral hospital located in the capital city, Nairobi. It serves as one of the two national referral facilities thus caters for a diverse populous seeking specialist review and follow-up in various fields of medicine. The facility also acts as a primary facility for the population within and around the capital city.

KNH also serves as a teaching hospital for the College of Health Sciences of the University of Nairobi and the Kenya Medical Training College. It has a 1500 bed capacity though hosts between 2500-3000 in-patients. Outpatient services cater to over 500,000 patients annually.

Patients with SCD are followed up in the haemato-oncology clinic with children under 12yrs seen as paediatric patients and those older than 12years seen with the adults. This clinic runs once a week and in 2011, 8959 patients were attended to with an average of 235 patients per clinic of who approximately 15 to 25 have SCD. Patients are referred from outpatient and lower level hospitals and former in-patients at the facility.

Care of these patients is not fully standardized. All patients are on folic acid supplementation as well as penicillin prophylaxis. Patients residing or travelling to malaria endemic areas are on proguanil and all patients are encouraged to get recommended vaccinations as well as emphasis on hydration and early hospital reviews in case of ailments. The unit however does not facilitate vaccinations. Analgesics prescribed for home use are
largely acetaminophen or/and non-steroidal anti-inflammatory agents with dosing for age and frequency for use advised to be titrated with symptoms. No transfusion program is in place. Patients are transfused as in-patients if anaemia is severe or symptomatic and when a patient is admitted in an acute crisis. Hydroxyurea use is instituted generally in patients over the age of 5 years with recurrent pain symptoms, recurrent admissions or a cerebral vascular event.

Monitoring of these patients is by history as reported on review and regular blood counts during clinic reviews. Those with emergent new symptoms also have specific work-up done such as radiographs or echocardiograms as indicated. Sub-specialty reviews are thus sought as required.

**Study Population:**
Children aged between 2-18yrs attending haematology clinics at Kenyatta National Hospital, as well as stable patients in the general paediatric wards
Sample size estimation

Using Fischer’s formula for sample size estimation in prevalence studies:

\[ n = \frac{Z^2(1-\alpha/2)p(1-p)}{\Delta^2} \]

Where:

- \( n \) = sample size
- \( p \) = estimated prevalence of microalbuminuria in SCD: 20.3% from Nigerian Study (19)
- \( \Delta \) = precision (7.5%)
- \( Z^2(1-\alpha/2) \) = the square of the standard deviation corresponding to a 7.5% precision ie. 1.78

\[ n = 1.78^2 \times 0.203 \times 0.797 \times 0.075^2 \]

\[ n = 92 \]

Inclusion criteria

- Children aged 2yrs - 18yrs, both on follow up as well as stable in-patients (afebrile, at least 2 weeks from last crisis) at KNH
- Hb SS diagnosed by electrophoresis (CAPE)
- Informed consent given from parents
Exclusion criteria

- Documented disease that can cause proteinuria independently eg. Diabetes
- Urinary tract infection (UTI) on urinalysis based on presence of nitrites and/or leucocyte esterase
- Acute febrile illness
- Evident cardiac compromise on examination
- Macroalbuminuria (ACR >300mg/g)
- Unable to provide urine sample

Sampling Method:

Consecutive recruitment of clinic attendants and stable in-patients who met inclusion criteria and gave consent was carried out until target sample was achieved.
**Study Procedure:**

Eligible patients on follow up at both paediatric and adult haematology clinics, and stable patients in the wards, were be enrolled by the principal investigator. Consent was sought from guardians or parents, and assent from older minors.

For the clinic setting this process of recruitment was carried out early before the start of the clinic, this was a one hour period that allowed for recording of vitals as well as sorting...
records. Those who were eligible and guardians gave consent had their records reviewed to confirm diagnosis of SCD by CAPE and latest Hb level.

Those recruited from the ward were fewer as an arbitrary two week period was to be allowed after a crisis so as not to interfere with urine protein determination. They were however similarly recruited with informed consent sought before any samples were taken. Patient information was collected using questionnaires (appendix 1) which included: age, gender, clinical history on medication use and frequency of admissions. Family history of renal disease and hypertension was also sought.

Physical examination was carried out with measures of weight and height from which BMI was calculated, as well as any signs of overt cardiac compromise as this was an exclusion criterion. These included examination for edema, pulses and precordium.

**Clinical Procedure:**

*Urine specimen collection*

A clean catch specimen was collected into sterile urine bottles. Guardians and older children had the procedure explained to them clearly by the principal investigator; the specimen was then collected during the course of the same clinic. Those unable to collect a specimen then were to have the same done at a subsequent appointment but this was not feasible due to study time constraints. They were thus not included in final analysis.

At least 10mls of urine was collected in bottles prelabelled with participants’ study number. The samples were analysed on the same day of collection as described below.

