THE PREVALENCE OF IRON OVERLOAD AND RELATIONSHIP BETWEEN BLOOD TRANSFUSION, IRON OVERLOAD AND HEPATOTOXICITY IN CHILDREN WITH SICKLE CELL DISEASE AT THE KENYATTA NATIONAL HOSPITAL- A CROSS SECTIONAL STUDY.

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DECLARATION

This dissertation is my original work and has not been published elsewhere or presented for a degree in any other university.

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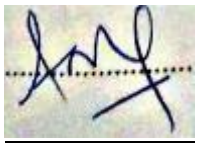
Signed:  Date: 28th April 2022

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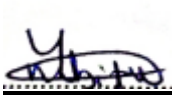
Signed:  Date: 28th April 2022

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ABBREVIATIONS

ALT	alanine amino transferase
CI	Confidence Interval
Dlw	dry liver weight
ERC	Ethics and Research Committee
Hb	haemoglobin
HBS	sickle haemoglobin
HBSS	homozygous sickle cell disease
HCC	hepatocellular carcinoma
HIC	hepatic iron content.
HSC	hepatic stellate cells
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital
LIC	Liver iron content
MOH	Ministry of Health
MRI	magnetic Resonance Imaging
Ng	nanogram
NTBI	non- transferrin bound iron
OPD	Outpatient Department
ROS	reactive oxygen species
SCD	sickle cell Disease
SF	serum ferritin
STOP trial	Stroke Prevention Trial in sickle cell anaemia.
Thal	thalassemia
TIL	transfusion iron overload
ULN	upper limit of normal
UoN	University of Nairobi
VOC	Vaso-Occlusive Crises
WHO	World Health Organization

DEFINITIONS OF TERMS

Sickle Cell Disease (SCD): Confirmed sickle cell disease e.g., by Hb electrophoresis, Peripheral blood film or sickling test.

Unit of blood transfusion: 200 ml of packed red blood cells or 525ml of whole blood.

Iron overload – Elevated serum ferritin levels > 300ng/ml

Hepatic dysfunction – Elevation of serum alanine aminotransferases as indicator of liver injury, with alanine amino transferase (ALT) levels greater than age-appropriate levels.

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ABSTRACT

Background: Blood transfusion, essential in the management of sickle cell disease ultimately leads to the accumulation of exogeneous iron. Iron overload which is often undetected is pathological to the body. Serum ferritin can be used a means of evaluation of body iron stores.

Study justification and utility : Children with SCD undergo repeated blood transfusions which exposes them to the risk of iron overload. Monitoring of iron levels enables preventive therapy as iron overload is treatable. There is a paucity of evidence on the magnitude of iron overload and its associated effects among children in long term care in our setting.

Objectives: To determine the prevalence of iron overload among children aged 1-18 years with sickle cell disease receiving care in KNH, determine the association between blood transfusion iron overload, and liver function in these children.

Study Design: This was a hospital based descriptive cross-sectional study .

Methods: The study population comprised of 145 children recruited from the Kenyatta National Hospital Haematology clinic, the inclusion criteria being any child aged 1-18 years with sickle cell disease and whose consent and assent (where applicable) was obtained. The exclusion criteria was anyone with a history of recent crises in the preceding 4 weeks or exacerbation of acute illness on day of screening. Sampling was by consecutive recruitment and once screened and enrolled, history of blood transfusions received was obtained. A retrospective abstraction of the patient's medical records was also carried out. A venous blood sample was delivered to the laboratory for assessment of serum ferritin and alanine aminotransferase levels. The outcomes of interest were serum ferritin measurement as evaluation of iron overload, where iron overload was defined as serum ferritin levels >300ng/ml, the number and volume of blood transfusions over a three-year period as well as assessment of serum alanine aminotransferase (ALT) levels as indicator of hepatic dysfunction, with hepatic dysfunction defined as serum ALT >42 IU/L.

Data management and analysis: All data was entered into a case record form. The prevalence of iron overload was computed by the number of children with

elevated serum ferritin levels as the numerator and the total number of children as denominator and this was converted to percentage. Logistic regression as well as the Mann Whitney test in which medians between groups was compared was used to analyse the association between number of blood transfusions and serum ferritin levels as well as serum ferritin levels and ALT levels .

Results: 145 children were enrolled into the study, 86 male and 59 female. The median age of the population was 8 years and the median duration of care in KNH was 7 years. The median number of blood transfusions was 2. The prevalence of iron overload as measured by serum ferritin was 64% (95% CI 407-584 IU/L), with 49 (39%), 27 (19%), 6 (4%) and 11 (8%) of the children having mild, moderate, high and severe overload respectively. The number of transfusions received influenced the level of ferritin in the serum and each additional unit of blood transfused conferred a 1.8-fold increased odds of iron overload (OR 1.8 95% CI 1.38,2.54.). Elevated serum ferritin conferred an 8.3-fold increased odds of hepatic dysfunction (OR 8.3 , 95% CI 1.05, 65.29). Serum ferritin was significantly associated with ALT levels (p value <0.01) and every 1 ng increase in serum ferritin increased the odds of elevated ALT level by 0.1% (OR 1.001, CI 1.001, 1.002)

Conclusions: Iron overload was highly prevalent in this population of children with SCD. Every additional unit of transfused blood increased the risk of iron overload by two-fold. There is a positive association between iron overload and hepatic dysfunction in these children with SCD

Recommendations: We recommend that children with SCD have careful monitoring of number and volume of blood transfusions, and routine monitoring of serum ferritin should be done. Further studies can be done to study the correlation between elevated serum ferritin levels and hepatic iron concentration.

CHAPTER 1: BACKGROUND

Sickle cell disease (SCD) is an inherited blood disorder with autosomal recessive inheritance. A defective form of haemoglobin arises from a single point gene mutation in the β (beta) globin chain resulting in a base pair exchange of thymine for adenine. Defective haemoglobin synthesis results in a sickle haemoglobin. Patients who are homozygous for the condition (HbSS) develop sickle cell anaemia. Several heterozygous mutations of the globin chains exist.

The majority of cases of SCD are found in Sub- Sahara Africa(1). It is found commonly in malaria endemic regions as an evolutionary mechanism to malaria parasites. 6-15% of all deaths in children less than 5 years in Africa are attributed to SCD, where it remains an issue of great public health significance.(2). In Kenya, the prevalence of SCD is reported as 3-4% and more than 80% of the patients are from Western Kenya in terms of ethnic origin. (3)

The signs and symptoms of sickle cell disease begin to manifest after infancy as the levels of foetal haemoglobin (HbF) begin to decrease. The clinical expression of disease found in SCD arises due to the alteration in the physical and molecular properties haemoglobin (Hb). The resulting sickle Hb develops the propensity to polymerize during deoxygenation, affecting the pliability of the red blood cell (RBC). This hampers its ability to flow within the microcirculation. The sickle shaped RBCs also exhibit increased adherence to vascular endothelium and are prone to haemolysis.

The complications of SCD include acute pain crises, acute chest syndrome, splenic crises, and ischemic stroke. A study done on the prevalence of complications of sickle cell disease in children in Saudi Arabia (4) (N=145, age 1-14 years) found vaso occlusive crises occurred most frequently in more than half of the cases (55.9%) and is the reason why most children required in patient management.

Most patients with SCD will therefore require regular red blood cell transfusions throughout their lives. This inexorably presents a substantial load of exogeneous

iron to the body, necessitating the need for monitoring and treating of iron overload, for which a variety of methods exist.

Cross-sectional studies done on children in other parts of Africa have an estimated prevalence of iron overload of 24%-33% amongst children with sickle cell disease. (5) However, the magnitude of iron overload in Kenya and its associated risk factors is unknown.

The focus of this study is thus to investigate the approximate amount of iron in children who have been diagnosed with sickle cell disease presenting to KNH and to address the role of serum ferritin as an initial step in identification and assessment of iron status. These findings will be useful to guide level of need for the monitoring of iron levels, and guide policies for prevention and treatment of iron overload in children with sickle cell disease in our setting.

CHAPTER 2: REVIEW OF LITERATURE

Blood Transfusion in Patients with Sickle Cell Disease

Blood transfusion is a key intervention which has been found to be an effective therapeutic modality in sickle cell disease. It is critical in the treatment of SCD because it increases the oxygen carrying capacity of blood and decreases the proportion of the sickle Hb. STOP (Stroke Prevention Trial in sickle cell anaemia)(6) is a large landmark research which demonstrated the effectiveness of transfusion therapy in children with SCD.

Blood transfusions in sickle cell disease commences at an early age (between 2-9 years) and the burden of iron overload increases over the years. Notwithstanding the STOP trial and other similar clinical trials which have demonstrated the efficacy of red cell transfusions in primary and secondary stroke prevention in children, there is a paucity of research and limited guidelines on the management of sequelae following recurrent transfusions in SCD.

Aimed at describing transfusion patterns and indications amongst sicklers in low resource settings, a study conducted in Yemeni (7) on 217 children aged less than 16 years (mean age 6.9 $\pm$ 4.6 years) found that within the study period (12 months), 169 children (77.9%) were transfused. 122 (72.2%) received one transfusion whereas 5 children (4.15%) experienced greater than 5 transfusions. The major indications for transfusion were anaemic crises (41.4%) and vaso-occlusive crises (13.8%). This study concluded that occasional blood transfusion is a prevalent practice for children when they develop acute complications of the disease.

Blood Transfusion and Iron Overload in sickle cell disease

Absorption of iron occurs in the duodenum. The body has no mechanisms for elimination of excess iron and 1mg is excreted daily. In essence, long term transfusion therapy loads the body with large quantities of exogenous iron which exceeds its elimination capability. It has been shown that patients with sickle cell disease who do not receive recurrent blood transfusions do not experience elevated

iron stores and total body iron does not surpass 2000mg.(8) A study by Mohanty in India (9) assessed iron deficiency in sickle cell disorders (N=8434) and iron deficiency was found in SCD (67%) as compared to the trait (20%) and the healthy population (22%) leading to the conclusion that iron overload is described in sickle cell disease only as an aftermath of repeated blood transfusions.

The extent of iron overload is determined by the age of onset of blood transfusions and the number of transfusions received since commencement. (10) Transfusion of 1 unit or 220 ml of packed RBCs delivers 1mg per ml transfused extra elemental iron, (~ 200mg of iron) (8). Iron loading has been shown to occur after approximately 10 lifetime transfusions. This correlates with serum ferritin levels of approximately 500ng/ml and hepatic liver concentration of 3.5mg/g dlw.(10)

Physiology of Iron

Iron levels in the body are normally maintained within the range of 40-50mg/kg body weight. Absorption of iron occurs in the duodenum and stored as ferritin. Daily excretion is 1mg with excess iron deposited in the liver.(5)

Multiple transfusions result in unbound iron which lead to cell damage. In the liver this is evident as fibrosis and eventual cirrhosis.(6)

Though iron overload can affect other organs, e.g. the heart , the liver is the most severely affected organ by iron overload. (6)(7).