*Blood specimen collection*

Venous blood was collected aseptically with appropriate infection control including skin sterilisation with medical spirit, clean gloves, sterile needles and proper disposal of soiled
materials. Two millilitres of venous blood was collected into plain vaccutainers for creatinine determination and another 2mls into EDTA vaccutainers for haemoglobin determination where not done in the past 1 month prior to data collection.

**Laboratory Procedure:**

**Urine processing**

The collected sample was transported to the lab (University of Nairobi, Department of Paediatrics and Child Health lab) and processed within 2 hours of collection.

Initial test was a urine dipstick using SIEMENS CLINITEK urine chemistry analyser. The Clinitek dipstick was immersed into the sample, blotted off, and then slotted into the analyser after priming with a control. This gave a print out analysing presence of nitrites, red blood cells and casts based on colour change of reagent pads on the strip. Quality control was ensured by running positive and negative controls.

Samples positive for nitrites or leukocyte esterase were not to undergo further analysis as this was used to indicate possible urinary tract infection.

Albumin and creatinine concentrations were then quantified similarly using dipsticks with reagent pads for the two parameters.

*Albumin:* This test is based on dye binding using a high affinity sulfonephthalein dye. At a constant pH, the development of any blue colour is due to the presence of albumin. The resulting colour ranges from pale green to aqua blue. Intensity of colour change as measured by spectrophotometry gave the albumin concentration.

*Creatinine:* This test is based on the peroxidase-like activity of a copper creatinine complex that catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3',5,5'-tetramethylbenzidine. The resulting colour ranges from orange through green to blue. The
same analyser was used to give readout of the concentrations in the sample as with albumin.

Controls were run similarly as stated earlier.

Albumin: creatinine ratio (ACR) read in mg/g with any result 30-300mg/g recorded as positive for microalbuminuria and those>300mg/g as macro albuminuria.

**Blood processing**

The collected blood samples were centrifuged to separate out the serum then creatinine was quantified using Biosystems Automated Chemistry A15. This is based on a chemical reaction, depending on analyte, that produces a coloured complex and measuring rate of formation by spectrophotometry.

**Estimated GFR**

The most commonly used method to assess and approximate the GFR in children is the Schwartz-Counahan method¹.

\[
\text{GFR (ml/min/1.73m²) = } k \times \frac{\text{body length (cm)}}{\text{serum creatinine (micromole/L)}}
\]

Where k (table 4) is a constant that is a function of urinary creatinine per unit of body size
Table 4: Values of K in paediatric patients

<table>
<thead>
<tr>
<th>Age Group</th>
<th>K values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm neonates</td>
<td>24</td>
</tr>
<tr>
<td>Term neonates</td>
<td>33</td>
</tr>
<tr>
<td>Infants 1-12 months</td>
<td>40</td>
</tr>
<tr>
<td>Children 2-12 years</td>
<td>49</td>
</tr>
<tr>
<td>Females 13-21 yrs</td>
<td>49</td>
</tr>
<tr>
<td>Males 13-21 yrs</td>
<td>60</td>
</tr>
</tbody>
</table>

The estimated GFR (eGFR) was used to determine the renal function of the children. Normal renal function for children (mean eGFR ± SD in mls/min/1.73 m$^2$) was taken as 133.0 ± 27.0 for males and females aged 2-12 years old, 140.0 ± 30.0 for males aged 13-21 years old, and 126.0 ± 26.0 for females 13-21 years of age (appendix 5). Values higher or lower than these reference ranges were classified as high or low eGFR respectively.
To ensure good quality, data were collected uniformly, quality assured laboratories were used for blood and urine testing and a statistician assisted in data analysis. Data recorded in the data collection tools were kept confidential and stored safely (under lock and key) by the principal investigator.

Data was collected at the clinic and wards and entered into a questionnaire (appendix 2). This was then cleaned and entered into a windows Excel data base. Further cleaning was carried out after entry using frequency distributions and cross-tabulations until no more errors could be detected.

We used statistical products and service solutions (SPSS) version 17.0. Univariate analysis was done for the categorical variables and descriptive statistics (means, medians, and standard deviations) for continuous or discrete variables.

Bivariate analysis was used to investigate any association between the response variable and other variables of interest. The chi-square ($\chi^2$) test was used to test association between two variables if categorical and satisfied all the conditions. Odds ratio was also calculated for all associations being tested. If some chi-square assumptions were not met, Fisher’s exact test was used instead.
RESEARCH ETHICS

The participants of this study were children aged 2-18 years. These are minors thus full explanation of the study was given to parents/guardians and written consent (Appendix 2) sought from them. Assent for participation was also sought from the older children.

Costs of lab tests were borne by the investigator. These included urinalysis, urine ACR and serum creatinine. All patient information was treated with strict confidentiality with all patient paper records kept in locked cabinets and electronic records within the database password protected. Only data the principal investigator and statistician involved in this project had access hence confidentiality was maintained.

In addition, patient names and identifiers were removed from all data tables and records prior to data analysis.

Approval for the study was received from the University Of Nairobi Department Of Paediatrics and Child Health as well as the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON-ERC). The study commenced after approval was granted.

Study results were communicated to the haematology clinic to facilitate follow up for patients positive for microalbuminuria.
5. RESULTS

During the study period 127 patients were interviewed at the haematology clinic and paediatric wards. Of these, 6 subjects opted out while another 2 required urgent admission from the clinic for acute infections, one with pneumonia symptoms while the other had acute osteomyelitis. On reviewing the medical records for the remaining patients, 3 were found to have sickle cell trait thus excluded. A further 4 patients were unable to provide a urine sample thus also got excluded.

**Figure 3: Study Profile**

- Patients at haematology clinic and paediatric wards assessed for eligibility (n=127)
- 6 opted out
- 2 admitted with acute infections
- 3 patients had Hb AS
- 4 no urine sample obtained

- Records reviewed
- Questionnaire administered
- Physical examination done
- Samples collected

- Urinalysis to exclude UTI
- Creatinine concentration in serum
- Urine albumin: creatinine ratio
  (n=112)

- 2 macroalbuminuria

- Study subjects data analysed (n=110)

Of the 110 analysed, 6 patients were recruited from the adult clinic and 21 stable ward patients while the rest were from the paediatric clinic. No distinction between the groups was made during analysis.
Prevalence of Microalbuminuria (MA)

We found microalbuminuria in 43(39.1%; 95% CI 29.9 to 48.9) of the 110 subjects (figure 4) with prevalence of 32% in <5 age group, 43% in those 5 – 10 years and 40% in those over 10 years (figure 5).

Figure 4: Prevalence of Microalbuminuria

Figure 5: Microalbuminuria prevalence by age
Population Characteristics

Our study population comprised 110 subjects. Gender distribution showed 62(56.4%) were male and 48(43.6%) were female giving a male to female ratio of 1.3:1. As shown in table 5, the mean age for the group was 7.53yrs (+3.76). Distribution in age categories had 34(31%) under 5 years, 51(46%) between 5 and 10 years and 25(23%) over the age of 10 years. Distribution of BMI showed most (83%) of the subjects to have normal BMI while 3.6% were overweight and the remaining 13.6% were underweight.

Of the population, 67(61%) were on hydroxyurea with 47(70%) having taken the same for more than one year. Fifty (47%) had not been admitted to hospital in the past one year preceding this study while 16(15%) had been admitted more than once. Nine (8.2%) of the subjects had a history of severe vaso-occlusive phenomena at the time of recruitment with 8 having had a cerebral vascular event and one had avascular necrosis of the hip.

Mean Haemoglobin level for the group was 7.91(+1.2) g/dl. Two (1.8%) of the subjects were found to have low estimated glomerular filtration rates (eGFR) while 49(44.5%) and 59(53.6%) had normal and high eGFR respectively.
### Table 5: Population Characteristics

#### Population Characteristics

<table>
<thead>
<tr>
<th></th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n= 110</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong> mean(SD)</td>
<td>7.53 (+3.76)</td>
</tr>
<tr>
<td><strong>Age categories</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;5 yrs</td>
<td>34 (30.9%)</td>
</tr>
<tr>
<td>5-10 yrs</td>
<td>51 (46.4%)</td>
</tr>
<tr>
<td>&gt;10 yrs</td>
<td>25 (22.7%)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>48 (43.6%)</td>
</tr>
<tr>
<td>Male</td>
<td>62 (56.4%)</td>
</tr>
<tr>
<td><strong>BMI (WHO)</strong></td>
<td></td>
</tr>
<tr>
<td>Thinness (-2 to -3SD)</td>
<td>15 (13.6%)</td>
</tr>
<tr>
<td>Normal (1 to -2SD)</td>
<td>91 (82.7%)</td>
</tr>
<tr>
<td>Overweight (1 to 2SD)</td>
<td>4 (3.6%)</td>
</tr>
<tr>
<td><strong>Admissions in the past year</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>52 (47.3%)</td>
</tr>
<tr>
<td>1</td>
<td>42 (38.2%)</td>
</tr>
<tr>
<td>&gt;1</td>
<td>16 (14.5%)</td>
</tr>
<tr>
<td><strong>Hydroxyurea use</strong></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43 (39.1%)</td>
</tr>
<tr>
<td>Yes</td>
<td>67 (60.9%)</td>
</tr>
<tr>
<td><strong>Duration of hydroxyurea use (yrs)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>20 (29.9%)</td>
</tr>
<tr>
<td>&gt;1</td>
<td>47 (70.1%)</td>
</tr>
<tr>
<td><strong>Severe vaso-occlusive phenomenon</strong></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>101 (91.8%)</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (8.2%)</td>
</tr>
<tr>
<td><strong>Haemoglobin</strong></td>
<td>7.91 (+1.2)</td>
</tr>
<tr>
<td><strong>estimated GFR(eGFR)</strong></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>Normal</td>
<td>49 (44.5%)</td>
</tr>
<tr>
<td>High</td>
<td>59 (53.6%)</td>
</tr>
</tbody>
</table>