The pathogenesis of iron overload in the liver is illustrated in Figure 1.

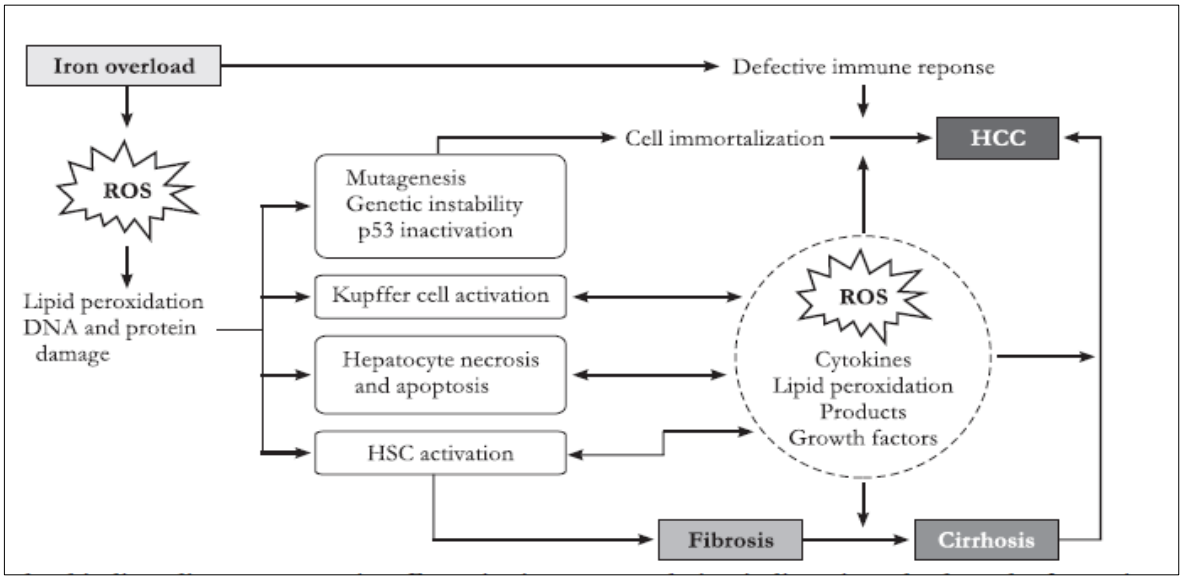

FIGURE 1: Effects of iron in the liver

Accumulation of iron resulting in hepatic cell damage

(Source: (15)*Hepatobiliary & pancreatic diseases International, 2016; 15:5,10).*

A multicentre study of the frequency of hospitalization in patients receiving multiple transfusions(25) (N= 263, 199- transfused, 64 -non transfused, mean age 25 $\pm$11 years) found that patients who received frequent transfusions were hospitalized more frequently. The magnitude of body iron stores (which were evaluated using SF) and the number of times the patient's required admission were found to be correlated. (r=0.20, p=0.009). Furthermore, following a 23.5-month followup,17 patients in the transfused group had died, compared to no deaths in the non - transfused patients. Autopsy, carried out in 6 subjects revealed the presence of exogenous iron in the lymphatic organs and hepatic parenchyma.(25)

A similar study by Ballas et al (26) sought to provide research on iron overload as a contributing factor to morbidity and mortality in SCD by actively following up 247 patients with SCD for 10 years (1987-1988). Iron overload was assessed by SF evaluation. Death as an outcome was substantially more in the population that had elevated iron stores (64% versus 5%).

Blood Transfusion and Iron Overload in Sickle Cell Disease

Iron overload is dependent on the number of blood transfusions received. (9) Transfusion of 1 unit or 220 ml of packed RBCs is estimated to result in 1mg per ml transfused iron, (~ 200mg of iron) (7). This correlates with serum ferritin levels of approximately 500ng/ml and hepatic liver concentration of 3.5mg/g dlw.(9)

Studies On Prevalence of Iron Overload

Studies have been carried out in resource limited settings where SF is utilized to establish the magnitude of high iron stores amongst children with sickle cell disease. In West Bengal state, India, Ray et al recruited 150 children with SCD aged 3-18 years in a prospective study aimed at assessment of iron status. Mean serum ferritin level was found to be 140.2ng/ml with range of 4.7-450ng/ml. 27 patients had low SF levels, 57 had normal levels and 24 patients presented with high SF levels. Those transfused with greater than 10 units has SF ranging from 120-450ng/ml, with mean of 256.8ng/ml. A linear relationship between the volume of blood transfused and SF levels was demonstrated (29)

A similar study conducted in Nigeria assessed in children who had been diagnosed with SCD (N=96, age 1-5 years). The average SF level was found to be 381.2ng/ml, range of 34.2-3282.9ng/ml. SF level greater than 300ng/ml were reported in 16 (33.3%).(30)

Finally, in Congo, the magnitude of elevated iron stores was assessed using serum ferritin levels (N=70, age 2-18 years). The study found SF 24-2584ng/ml, with a fifth of the children having high SF level. This sub group was also found to have a history of poly transfusions(>3 in the last year) ,concluding that elevated SF levels that indicated iron chelation was associated with the frequency of transfusions. (31)

Table 1 summarises studies that used serum ferritin to investigate iron overload prevalence in children in various parts of the world.

Table 1 : Research work on iron overload .

STUDY DESCRIPTION		KEY FINDINGS
Country, Author, Year	Study Population Age (yrs.)	Findings
India, 2014	150 children	S.F levels 4.7-450ng/ml

STUDY DESCRIPTION		KEY FINDINGS
Country, Author, Year	Study Population Age (yrs.)	Findings
Ray (10)	with SCD 3-18.	Number of blood transfusions and serum ferritin levels were found to be significantly related.
Nigeria, 2017 Odunlade (11)	96 children with SCD 1-15	SF levels 34.2-3282ng/ml, mean of 381.2 SF levels>300ng/ml in33%.
Congo, 2019 Makulo (12)	70 children with SCD 2-18	SF levels 24-2584ng/ml SF levels >300ng/ml in 21.4% Increased SF levels in > 3 transfusions
Egypt, Saied, 2017	70 children with SCD 2-18	Hepatobiliary complications in 47% with increasing age(p=0.003) Number of blood transfusions (p=0.002) and higher SF levels p=0.0047

Histo-Pathologic Changes Seen with Iron Induced Hepatic Injury

The sequelae of hepatic iron loading initially presents as fibrotic changes in children but in older patients iron overload significantly contributes to sickle hepatopathy(16). In a review of the autopsy results of 141 patients with sickle cell disease who died in adulthood (mean age 36 $\pm$ 11 years,)16 patients (11.4%) had cirrhosis as their cause of death. Of this, 7 (43.8%) had evidence of iron overload ($p<0.001$)(17)

As assessment of hepatic iron overload was conducted on a cohort of 27 children (mean age 10.95 $\pm$ 3.34 years,) with SCD undergoing regular transfusion therapy (total duration of transfusion 50 $\pm$ 26.6 months) but not on chelation therapy. Liver biopsy was done for histologic scoring and determination of HIC. In this study 10 children were found to have liver fibrosis and 9 had lobular inflammation. Hepatic iron content was higher in biopsies with fibrosis (28.2 $\pm$ 3.8mg/g dlw), p=0.012. (18)

Vichinksy et al (19)assessed 73 patients on chronic transfusion therapy, 43 with SCD, using serial liver biopsies to assess iron overload and liver injury. This study

found high serum ferritin levels (mean 2916 ng/ml), 7% had abnormal levels of ALT (>65 IU/L) and 39% had an abnormal fibrosis score and evidence of severe iron overload using quantitative liver iron measurement. This study determined that transfusion results in hepatic injury in SCD, and the amount of hepatic iron was related to the presence of liver fibrosis (p=0.04)

Changes In Hepatic Transaminases Indicative of Iron Induced Injury Toxicity

Amino transferases are enzymes found in the liver which are released into the blood during hepatic injury. Alanine amino transferase (ALT) has been found to be more specific to the liver.

The normal values for ALT are shown in the table below:

Table 2 : Normal reference values for alanine amino transferase (ALT) by age group.

AGE GROUP	REFERENCE RANGE (IU/L)	
(YEARS)	MALE	FEMALE
0 - 6	11 - 39	10 - 32
6 - 10	12 - 34	11 - 28
10 - 20	8 - 36	8 - 29
>20	11 - 47	7 - 30

(Source: Kirsty England et al, Ranges of alanine aminotransferase levels in children (20)*European Paediatric HCV Network. Journal of Paediatric Gastroenterology and Nutrition,2009,49(1):71-7)*

Quantification of the severity of liver test abnormalities can be quantified by use of grading systems developed by the Division of AIDS (DAIDS) Table for Grading the

Severity of Paediatric Adverse Events which are similar to the guidelines provided by the clinical Trials Group (CTG).(23)

Severity of liver dysfunction can be classified into mild, moderate, severe, and potentially life threatening according to the severity of increase in serum ALT above the upper normal limit for age as shown in the table below.

Table 3 : **Elevation of serum Alanine transferase.**

Grade 0	Normal	<1.25 ULN
Grade 1	Mild	1.25 to <2.5 x ULN
Grade 2	Moderate	2.5 to <5.0 x ULN
Grade 3	Severe	5.0 to <10.0 x ULN
Grade 4	Potentially life threatening	>10.0 x ULN

(Source : Grading Table Working Group;(24) *National Institute of Allergy and Infectious Diseases, National Institutes of Health , US Department of Health and Human Services; Grading of Paediatric Adverse Events, 2017)*

Prieto et al studied the relationship between serum ferritin and ALT levels and found a linear correlation between SF and ALT (N=20, r=0.73) in patients who had iron overload and clinical characteristics of liver disease.(21)

Adamkiewicz et al (22) utilized the patients enrolled in the landmark Stroke Prevention Trial in sickle cell anaemia(STOP and STOP 2) (3) trials to evaluate the measures of iron overload in the children on routine transfusion therapy. During the course of these trials (N= 271, average age 8.5 $\pm$3.4 years) serial assessments were made of serum ferritin (SF), serum alanine amino transferases (ALT), and iron content in the liver. Mean LIC was found to be 13.6mg/gdlw, with a range of 1.8-41.3. There was a correlation between serum ferritin and transfusional iron load, (r=0.55) with all measurements of SF of 4000ng/ml or more corresponding with LIC of 10mg/g dlw. SF measurement was also useful in the assessment of liver injury. Fifty-seven percent (57%) of children had at least 1 ALT measurement of 40IU/L or more. The study found a correlation (r=0.53) between SF and ALT; with peak ALT

elevation (40-329IU/L) occurring on average after a mean 43 transfusions. In this study, SF levels were associated with ALT p=0.025 and liver iron content (LIC) p=0.006.

The relationship between SF and ALT levels is shown in Figure 2 below.

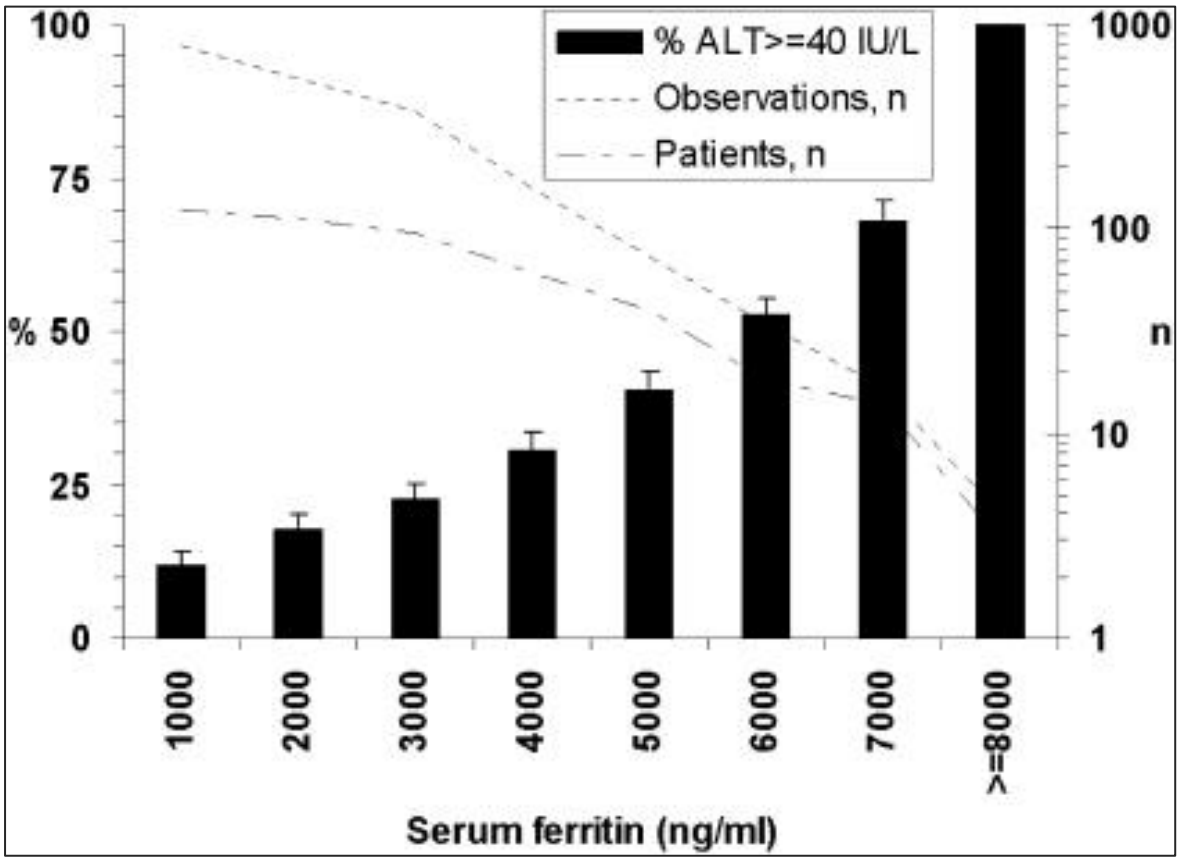

FIGURE 2*:* Rise of serum alanine transaminase levels with increase in Serum Ferritin

Association between serum ferritin and serum alanine transaminase levels as assessed in 271 children aged 2-16 years (average 8.5 $\pm$3.4) with sickle cell disease undergoing episodic blood transfusions. This was a randomised, phase 111 multicentre trial that ran from 1994-2000.

(*Source: Adamkiewicz et al, Serum ferritin level changes in children with sickle cell disease on chronic blood transfusions are nonlinear and associated with iron load and liver injury* (22) *Blood (2019) 114 (21)4032-8)*

Assessment of Iron Overload

Different parameters have been used to approximate body iron load. These tests are also useful in establishing the need for chelation. Furthermore, an accurate assessment of total body iron requires a combination of methods. The various methods which have been used to estimate body iron status include the serum ferritin assay, measures of transferrin iron binding capacity, magnetic resonance imaging (MRI) of the liver, and liver biopsy.

These methods are summarised in the table below:

Table 4 : Methods of assessment of total body iron.

Investigation	Advantages	Disadvantages
1. Serum Ferritin Assay	• Least costly • Easy to measure. • identifies trends	• Acute phase reactant.
2. Transferrin iron binding capacity (TIBC)	• Consistent with iron overload in adults	• Affected by diet and comorbidities
3. MRI (T2*) of liver	• Well-validated predictor of HIC • Non-invasive	• Costly • Not widely available
4. Liver biopsy	• Accurate • Gold standard	• Invasive. • risk complications

Use Of Serum Ferritin Levels to Assess Iron Overload

Serum ferritin is a simple widely available laboratory test useful in monitoring, identification and assessment of iron overload in patients undergoing blood transfusion especially where resources are inadequate.(5)

Other than intracellular ferritin, plasma also contains limited amounts of ferritin, the amount of which compare to the body store. With high body iron load, there is an enhanced overflow from the liver, coupled with increased seepage of hepatic enzymes. It has a sensitivity and specificity of 60-80% and the correlation with iron

storage is reliable up to 3,000-4,000 ng/mg. Normal SF concentration is normally in the range of 25-300ng/ml.(27) Serum ferritin, as an acute phase reactant protein increases during infection or inflammation. Studies have however demonstrated that ferritin concentrations reach a maximum level after 3 -7 days and gradually return to normal over a period of 10 days. Furthermore, the increments are relatively small, averaging 20ng/ml every 24 hours.(28)

Table 5: Defining age and sex specific reference values for serum ferritin levels.

Age group	Reference interval	
	Male	Female
10 days to < 1 year	9-139	10-271
>1 year to < 3 years	6-9	7-89
>3 years to 6 years	12-74	12-81
>6 years to 10 years	15-87	13-109
>10 years	18-270	18-160

(Source: Parkin PC et al,(29) *Laboratory Reference internals in the assessment of iron status BMJ Paediatrics, 2017 (1)e000074)*

Brittenham et al(5) examined found that the correlation between serum ferritin levels and hepatic iron stores was significant ($R=0.75$, $p<0.0001$). (5). Therefore, although the gold standard for assessment of body iron status is by hepatic magnetic resonance imaging (MRI) serum ferritin has been revealed to be an accurate indicator of body iron.

Table 6: Definition of iron elevation using serum ferritin level.

Degree of iron elevation	Ferritin ng/ml	Liver iron concentration mg/g dlw
Normal	25-300	0.8-1.5
Low elevation	300-800	1.5-3.5
Moderate elevation	800-1700	3.5-5.0
High elevation	1700-2500	5.0-8.0

Severe elevation	>2500	10-20

(Source: Coates DT, Wood JC (30)*How we manage iron overload in sickle cell patients, BJH (2017)177, 703-716)*

Other Methods of Measurement of Iron Overload

Magnetic resonance imaging (MRI) is the best available technique for examining in three dimensions the iron content of the body as multi- echo gradient sequences of the whole liver can be examined. Measurements are however qualitative, and bio magnetic spectrometry is required for quantitative measurements. Though it is non-invasive, it is costly and not widely available.

Total iron binding capacity is also known as serum iron to transferrin iron binding capacity. It has been found to correlate with serum iron content, especially in adults. In children, it has the major drawback of elevation in absence of iron overload and is unduly affected by diet and illness.

The liver biopsy is the gold standard of accurate assessment of hepatic iron content. It allows for histochemical determination of cellular iron distribution and pathological examination of liver injury. It is however limited in the presence of severe liver disease such as cirrhosis or fibrosis. It also runs the risk of sampling error. Furthermore, it is invasive and poses the risk of complications arising from infection and bleeding. (6)

Treatment of Iron Overload

Chelation is the process of administering a pharmaceutical agent to prevent or reverse the toxicity of iron on the cell and facilitate its excretion from the body. Chelation eliminates the non-transferrin bound iron (NTBI) and labile plasma iron, which are the toxic forms of iron, from the body.(30)

Iron chelation results in an increase of urinary and biliary excretion of iron, decrease in serum ferritin levels and normalization in plasma ALT levels (31). Accordingly , iron depletion strategies have been shown to be effective in reducing the

progression of liver disease and resultant complications including cirrhosis and portal hypertension (32)

There are currently three available chelators, Deferoxamine, given as daily subcutaneous injection, and Deferasirox and Deferiprone which are oral.

In our setting, Deferasirox is available as 400 mg tablets and is given at a dose of 20mg/kg for one month. Use of chelating agents is associated with gastrointestinal side effects such as diarrhoea, nausea and vomiting and abdominal pain. Systemic effects include liver dysfunction, haematological disturbances, growth delay and renal failure including a reversible increase in creatinine which necessitates frequent monitoring of renal function.

Use of iron chelation has led to a decrease in the mortality and morbidity associated with iron overload.(33) Control of transfusional iron overload is also possible through sequential phlebotomy and regular exchange transfusion.

The clinical indications and laboratory parameters for commencement of chelation therapy are as shown in table 7 below:

Table 7 : Indications for chelation therapy

Parameters	When to give preventive therapy	When to give treatment
Transfusional iron load	1-2 years of chronic transfusion therapy (10-12 transfusion) 120-200ml of transfused RBCs /kg	When a threshold of >200ml/kg of transfused RBCs/kg is reached.
Serum ferritin	To maintain serum ferritin <1000ng/ml target	>1500ng/ml
Hepatic iron content	>7mg/g dry weight	>7-9mg/g dry weight

(Adapted from Salem Ahmed.(34) *Medical and Surgical Complications of Sickle Cell Anaemia, Springer (2016) 328-332)*

CHAPTER 2: STUDY JUSTIFICATION, RESEARCH QUESTIONS AND STUDY OBJECTIVES

Study Justification

Children and adolescents with SCD frequently undergo repeated blood transfusions as a way of countering the various complications of the disease. This however exposes them to the risk of excessive body iron. Iron overload has pathologic effects especially to the liver contributing to further morbidity.

Monitoring the accumulation of iron in SCD is useful to identify the patients at risk of accumulating excess iron, however, this is rarely done in our setting. Serial estimations of iron status are useful to enable timely preventive therapy in children with SCD and treatment of iron overload to minimize progressive organ damage. Serum ferritin is an available, non-invasive cost effective widely accepted marker of body iron status.

This study researches the extent to which children with sickle cell disease have excessive iron body stores by laboratory estimation of the amount of ferritin present in serum. Currently, there is paucity of evidence on the magnitude of iron overload and its associated effects among children in long term care in our setting.