1 cerebral vascular accident (CVA), avascular bone necrosis, priapism, retinopathy, pulmonary infarct

ii eGFR: low;<mean-1SD for age, normal; mean+1SD, high;>mean+1SD (appendix 5)
Factors associated with Microalbuminuria

In the bivariate analysis comparing those positive to those negative for microalbuminuria, we found no statistically significant association with age, gender, mean Hb level, BMI, number of in-patient visits, hydroxyurea use or duration of use (table 6). There was however a significant association between a high eGFR and testing positive for microalbuminuria; p=0.006, odds ratio (OR) 3.066 95% CI 1.358 to 6.922. We also found a trend towards significance in the association with severe vaso-occlusive event; p=0.072, OR 0.175 95% CI 0.021 to 1.457. The number of subjects was however too small to make a conclusive assessment of this relationship as evidenced by the confidence interval.

Figure 6: Association between eGFR and Microalbuminuria
Table 6: Factors associated with Microalbuminuria

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive n=43</th>
<th>Negative n=67</th>
<th>P-Value</th>
<th>OR</th>
<th>95% CI for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age {mean(SD)}</strong></td>
<td>7.64(±3.42)</td>
<td>7.46(±4.0)</td>
<td>0.805</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hb {mean(SD)}</strong></td>
<td>7.83(±1.49)</td>
<td>7.96(±0.9)</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>19(39.6%)</td>
<td>29(60.4%)</td>
<td>0.926</td>
<td>1.037</td>
<td>0.474-2.259</td>
</tr>
<tr>
<td>Male</td>
<td>24(38.7%)</td>
<td>38(61.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thinness (&gt; -2SD)</td>
<td>10(67%)</td>
<td>5(33%)</td>
<td>0.538</td>
<td>1.434</td>
<td>0.453-4.535</td>
</tr>
<tr>
<td>Normal(1 to -2SD)</td>
<td>53(58.2%)</td>
<td>38(41.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Admissions in past year</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or 1</td>
<td>36(38.3%)</td>
<td>58(61.7%)</td>
<td>0.679</td>
<td>1.253</td>
<td>0.429-3.659</td>
</tr>
<tr>
<td>&gt;1</td>
<td>7(43.8%)</td>
<td>9(56.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>eGFR {mean(SD)}</strong></td>
<td>166(±56.2)</td>
<td>141(±50)</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>eGFR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>31(52.5%)</td>
<td>28(47.5%)</td>
<td>0.006</td>
<td>3.066</td>
<td>1.358-6.922</td>
</tr>
<tr>
<td>Normal</td>
<td>13(26.5%)</td>
<td>36(73.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hydroxyurea use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25(37.3%)</td>
<td>42(62.7%)</td>
<td>0.633</td>
<td>0.826</td>
<td>0.378-1.808</td>
</tr>
<tr>
<td>No</td>
<td>18(42%)</td>
<td>25(58%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration of hydroxyurea use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>19(40.4%)</td>
<td>28(59.6%)</td>
<td>0.419</td>
<td>1.583</td>
<td>0.516-4.852</td>
</tr>
<tr>
<td>&lt;1</td>
<td>6(30%)</td>
<td>14(70%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Severe vaso- occlusive phenomenon</strong>ii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1(11%)</td>
<td>8(89%)</td>
<td>0.072</td>
<td>0.175</td>
<td>0.021-1.457</td>
</tr>
<tr>
<td>No</td>
<td>42(41.6%)</td>
<td>59(58.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* eGFR: low; <mean-1SD for age, normal; mean±1SD, high; >mean+1SD (appendix 5)