Data obtained from the study will be useful to guide the need for monitoring of iron levels and propose if there is a need for policies into the prevention and treatment of iron overload.

KNH was chosen as a study site because of its accessibility to the principal investigator. It also has a busy specialised Paediatric Haematology clinic which runs on a specific day and time of the week, allowing it to cater to the needs of several children with SCD. Furthermore, the children who attend this clinic do so regularly and have their disease needs met here. As a result, the data from most of the children's records was detailed, robust and encompassing their journey with SCD.

Research Question

What is the magnitude of, and factors associated with iron overload in children aged 1-18 years with sickle cell disease receiving care at Kenyatta National Hospital?

Study Objectives:

Primary objective

1. To determine the prevalence of iron overload as measured by serum ferritin levels among children aged 1-18 years with sickle cell disease receiving care in Kenyatta National Hospital.

Secondary objectives:

1. To determine the association between blood transfusions and serum ferritin levels in children with sickle cell disease at Kenyatta National Hospital.
2. To evaluate the association between serum ferritin levels and liver function in children with sickle cell disease at Kenyatta National Hospital.

CHAPTER 3: RESEARCH METHODS

Study Design

This is an observational cross-sectional study.

This design was chosen because it is not costly to perform, does not take a lot of time and would allow for calculation of prevalence.

The study was carried out between June 2021 to October 2021.

Study Setting

The study was carried out in Kenyatta National Tertiary and Referral Hospital, the teaching hospital of the University of Nairobi. KNH is situated in Nairobi County and serves as a national referral centre. It has an inpatient capacity of 2000 and annual patient turnover of approximately 600,000 patients.

The haematology Clinic is run every Monday and Tuesday at the Out-Patient clinics. Approximately 500 children are reviewed every month. The clinic is involved in the management and follow up of patients with different haematological ailments such as aplastic anaemia and sickle cell disease, haematological malignancies such as the various forms of leukaemia, and bleeding and coagulation defects such as haemophilia. The medical personnel running the clinic include consultant haematologists, consultant paediatric haematologists, pathologists, radio-oncologists as well as residents, clinical officers, and nurses. There is also a support team composed of nutritionists, palliative- oncology team, counselling, and psychosocial care. The patient's records are stored in the Health Information Records Office in hard copy and physically availed on the day of appointment.

Study Population

The study population are children aged 1-18 years with sickle cell disease who attend the Haematology Clinic at Kenyatta National Hospital and meet the inclusion criteria.

Inclusion Criteria

- ✓ All children aged 1-18 years with sickle cell disease.
- ✓ Receiving care at KNH
- ✓ Have given consent
- ✓ Additionally, assent has been obtained for those aged >7 years.

Exclusion Criteria

- ✓ Recent sickle cell crises in preceding 4 weeks.
- ✓ Exacerbation or acute illness on day of screening.

Children who have a history of recent sickle cell crisis or acute illness were excluded to limit the impact of inflammation, haemolysis, infection or recent transfusion on serum ferritin.

Case Definition

A child was defined as having sickle cell disease based on presence of any one of the following: identification of Haemoglobin SS through Hb electrophoresis, or a peripheral blood film showing sickle cells or a positive sickling test.

Key Outcomes of Interest

- **Iron overload** is defined as elevated serum ferritin levels >300ng/ml.
- **Severity of iron overload** is classified as follows:
  - ✓ Low elevation >300-800ng/ml
  - ✓ Moderate elevation >800-1700ng/ml
  - ✓ High elevation >1700-2500ng/ml
  - ✓ Severe elevation > 2500ng/ml

- **Normal liver function** is defined as serum alanine transaminase within the normal range by age group as follows:

Table 8 : Normal liver function as designated by serum alanine transferase

AGE GROUP	REFERENCE RANGE (IU/L)	
(YEARS)	MALE	FEMALE

0-6	11-39	10-32
6-10	12-34	11-28
10-20	8-36	8-29

- **Severity of liver dysfunction** is defined according to severity of increase in serum ALT level as follows:

  - ✓ Grade 1 (mild dysfunction) is ALT of 1.25-2.5 times the upper normal limit for age.
  - ✓ Grade 2 (moderate liver dysfunction) is ALT of 2.5-5 times the upper normal limit for age.
  - ✓ Grade 3 (Severe liver dysfunction) is ALT of 5.0-10 times the upper normal limit for age.
  - ✓ Grade 4 (potentially life-threatening dysfunction) is ALT level >10 times the upper normal limit for age.

- **Number and volume of Blood Transfusion**

One unit is defined as 200 ml of packed red blood cells or 525 ml of whole blood.
Information on this variable was sought up to a maximum of 3 preceding years.
The methods that were used to obtain blood transfusion data were:

- ✓ Parent recall, teenager (patient) recall (up to 3 years recall),
- ✓ Review of patient medical records for documentation of all transfusions within the previous 3 years

Sample Size Calculation

Sample size calculation is based on the first objective (prevalence of iron overload as measured by serum ferritin).
Fischer's formula is used with the following assumption:

Prevalence (p) estimate is drawn from a similar study in Nigeria by Odunlade et al where they found a prevalence of iron overload as defined by elevated serum ferritin levels (>300ng/ml) in children with SCD at 33%.

$$n = \frac{Z\alpha^2\, p\,(1-p)}{d^2}$$

n = estimated minimum sample size

$Z\alpha$ = 1.96, with level of significance set at 95% confidence level (two sided)

P = 0.33

d = 5% precision.

$$n = \frac{1.96*1.96*0.33(1-0.33)}{0.05*0.05}$$

Minimal required sample size =138

To cater for possible missing key data for some children, an additional 5% was added to make a sample size of 145.

Sampling Technique

Consecutive sampling was conducted. All eligible children who present to the Haematology clinic were enrolled consecutively.

Study Procedure

Patient recruitment

All children aged 1-18 years with sickle cell disease presenting to the Haematology clinic were screened for eligibility. The principal investigator explained to the participants the procedure and ensured comprehension before proceeding, as well as answering any questions.

Informed written consent of the caregivers or parents, describing the objective of the study, the process, potential benefits, and risks as well as assent from a child aged more than 7 years was sought. There was no coercion and patients who did not enrol in the study were still attended to. Eligible patients were then enrolled, and study number attributed to each.

Clinical evaluation of patient

A detailed history demonstrating demographic characteristics, including age, sex, and education status; as well as drug and transfusion history was obtained from the patient and recorded in a standardized hard copy case record form. History of blood transfusion was obtained from patient recall and review of the patient's records. A period of 3 years prior to the period of study was chosen to enable the assessment of the effect of blood transfusions on the serum ferritin level. Three years was chosen in consideration of the patient's or caregivers recall ability. Where the child's companion was not conversant with the blood transfusion history, the care giver's telephone number was obtained and contacted at a later time. In this study ten (10) children were accompanied by caregivers not conversant with the transfusion history. Seven were contacted by telephone and three were able to avail themselves to the clinic in the next month's appointment.

Medical Record Data Abstraction

The patient's hard copy records were perused to obtain the blood transfusion history. The data abstracted from the files was date of commencement of blood transfusion, number and date of blood transfusions received as well as the volume of blood transfused (ml/kg). The Haematology Outpatient department uses the same file for out-patient visits as well as when the patient is hospitalised (in-patient). These files are then stored in the records department until the patient presents to the hospital again, either as an in-patient or as an outpatient in the Haematology or out-patient clinic.

To enable assessment of the relationship between serum ferritin and blood transfusions, the number of blood transfusions received in the prior 3 years was also obtained.

Procedure for Blood Specimen collection and handling

3 ml of venous blood from an accessible peripheral vein was drawn using aseptic technique from the patient and put into a plain vacutainer tube for laboratory

assessment of serum ferritin (SF) and serum alanine aminotransferase (ALT) levels. This procedure was explained to the study participants and consideration for their safety and comfort undertaken. The vacutainers were labelled and put into a cooler box. Samples were transported to the laboratory within 1 hour of collection. The time of obtaining samples has no effect on the results. The laboratory samples were given a special identification number and initials used to maintain the patient's confidentiality. Samples were then delivered to the UoN Paediatric Department Laboratory within an hour of collection. Each specimen was accompanied by a laboratory request form also with the initials of the patient and a specific study identification number.

Study procedures (recruitment, clinical evaluation, abstraction from files and obtaining blood samples) was carried out by the principal investigator.

Laboratory Procedures for serum ferritin assay

The serum ferritin assay was performed using the LIAISON^{R} analyser machine. In the laboratory, the samples were centrifuged and refrigerated at 2-8 ^{0}C for at most 72 hours or at room temperature for not more than 24h as they awaited analysis. The method which is used for the determination of ferritin is a sandwich chemiluminescence immunoassay technique. The laboratory personnel then issued a printout of the results.

Laboratory Procedures measuring serum alanine transferase levels.

Serum aminotransferase (ALT) was run on the HumaStar600^{R} machine which utilizes a kinetic method to measure ALT activity in serum.

The samples upon delivery to the laboratory were centrifuged and refrigerated to +4^{0}C for storage before analysis and prior to analysis thorough mixing with a Vortex mixture done. The loss of activity within 3 days is ~ 10% at this temperature. The reagents are ready to use and were directly applied to the analyser. Calibration and quality control were employed prior to the first run. The laboratory subsequently issued a printout of the results obtained.

All the laboratory reports were transferred to the case record form and a hard copy sent to the patient's file. Where an elevated serum ferritin was found, the patient was flagged for follow-up and further evaluation by the consulting physician.

Quality control in the UON Paediatric Laboratory is assured by the presence of experienced technicians and adherence to the laboratory protocols as concerns timely centrifuging, prompt separation, storage at recommended temperature and availability of results in timely manner. The UON Laboratory also runs daily quality control on the tests and carries out daily calibration of their machines.
Additionally, all samples are properly labelled and accompanied by a correctly filled in laboratory request form.

The diagram below summarizes the study procedure employed:

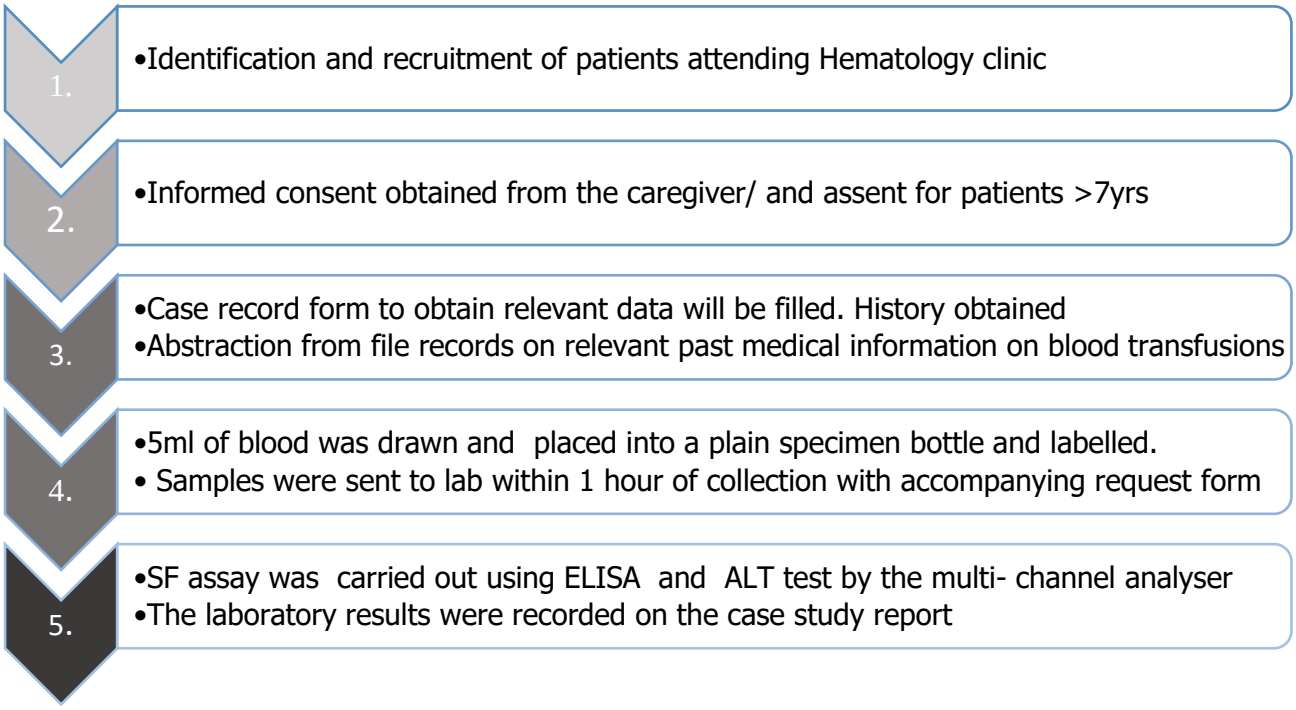

FIGURE 3 : Study procedure indicating patient and laboratory samples flow.

Study Instruments:

The Case Record Form

This was used to record the participants' demographic characteristics, including age, sex, and education status, as well as drug and transfusion history. This standardised form was also used to record the history of blood transfusion as obtained from patient recall and a review of the patient's records. The laboratory results obtained (serum ferritin assay results and serum alanine transaminase levels) were also transcribed into each participant's case record form.

Data Management

The data collected from the patient was entered into a case record form. The hard copies were stored securely until the end of the data collection period. Cleaned data was typed into Microsoft Excel and stored in a hard drive which was secured with a password and kept safely.

The collected data was computed by utilizing SPSS version 23.

Data Analysis

Descriptive analysis for the study population (measures of central tendency and dispersion) were computed. Descriptive data was presented using bar graphs and tables.

Comparative analysis was carried out for the grouped data as percentages and chi square test applied. Means or medians computation for continuous variables was done (e.g. - mean age of the participants). Frequencies and proportions were described for categorical variables. The association between level of serum ferritin and age, number of blood transfusion and liver function test (ALT level) was defined. Statistical significance was defined by a p value of < 0.05.

- Prevalence of iron overload was computed by number of children with elevated SF level as the numerator and with the total no. children as denominator and converted to percentage.
- Association between blood transfusions which is the exposure and serum ferritin levels as the outcome was determined. The total number of blood transfusions as a continuous variable was compared with the presence of overload. Blood transfusion history within the previous 3 years was used to

assess the relationship between measured serum ferritin level and the effect of blood of blood transfusion. The Mann Whitney U Test. which compares medians between the two outcome groups as well as binary logistic regression was used.

- Association between SF as continuous variable and outcome of liver tests as normal/abnormal was assessed by use of Mann Whitney U Test comparing the median ferritin levels in each outcome group.

Ethical Considerations.

Approval to proceed with this study was sought from the KNH-UoN- Ethics and Research Committee (KNH-UoN ERC) as well as from the KNH administration. Only once permission was formally granted did the study proceed as stipulated in the study procedure protocol.

To ensure autonomy, informed parental consent, and assent for children more than 7 years in age was obtained. The principal investigator explained the objective of the study, the procedure, benefits and risk to the parents or guardians together with their children as well as answered any pertinent questions as concerns the study. All participation in the study was voluntary and there was no coercion.

Participants who decline to participate in the study were availed the usual standard of care.

Non maleficence was ensured by conducting all the procedures in a safe and sterile environment. As the procedure for drawing blood may be uncomfortable utmost care was taken to assure the patient, minimise discomfort and ensure safety. Covid-19 precautions were also adhered to. At all times the PI was gloved, had adequate PPE on, washed hands before and after attending to the patients and ensured procedures were carried out in an airy room.

Patients were assured of confidentiality, and this was strictly adhered to. The study only used the patient's initials and a specific study identification number on all records involving the patient including the laboratory specimens and forms.

To ensure justice, all children attending the haematology clinic and fulfilled the inclusion criteria had a fair chance of participating in the study as consecutive sampling was carried out. A copy of the results was sent to the patient's file to form part of the management plan. It was discussed with the clinicians that children who were found to have high levels of serum ferritin would be evaluated further by the haematologists.

Study Dissemination Plan

Study findings will be disseminated to the relevant authority at KNH as well as presentation of findings as part of the thesis defence to the Department of Paediatrics, University of Nairobi in both hard and soft copies.

Control of Errors and Biases

The following measures were undertaken to reduce occurrence of bias and errors.

- Participant enrolment was done using consecutive sampling on all eligible patients from the minute the clinic opened so as to reduce sampling bias.
- Standard case record form was used on every study participant to ensure uniformity and standardization.
- The principal investigator ensured validity of collected data and accurate transcription of the laboratory reports by utilizing a cross checking method.
- Thorough perusal of the patients records was done as it created an objective record of history of past transfusions, and was an attempt to overcome recall bias as pertains to history of blood transfusion .
- Efforts to obtain data on blood transfusion for transfusions obtained outside of KNH involved specifically inquiring of the same from the parent and relied on patient recall.

CHAPTER 4: RESULTS

We screened all the 203 children who attended the Haematology Clinic at KNH during the period June 2021 to October 2021. At screening , 34 were not eligible due to recent sickling crisis (n=25), or current acute illness (n=9). This left 169 eligible subjects, and after explaining the study to their caregivers, 154 were willing to participate (15 declined). We obtained consent from 154 participants and enrolled them into the study (as shown in figure 4 below). Medical record abstraction was done for all, clinical assessment, and blood samples obtained from all 154 participants. Of the samples sent to the laboratory, 9 were rejected for quality reasons, leaving 145 as the sample size.

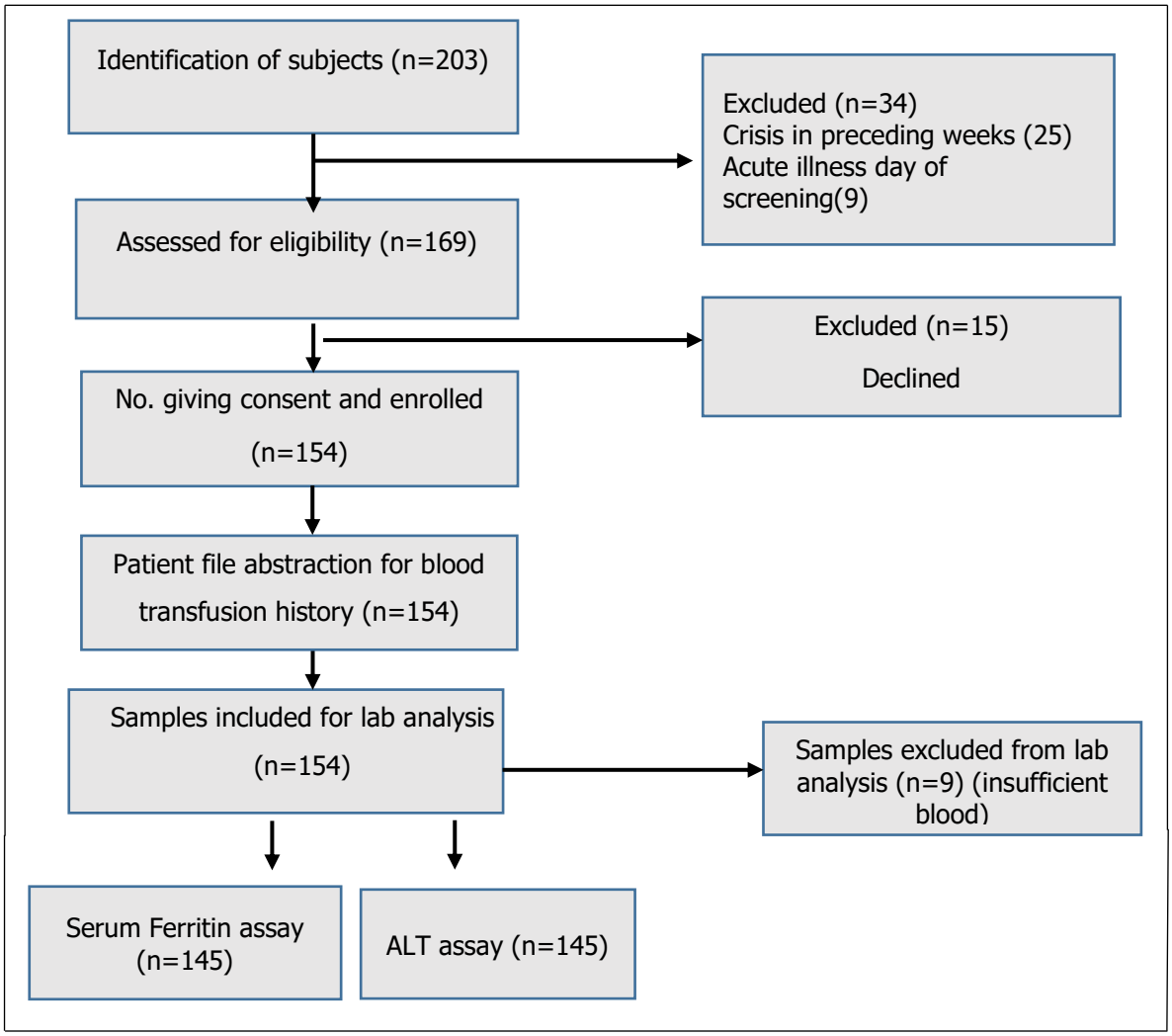

Figure 4 : **Study screening and enrolment procedure**

Socio- Demographic Characteristics of the study population

There were 86 males while the females were 59. The median age of the population was 8 years, the 25th centile being 4 years and the 75th centile 11 years. The largest age group encountered was the 0 - 4-year-olds (36%). Forty-four percent (44%) of the children attend primary school and there was none at the tertiary level of education. The median duration of care in KNH was 7 years (25th centile was 2 years and the 75th centile was 10 years.) Furthermore, 64.1% (93) children had been under treatment for sickle cell disease for more than 5 years. The socio-demographic characteristics of the study population are shown in Table 9 and Figure 5 below.