** ii cerebral vascular accident (CVA), avascular bone necrosis, priapism, retinopathy, pulmonary infarct
6. DISCUSSION

In this study we found the prevalence of microalbuminuria to be 39.1% (95% CI 29 to 48%) with prevalence in <5 years at 32%, 5-10 years at 43% and 40% in >10 years. In comparison previous studies\(^6\)-\(^{13,20,34}\) found a prevalence ranging from 16 to 36% in paediatric populations with higher prevalence of up to 43% in adult populations\(^15\). Dhanidharka et al\(^12\) in the US looking at 102 subjects aged 2 - 18 years found a prevalence of 26.5% that rose to 46% when looking at those >10 years. This result was thought to be due to a higher number of subjects between 16 and 18 years of age. This increasing prevalence has been replicated in subsequent studies\(^6,9,17,20,37\) showing higher prevalence in >9 years age group. This rising incidence with age has been attributed to more ischaemic episodes thought to be the initial insult in the cascade of renal damage. This is further illustrated by McKie et al\(^21\) who in a longitudinal study in Georgia USA involving 191 Hb SS subjects with average 2.19+ 2.05 years follow up showed development of MA being at an average age of 11.8+ 3.93 years. They thus concluded point prevalence studies would be influenced by mean age of the study population. This may be one of the explanations for the wide variations in prevalence. The study population mean age was 7.53 +3.76 years which is relatively low but showed higher prevalence in those over 5 years of age at 43% in the 5-10 year age group and 40% in those >10 years. Likely explanations for the difference in prevalence are that this study was based at a tertiary referral centre. This may have selected subjects with severe disease. In comparison, most reports on MA prevalence in this patient group are from specialised sickle cell centres\(^9,11,17,21,37\) some of which start follow up from the time of diagnosis after neonatal screening\(^10\). Datta et al\(^6\) in India carrying out his study at a general paediatric centre a similar population to ours found a prevalence of 18.8% with a higher figure of 33.3% in those >9
years old. He was however working with a population <14 years of age compared to our population that was up to 18 years. This may account for the slightly higher prevalence.

Secondly, care of our SCD patients is not fully standardised in addition to introduction of hydroxyurea after the age of 5 years which from intervention studies\textsuperscript{21} reverses or delays renal damage in SCD. From our interaction with the subjects we also realised most of them are symptomatic but diagnosed late after several admissions and blood transfusions. This is likely to expose them to more episodes of sickling with subsequent ischaemic damage before any interventions are instituted also contributing to a higher prevalence.

The youngest subject with MA was 2 years old. In previous US studies Dhanidharka\textsuperscript{6} found no child <7 years with MA, Marsenic\textsuperscript{9} had his youngest subject with MA being 4 years old and Mc Kie’s\textsuperscript{21} youngest MA positive subject was 5 years old. King et al\textsuperscript{10} in Jamaica found MA in a subject 2.8 years old while studies in Nigeria reported MA at 4 years\textsuperscript{37} and in 2 subjects <5 years\textsuperscript{20}. Late diagnosis may account for this observation in our population and since the population was 2 to 18 years of age, we cannot be certain MA doesn’t appear even earlier.

Factors associated with MA have been investigated in previous studies with varying results. In this study we found significant association with a raised eGFR (hyperfiltration). This was also reported by King el al\textsuperscript{10}. However studies by Datta\textsuperscript{6} in India, Alvarez in US and Eke\textsuperscript{37} in Nigeria found no association. On average eGFR was also significantly higher in the group testing positive for microalbuminuria

There was no association with average age a finding also reported by Imuentinyan\textsuperscript{20} in Nigeria. Some studies however do report association with age\textsuperscript{6,9,12,21} with increasing age showing higher prevalence of MA.
No association was demonstrated with body mass index (BMI), number of hospitalizations, hydroxyurea use or duration of use. In investigating association with hydroxyurea the results suggest no significant association but we recognise that we only looked into use of the drug though some studies have suggested an effective dose range\textsuperscript{35}. Adequate dosing was not accounted for in our study.

Previous studies have shown association with lower haemoglobin concentration\textsuperscript{10,21,37} though others have not demonstrated this\textsuperscript{6,20}. There have been no studies showing association with gender or BMI. Dhanidharka\textsuperscript{12} in US and Datta\textsuperscript{6} in India also reported no association with number of hospitalizations but Eke et al\textsuperscript{37} in Nigeria found significant association with hospitalizations due to pain crisis. Duration of disease was initially part of our study but recall proved inaccurate for many of the first respondents and was thus excluded. We could not include it reliably as an indicator of disease severity.

7. STUDY LIMITATIONS

This study was based at a national referral hospital thus the selected patients may not represent the general population. This may in part account for our high prevalence of microalbuminuria as stated earlier.

We also relied on history in some of the data collected though this was corroborated by documentation in patient files. This was so for ruling out previous diagnosis of diabetes mellitus as we did not carry out random blood sugars though we did look for glucose in urine by dipstick. The same applied for previous diagnosis of vaso-occlusive phenomena such as CVA, priapism, pulmonary infarcts or AVN. We also did not carry out retinal examination to rule out retinopathy though we did ask about visual symptoms.
UTI as exclusion criteria was ruled out by dipstick examination, this may have been adequate but the gold standard is urine culture. Given our budget and timeline we limited our screening to use of the dipstick test.

8. CONCLUSION
   a. This study revealed a high prevalence of microalbuminuria. The youngest subject testing positive for microalbuminuria was 2 years old suggesting early onset of kidney damage.
   
   b. We found significant association between hyperfiltration and MA indicating that this is part of the cascade in renal damage.

9. RECOMMENDATIONS
   a. Once a diagnosis of SCD is made, testing for MA should be instituted with yearly testing. MA positive testing should then be an indicator for hydroxyurea use with those having persistent MA referred for nephrologist reviews and follow up.
   
   b. Renal function testing, including calculating eGFR, should be routine as there is positive association with MA. Both hyperfiltration and MA indicate progression of renal damage.
10. REFERENCES


Appendix 1: QUESTIONNAIRE

1. Biodata:
Study number:_______________       Age (yrs):   ___________          Gender:  M/F

2. Anthropometric measures:
Weight:____________kg   Height:________cm BMI:   ________________kg/m²
( ) underweight     ( ) healthy weight    ( ) overweight    ( ) obese  *(tick one as appropriate)*

3. Clinical severity of disease

At what age was SCD diagnosed?

☐ <12 months  
☐ 12-24 months  
☐ 25 months – 5 years  
☐ >5 years  

How many times have you been hospitalised in the past year?

☐ 5 or more  
☐ 2-4  
☐ 1  
☐ Nil

Have you been diagnosed with any of the following? *(tick as appropriate for positive response)*

☐ Cerebrovascular accident  
☐ Pulmonary infarct  
☐ Leg ulcer  
☐ Congestive cardiac failure  
☐ Renal disease  
☐ Avascular bone necrosis  
☐ Priapism  
☐ Retinopathy

4. Medication history

Are you taking hydroxyurea?  
☐ Yes  
☐ No  

If yes, for how long have you been taking hydroxyurea?

☐ <1year  
☐ >1year

5. Family history

Do you have any relative with renal disease?  
☐ Yes  
☐ No  

If yes, what is their relation to you?.................................

Do you have any relatives with hypertension?  
☐ Yes  
☐ No  

If yes, what is their relation to you?.................................
6. Physical examination:

General: Periorbital edema

- Yes
- No

Cardiovascular:

- Yes
- No

Pulse:

- Normal
- Low volume
- High volume
- Irregular

Precordium:

- Normal
- Hyperactive

Apex beat:

- Normal
- Displaced

Summary Cardiovascular system

- Normal
- Abnormal

7. Lab results:

Haemoglobin - _________g/dl

Creatinine - _________mmol/l

eGFR - _________ml/min/1.73m² (normal) (high) (low)

Albumin:creatinine ratio

- _________mg/g (negative) (positive)
Appendix 2: CLIENT INFORMATION AND CONSENT FORM

Title: PREVALENCE AND CLINICAL FACTORS ASSOCIATED WITH MICROALBUMINURIA IN CHILDREN AGED 2-18 YEARS WITH SICKLE CELL DISEASE ON FOLLOW UP AT KENYATTA NATIONAL HOSPITAL

Principal Investigator: Dr. Martin Mbiata Muthiga, Masters student; Department of Paediatrics and Child Health, University of Nairobi. Tel. No. 0720899221. email:mmmbiata@gmail.com
Supervisors: Prof. Rachel Musoke; Associate professor, Consultant Neonatologist and Senior Lecturer Dr. Dalton Wamalwa; Consultant Paediatrician; Senior Lecturer Dr. Bashir Admani; Paediatric Nephrologist; Consultant Paediatrician; Senior Lecturer
KNH Ethics Review Committee: Chairperson Prof. Guantai 2726300 ext. 44102

Introduction:
I am a postgraduate student pursuing a degree in Paediatrics and child health. As part of this degree I am carrying out a study investigating prevalence of microalbuminuria, which is trace levels of proteins in urine as a marker of kidney damage. My group of interest is children with sickle cell disease.
I wish to request your child’s participation in the study.

What is the purpose of this research?
Sickle cell disease is a chronic condition where the patient’s blood cells tend to get deformed within blood vessels resulting in pain and early destruction of the cells. This process takes place in various organs including the kidneys, which is the subject of this study. I am asking you to participate in this study aimed at establishing how common kidney involvement is in our sickle cell patients.

This study will help us in the long term management of sickle cell patients by informing on need for screening and thus early detection of kidney involvement allowing for early intervention hence improving quality of life.

Voluntary participation:
Taking part in this research project is voluntary. You can agree or decline to participate and this will not interfere in any way with the ongoing care of your child.

What will be done to my child if I agree?
If you agree to have your child in this study, we will request to:
- Review your child’s medical records
- Take a brief history from you concerning the child
- Do a physical examination including height and weight
- Collect a urine sample as instructed
- Withdraw only 4mls of blood from the child’s forearm. This will involve a needle prick and will cause pain though brief

Duration:
This research project will run for 3 months, but unless specified we will only meet your child once.
**Benefits from participating in the study:**
During the history taking and examination, any new information will be relayed to the clinic or ward doctor for any necessary action. Results from your child’s tests that also need any further action will also be communicated appropriately.