Table 9 : **Socio-demographic characteristics of the study population. (N=145)**

Characteristic	Detail	Frequency Or Median	Percent Or IQR*
Sex	Male	86	41
	Female	59	59
Median age		8	4, 11
Age group in years	0-4	52	36
	5-9	48	33
	11-14	27	19
	15 – 18	18	12
Education	Tertiary	0	0
	Secondary	18	13
	Primary	64	44
	Pre-school	63	43
Duration in care at KNH(yrs)	Median	7	2, 10
Years in care	<3	37	26
	3-5	15	10
	>5	93	64

*IQR = interquartile range, or 25th, 75th centile.

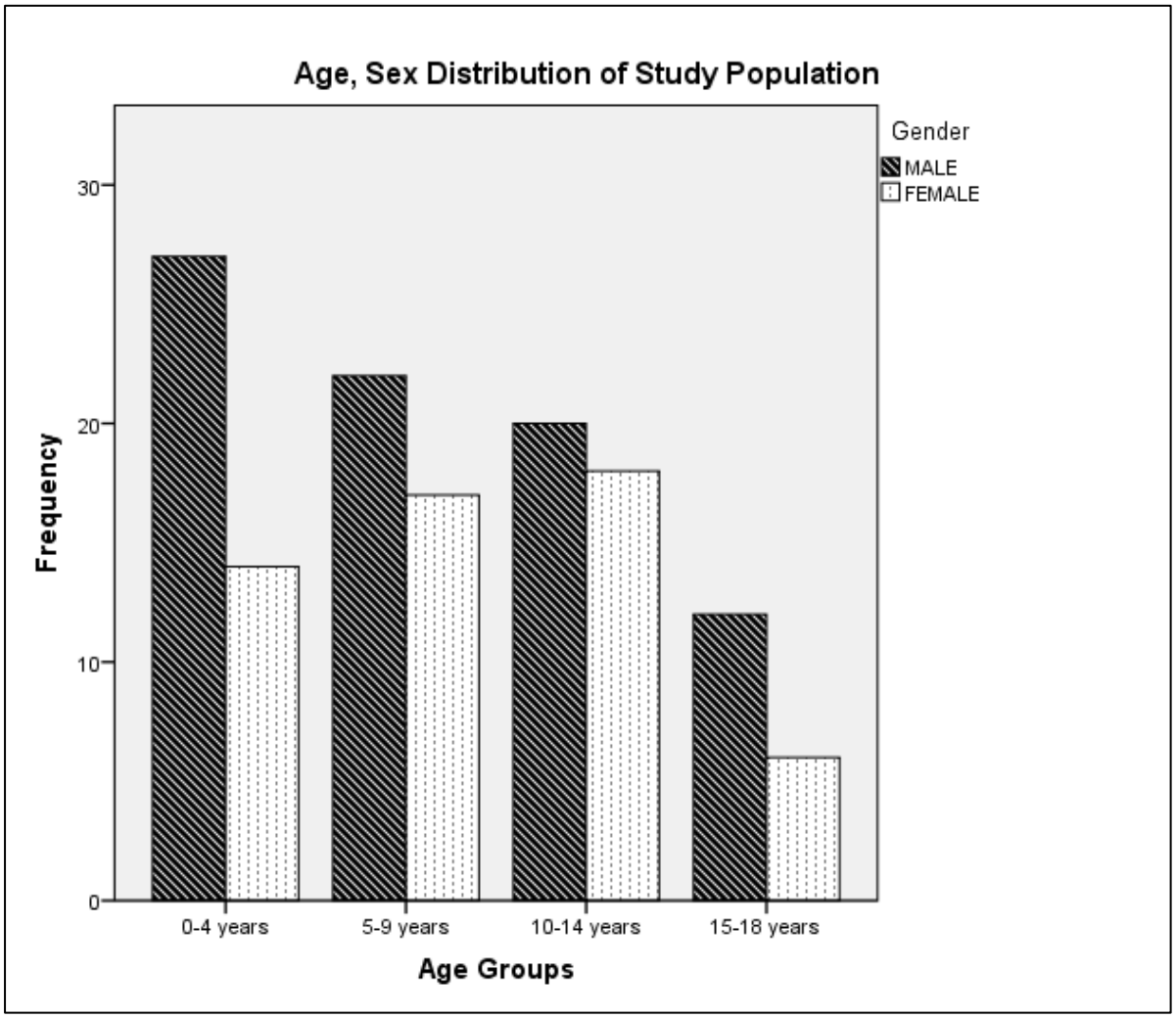

Figure 5 : **The age and sex distribution of the study population**

Treatment received by the study population

Majority of the patients were on folate (99.3%), hydroxyurea (97.9%) and Penicillin V (phenoxymethylpenicillin) (97.9%) as part of their treatment plan. Only 1 patient was taking iron and 4 patients (2.8%) were on analgesic drugs. Nearly all (86.2%) of the patients received blood transfusions in the previous three years. Only 20 children, representing 13.8% of the study population did not receive a blood transfusion (Table 8)

Table 10 : Treatment Received by the Study Population. (N = 145)

Characteristic	Detail	No. of children	Percent (%)
Medication	Folate	144	99
	Penicillin V	142	98
	Hydroxyurea	142	98
	Iron	1	1
	Analgesic	4	3
Blood Transfusion in past 3 years	Yes	125	87
	No	20	14

Clinical characteristics of the study population

The maximum number of blood transfusions received was 7 while the minimum was 0. The median was 2 transfusions, 25th centile was 1 and 75th centile was 3.

The minimum serum ferritin levels were 10ng/ml whereas the maximum level was 3070ng/ml. The median serum ferritin was 489ng/ml with 199 being the 25th centile and 1051 being the 75th centile, with 95% CI of 407-584IU/L.

The minimum level of serum alanine transferase was 9 IU/L whereas the maximum level was 205 IU/L . The median level was 24IU/L, with 25th centile being 17 IU/L and 32IU/L as the 75th centile. The 95% CI was 21-26IU/L.

Table 11:Clinical characteristics of the study population

N=145	Serum ferritin levels (ng/ml)	Frequency of Blood transfusions	Serum ALT levels IU/L
Minimum	10	0	9
Maximum	3070	7	205
Median	489	2	24
25th Percentile	199	1	17
75th Percentile	1051	3	32
95%CI	407-584	2-2	21-26

Blood Transfusion History of The Study Population

Twenty children (13.8%) had not received any transfusions in the prior three years, whereas 40 children (27.6%) had received only one transfusion. The highest number of transfusions received was 7 in 1.4% of the study subjects, as depicted in Table 12 and Figure 6 below.

Table 12: Frequency of transfusions in the study subjects in the preceding 3 years

No of transfusions in past 3 years	No. of children Or Median	Percent Or IQR
0	20	14
1	40	28
2	34	23
3	27	19
4	12	8
5	7	5
6 or more	5	3
Median No. transfusions	2	1, 3,

*IQR = interquartile range, or 25th, 75th centile.

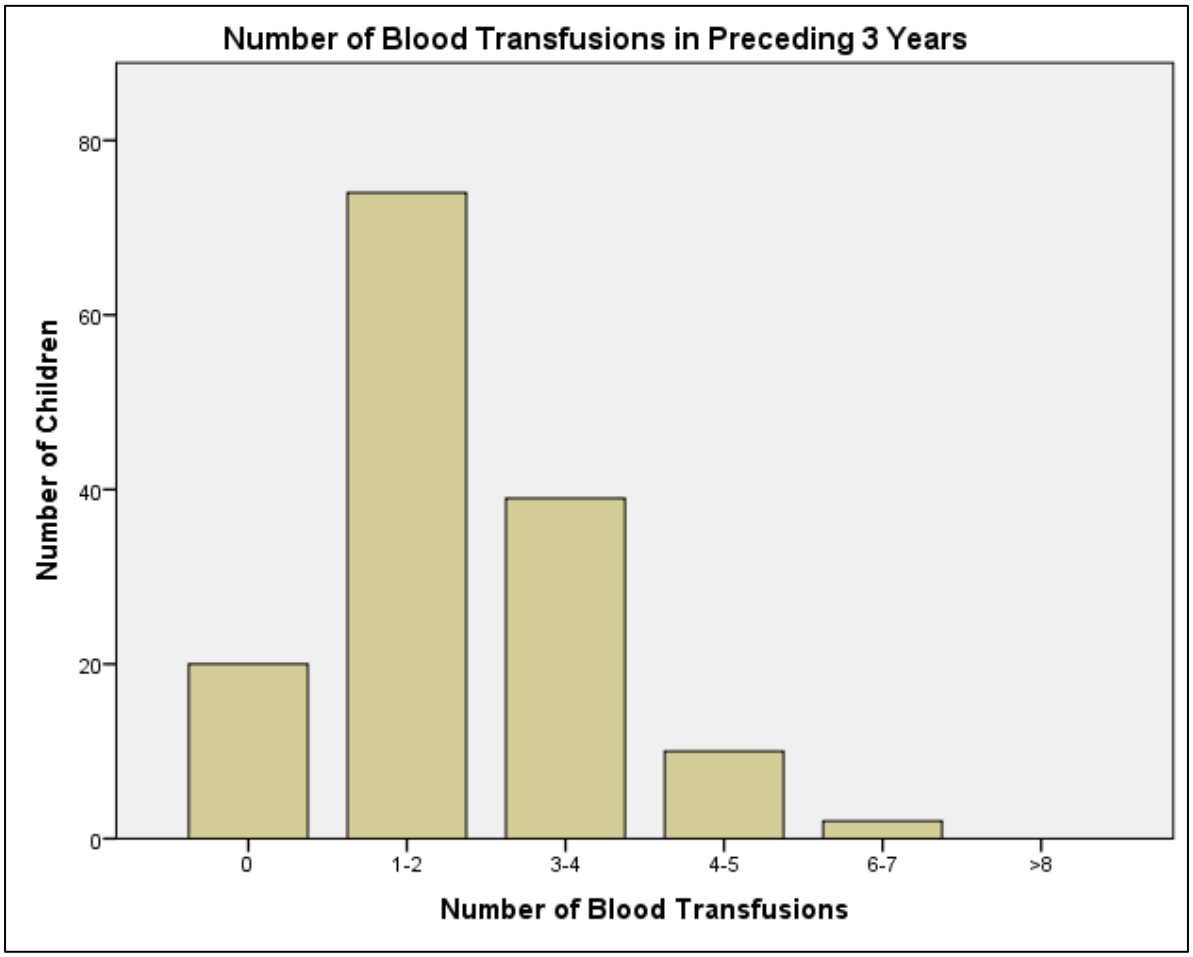

Figure 6 Number of blood transfusions in children in the preceding 3 years

Prevalence of Iron Overload in Children with SCD

Serum ferritin levels above the normal reference range (300ng/ml) were found in 93 (64%) of 145 children whereas 52 (36%) children had normal serum ferritin levels. The prevalence of iron overload can therefore be estimated at 64% (95% CI for this estimated prevalence is 407-584ng/ml) as shown in Table 13 below.

Elevated serum ferritin levels have been further categorised into low elevation (300-800ng/ml), moderate elevation 800-1700ng/ml), high elevation (1700-2500ng/ml) and severe elevation (>2500). 36 % of the population was found to have normal levels.

Table 13 : Prevalence and severity of iron overload as measured by serum Ferritin in children with sickle cell disease

	Serum Ferritin in ng/mL	**No. of children**	**Percent**
Normal Se Ferritin	Normal Iron Levels (< 300)	52	36
Elevated Se Ferritin	Low elevation: 300-800	49	39
	Moderate: 801-1700	27	19
	High: 1701-2500	6	4
	Severe: >2500	11	8
	Total with elevated Se Ferritin	*93*	*64*

Severe iron elevation in this population was only found in 11 patients (8%) and 6 patients had high elevation (4%) Majority of the iron elevation in this population was found to range in the low to moderate elevation, with 27 patients (19%) having moderated elevation and 49 patients (39%) having low elevation. (Figure 7)

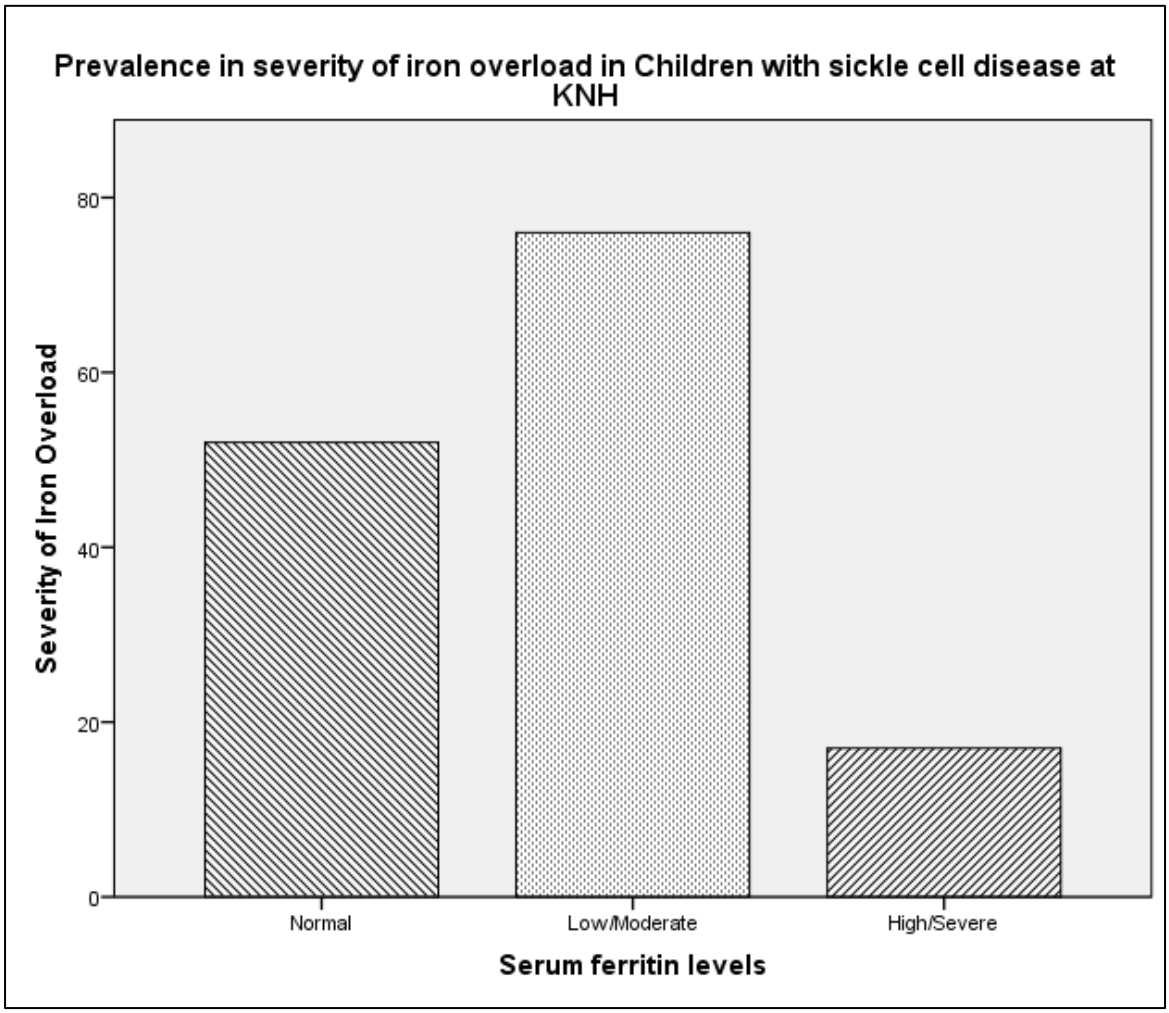

Figure 7 : Prevalence and severity of iron overload in children with sickle cell disease at KNH

Association Between Blood Transfusions and Serum Ferritin Levels.

We conducted binary logistic regression of blood transfusion against normal versus elevated serum ferritin. The p-value was <0.01 at 5% significance level which indicates that there was a significant statistical association between blood transfusion and serum ferritin. The Odds Ratio (OR) was 1.8 (95% CI 1.38, 2.54) which reveals that each additional unit of blood transfused conferred a 1.82-fold increased odds of elevated serum ferritin (or increased the odds of elevated serum ferritin by 82%).

The relationship between the number of blood transfusions and serum ferritin levels can be expressed in the form of a scatter diagram as below. Linear Regression was used to calculate the line of best fit, with the number of blood transfusions received as the independent variable and the serum ferritin level as the dependent variable. A positive relationship is found, as the number of transfusions received increases, the serum ferritin levels also tend to increase as shown in Figure 8 below.

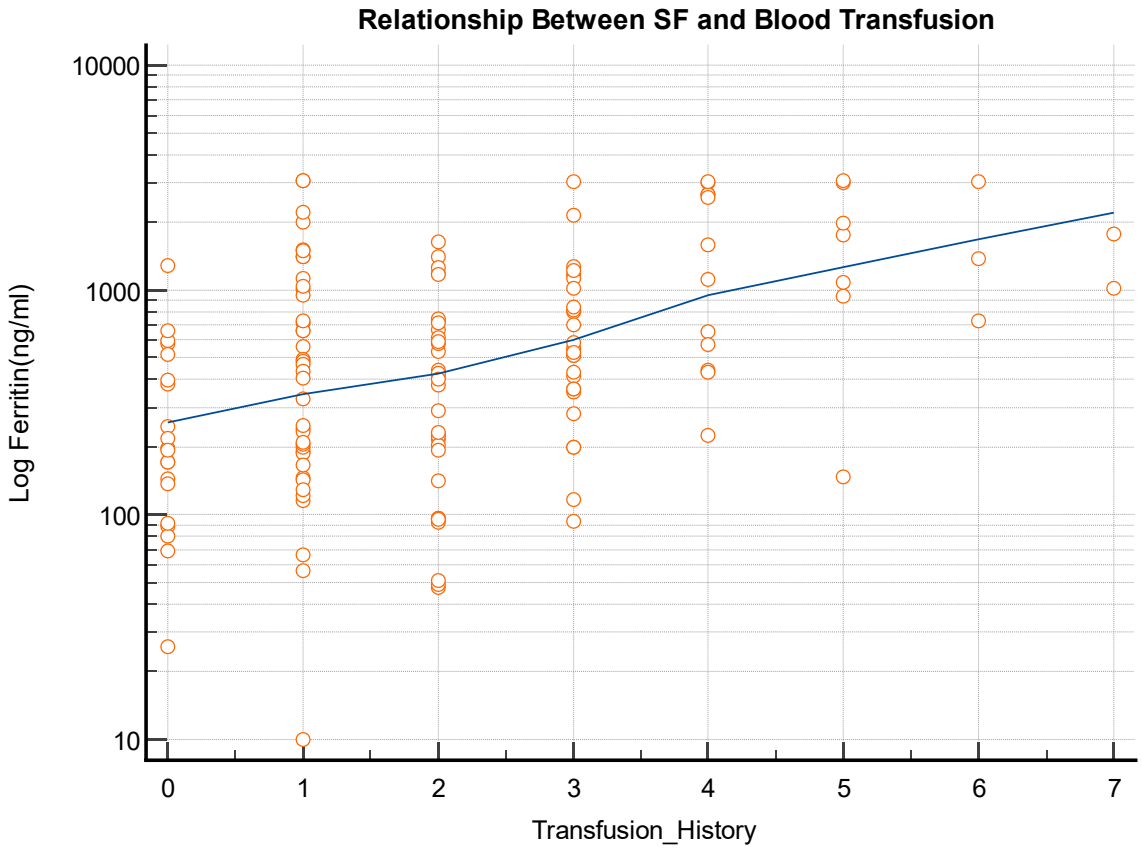

Figure 8 : Relationship between level of serum transferase and blood transfusion in children with sickle cell disease in KNH

Difference In Median number of Blood Transfusions Between Normal Vs. Mild-Moderately elevated Ferritin Levels

Mann-Whitney test was used to compare median number of blood transfusions among those who have normal versus mild-mod elevated ferritin.