**Cost:**
I will incur all costs involved in the study thus the tests involved will be at no cost to you. However no monies will be paid for your participation.

**Risk:**
The research will not involve any treatment that is not part of what the doctor will be giving. Save for the discomfort of blood sampling, there is no risk in participation.

**Confidentiality:**
All the information that we will gather about your child will be kept highly secret. Your name or that of the child will not be used at any time in the report of this research.

**Sharing of the results:**
The results, which will come from this research, will be used broadly and may be sent to medical journals to be published.

**Right to refuse or withdraw:**
You may refuse your child to participate at any time or even withdraw after agreeing to consent.

**Who has allowed this study to take place?**
The ethics and research committee of University of Nairobi/Kenyatta national hospital have studied the proposed study carefully and given permission for it to be done

**What if have questions to ask about this study?**
Dr. Martin Mbiata Muthiga Tel. No. 0720899221
If you have any questions on your rights as a research participant you can contact the Kenyatta national hospital/university of Nairobi Ethics and Research committee (KHH/UON-ERC)by calling 2726300 ext.44355. email:uonknh erc@uonbi.ac.ke.
Fax: 725272

To indicate that you understand the conditions of this study and that you consent your child to participate in it, please sign or put your thumb print in the space provided in the consent certificate._____________________

**ENGLISH CONSENT**
A study to determine prevalence and clinical factors associated with microalbuminuria in children aged 2-18years on follow up at KNH
I as the guardian/parent, voluntarily agree to participate in this study. I understand that participation in the study does not entail financial benefit. I have been informed that information obtained will be
treated with utmost confidentiality and my treatment will not be compromised if I decline participation or withdraw from the study. The results of this study, including laboratory or any other data, may be published for scientific purposes but will not give my name or that of my child or include any identifiable reference to me.

I have had a chance to ask questions. If I have questions later, I can ask the researcher. No coercion has been used to influence my decision to participate in the study whose nature, benefits and risks have been explained to me by Dr/Mr./Mrs. ……………………………. Signature:…………………………

Name of child …………………………………………………………………. Parent/guardian Signature ……………………………………………………….. Tel No. …………………………………

THE KISWAHILI CONSENT CERTIFICATE

MAELEZO KWENYE CHETI CHA RIDHAA

Kichwa cha habari: kubaini idadi ya watoto kati ya miaka miwili na miaka kumi na nane wanao ugu ugonjwa mundu kiini wanao patikana na microalbumin katika kipimo cha mkojo. Wasimamizi: Prof. Rachel Musoke; Associate professor, Consultant Neonatologist and Senior Lecturer Dr. Dalton Wamalwa; Consultant Paediatrician; Senior Lecturer Dr. Bashir Admani; Paediatric Nephrologist; Consultant Paediatrician; Senior Lecturer Utangulizi: Mimi ni daktari katika chuo kikuu cha Nairobi kule ambako ninafunzwa taluma ya matibabu ya watoto. Ninahitajika kufanya utafiti ili taluma hii. Ningependa kufanya utafiti kwa watoto walio na ugonjwa mundu kiini yani sickle cell. Lengo langu ni kubaini idadi ya wanopatikana na dalili ya ugonjwa wa figo katika kundi hii ya watoto. Ningependa kuomba kushiriki kwa mtoto wako kwa utafiti huu. Uko na uhuru wa kuuliza maswali yeyote yale yanoyoambatana na utafiti huu. Kusudi la utafiti huu: Utafiti huu utasaidia kufahamu idadi ya watoto ambao zimeanza kupata madhara ya ugonjwa mundu kiini ili kuwezesha shida hii kupelelezwa mapema na madawa ya kusitishe kudhoofika kwa figo kuanzisha mapema.
Kujitolea kushiriki:

Kukubali mtoto kushiriki kwa utafiti huu ni kwa hiari yake mtu mwenyewe.Hakatawepe na kulazimishwa ama kushurutishwa.Ni haki ya kila mshiriki kujiudhaa kwenye utafiti huu wakati ule wowote.kujiondoa kwa mshiriki kwemwe utafiti huu hakutapelekea yeye kunyimwa matibabu anayostahili.

Yale ambayo mtoto atafanyiwa:

Mtoto yeyote ambaye atashiriki kwenye utafiti huu atafanyiwa yafuatayo.

- Rekodi zitapitiwa
- Kuulizwa maswali ili kufahamuhu ukali ya maradhi au dalili za ugonjwa huu
- Kupimwa usito
- Kupimwa urefu.
- Kutolewa kwango kidogo cha damu kwa minajili ya kuchunguza kwa kudumu na figo na kiasi cha damu
- Mtoto atahisi uchungu kwa muda mfupi tu.

Muda wa utafiti:

Utafiti huu unakusuguliwa muda wa miezi mitatu tu.Mtoto wako atanufaidika kwa mtafiti mkuu mara moja tu.

Je mtoto wangu atanufaidika vipi kwa kushiriki kwa utafiti huu?

Katika ile halu ya kueleza shida za mtoto wako na pia pale nitakapo mpima mtoto wako kama kuna mambo mapya nitapata, nitamfahamisha muuguzi wako ili mtoto atanufaidika. Hapatawako na malipo yeyote kwa kushiriki kwa mtoto wako,lakini nitaghamisha malipo yote ya kupimwa damu kama ulivyo elezewa.

Je kuna uwezekano wa madhara yeyote kwa mtoto wangu?

Hapatawepe na madhara yeyote kwa mtoto wako.Utafiti huu umeidhinishwa baada ya kukaguliwa na baraza la ubora wa utafiti kwa binadamu la hospitali ya kenyatta na chuo kikuu cha Nairobi.

Hakikisho la siri kwa mhusika:

Yale yote ambaye ya kushiriki kujiudhaa kwa yatakipa mtoto wako yatakipa kwa hakuna majina ambayo yatakimika ambaye yanaweza kukutambulisha wewe au mtoto wako.

Utumizi wa matokeo ya utafiti huu:

Matokeo ya utafiti huu yanaweza kuchapishwa kwa majarida ya kisayansi lakini siri ya mshiriki itadumishwa.
Haki yako ya kujiondoa kwa utafiti huu:

Una haki ya kujiondoa kwenye utafiti huu wakati wowote ule.

Ruhusa ya kufanya utafiti:

Ruhusa ya kufanya utafiti huu imetolewa na idara ya hospitali ya taifa Kenyatta na idara ya watoto chuo kikuu cha Nairobi.

Mawasiliano: Jwapo una swala au unahitaji maelezo kuhusu utafiti huu tuwasiliane kupitia:

Dr. Martin Mbiata Muthiga; nambari ya simu: 0720899221, bara pepe: mmmbiata@gmail.com

Kenyatta national hospital/university of Nairobi Ethics and Research committee (KHH/UON-ERC) by calling 2726300 ext. 44355. email: uonknh erc@uonbi.ac.ke.

Cheti cha ridhaa:


Jina la mtoto-----------------------------------------------

Sahihi ya mzazi kwa niaba ya mtoto-----------------------------------

Tarehe-------------------------------------------------------------------

Sahihi ya anayeuliza maswali.--------------------------------------------

Tarehe-------------------------------------------------------------------
Appendix 3: ETHIC APPROVAL

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Dr. Mbati Martin Muthiga  
Dept. of Paediatrics & Child Health  
School of Medicine  
University of Nairobi

Dear Dr. Muthiga

RESEARCH PROPOSAL: “PREVALENCCE AND CLINICAL FACTORS ASSOCIATED WITH MICROALBUMINURIA IN CHILDREN AGED 2-18 YEARS WITH SICKLE CELL ANAEMIA ON FOLLOW UP AT KENYATTA NATIONAL HOSPITAL”  
(P214/04/2012)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above revised research proposal. The approval periods are 10th August 2012 to 9th August 2013.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.

b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.

c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.

d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.

e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal).

f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an executive summary report within 90 days upon completion of the study  
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

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

“Protect to Discover”
Yours sincerely

[Signature]

PROF. A.N. GUANTAI
SECRETARY, KNUON-ERC

c.c.
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Dean, School of Medicine
The HOD, Records, KNH
Supervisors: Prof Rachel Museke, Dr. Dalton Wamalwa, Dr. Bashir Admani

"Protect to Discover"
Appendix 4: BMI CHARTS

BMI-for-age BOYS
Birth to 5 years (z-scores)
BMI-for-age GIRLS
5 to 19 years (z-scores)

2007 WHO Reference
Appendix 5: NORMAL GFR IN CHILDREN AND YOUNG ADULTS²

**Table 24. Normal GFR in Children and Young Adults**

<table>
<thead>
<tr>
<th>Age (Sex)</th>
<th>Mean GFR ± SD (mL/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week (males and females)</td>
<td>40.6 ±14.8</td>
</tr>
<tr>
<td>2–8 weeks (males and females)</td>
<td>65.8 ±24.8</td>
</tr>
<tr>
<td>&gt;8 weeks (males and females)</td>
<td>95.7 ±21.7</td>
</tr>
<tr>
<td>2–12 years (males and females)</td>
<td>133.0 ±27.0</td>
</tr>
<tr>
<td>13–21 years (males)</td>
<td>140.0 ±30.0</td>
</tr>
<tr>
<td>13–21 years (females)</td>
<td>126.0 ±22.0</td>
</tr>
</tbody>
</table>

*Data based on three studies.⁶⁹-⁷¹

*Abbreviation: SD, standard deviation*