A comparison of median transfusions between the children with normal serum ferritin levels vs. mild to moderately elevated serum ferritin showed significant difference (median 1.0 vs 2.0 respectively, p-value <0.01). (Figure 14)

Association between Serum Ferritin Levels (normal vs. Moderate) and Number of Transfusions

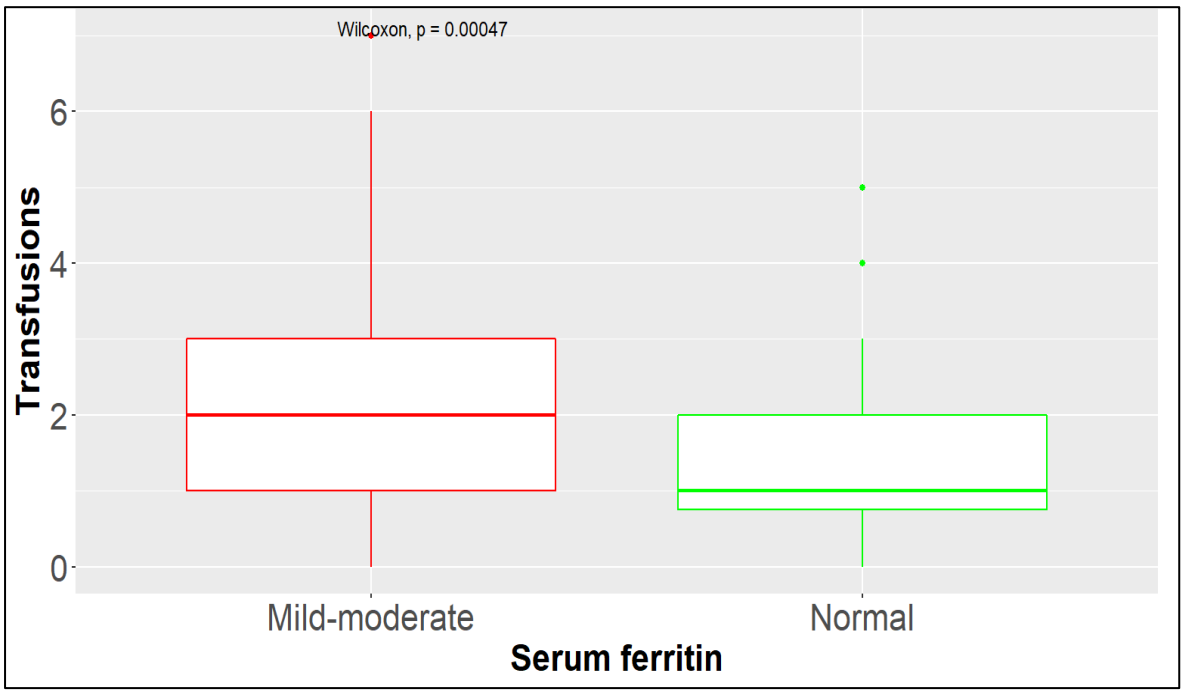

Figure 9: Box plots for difference in median transfusions between children with normal vs. mild-moderate serum ferritin levels.

Difference In Median number of Blood Transfusions Between Normal Vs. High-Severe Ferritin Levels

There was a significant difference in median transfusions between children with normal ferritin levels vs. high-severely elevated serum ferritin levels, (median 1.0 vs 4.0 respectively, (p-value <0.01) (Figure 15).

Association between Serum Ferritin levels(Normal vs. Severe) and number of transfusions:

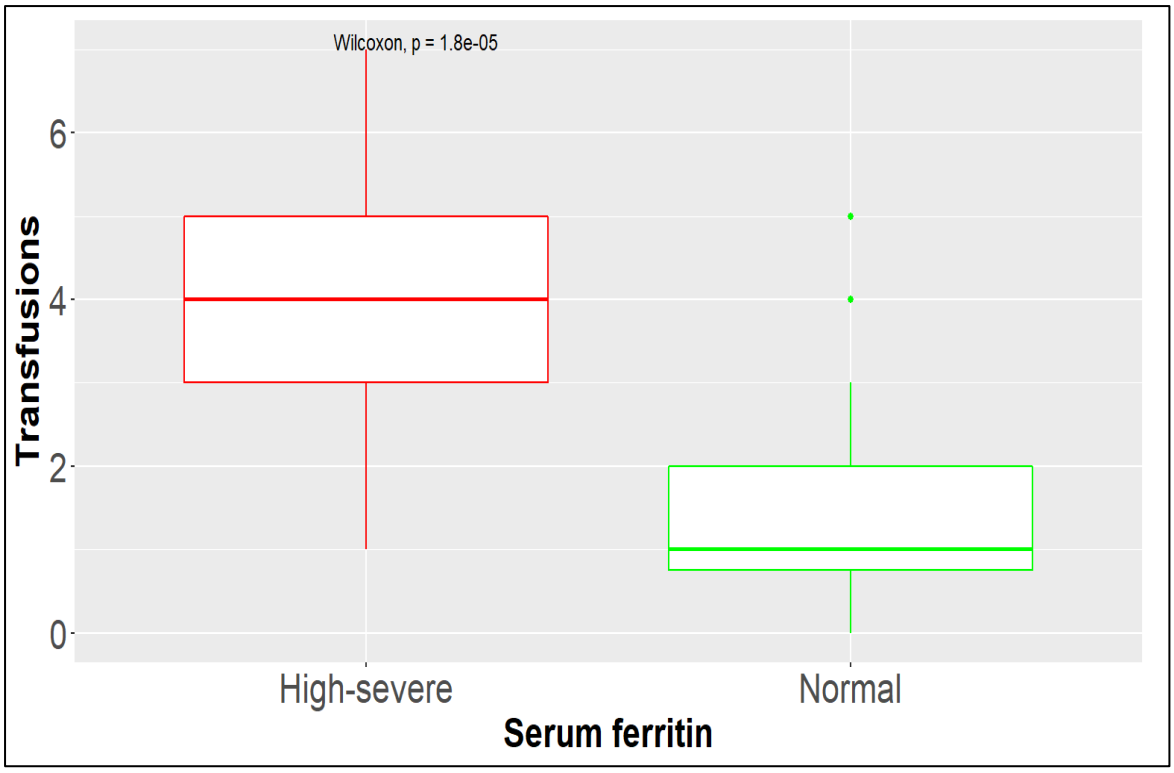

Figure 10 : Box plots for difference in median transfusions between children with normal vs. high-severe serum ferritin levels.

Association Between Serum Ferritin Levels and Liver Function

Serum Alanine Transferase Levels (Hepatic Dysfunction) in The Study Participants

Elevated levels of alanine transaminases were found in 14 of the children, representing 9.7% whereas 90.3% had normal transaminase levels. Of those with elevated levels, 10 (6.9%) were found to have grade 1 liver dysfunction and only 4 (2.8%) had Grade 2 dysfunction. None of the children had severe liver dysfunction as represented by Grades 3 and 4.

Elevation of serum alanine transferase was used to categorise liver dysfunction into Normal (Grade 0), Mild (Grade 1), Moderate (Grade 2), Severe (Grade 3) and Potentially life threatening (Grade 4). Most (131) of the subjects had normal liver

function and none of the subjects were found to have serum alanine levels suggesting severe or potentially life-threatening liver dysfunction. (Table 15).

Table 14 : Severity of liver dysfunction as measured by serum ALT in children with sickle cell disease in KNH(N=145)

	Detail	**No. of children**	**Percent**
Measured serum alanine transferase Levels in IU/L	Normal levels 1-42 IU/L	131	90
	Elevated levels >42IU/L	14	10
Severity of ALT Elevation	Grade 0	131	90
	Grade 1	10	7
	Grade 2	4	3
	Grade 3	0	0
	Grade 4	0	0

As a grouped variable , ALT levels (liver abnormal yes/no) a chi-square test was used as shown below (Table 16) to test the relationship between S.F. levels and ALT levels. The OR >1 indicates greater odds or likelihood of association between the iron overload and high ALT levels, suggesting liver dysfunction. The odds of high alanine transferase for the children with elevated serum ferritin was 8.3 times that of the children with normal serum ferritin.

(Table 16).

Table 15 : Association between serum ferritin and alanine transferase

Variable	**Alanine transferase**		**OR (95% CI)**	**p-value**
	Normal (%) N = 131	High (%) N = 14		
Serum ferritin Normal (ref)	39	7	8.3 (1.05, 65.29)	0.02
Elevated	61	93		

Effect Of Increase in Serum Ferritin on Alanine Transferase Levels .

Table 16: Effect of increase of Serum ferritin as a continuous variable on ALT level

Variable	**Alanine transferase**		**Crude OR (95% CI)**	**p-value**
	Normal(ref) N = 131	High N = 14		
Serum ferritin (ng/ml)			1.001 (1.001, 1.002)	<0.01

Every 1 ng/ml increase in serum ferritin as predictor variable was tested against normal vs high ALT as outcome variable. Serum ferritin was significantly associated with normal versus high alanine transferase levels, p-value <0.01 at 5% significance level. In addition, every 1ng/ml in serum ferritin increased the odds of high alanine transferase by 0.1%. This is represented by Table 17 above.

CHAPTER 5: DISCUSSION

Results

In this study, serum ferritin levels above the normal reference range (300ng/ml) were found in 64% of children reflecting a high prevalence of iron overload. The number of transfusions received was positively associated with the level of serum ferritin. There was a significant statistical association between serum ferritin and alanine transferase levels, implying hepatic function. Additionally, a unit increase in serum ferritin increased the odds of high alanine transferase.

Similar studies in other parts of Africa have found a high prevalence of raised serum ferritin levels amongst children with sickle cell disease. (12). The differences in ferritin levels amongst children with sickle cell disease in African studies has been attributed to differences in nutritional status, age, clinical condition and environmental influences.(12) In a study conducted amongst Nigerian children aged 1-15 years by Odunlade et al (11),serum ferritin levels above 300ng/ml were found in 33.3%. A similar study in Congo by Makulo et al estimated the prevalence of iron overload amongst children aged 2-18 years as being 21.4%.(12). It is likely that in our centre there is expanded use of blood transfusion in the management of sickle cell disease. This study suggests that there is need for more judicious use of blood transfusion in sickle cell disease as this inevitably leads to iron overload(35).

Severe iron elevation in this population was found in 11 patients (7.6%). This is similar to the study by Makulo et al (12) where serum ferritin levels in 70 Congolese children with sickle cell disease from two specialised centres was analysed and 5.7% (4 children) were found to have severe iron overload. Frequency of blood transfusions in this study was found to be strongly associated with the level of serum ferritin, (p-value 0.01, OR 1.8 ,95% CI 0.68, 0.84). This is similar to a study conducted in India(10) amongst 150 children with sickle cell disease aged 3-18 years by Debkumar et al in 2014 where a linear relationship between blood transfusion and serum ferritin level was found .

The deleterious effect of iron overload on the liver as well as the association with serum ferritin was analysed by determining the level of serum alanine transaminase. The level of serum alanine, which was used as a marker of liver toxicity was found to be significantly associated with the serum ferritin level . Similarly, Saied et al(36)in Egypt found that rising ALT levels amongst children with sickle cell disease were associated with frequency of blood transfusion($p=0.002$ and higher serum ferritin levels($p=0.0047$).Adamkiewicz et al showed that S.F. levels changes in children with SCD were associated with liver injury ($p=0.025$) (22). Furthermore, his study found SF to be an independent predictor of histologic severity where a threshold level of 300ng/ml was significantly associated with elevated serum ALT and hepatic iron deposition.

Serum ferritin is an assay which can be used in children with SCD who undergo regular blood transfusions as part of the management of the disease. (12). It is an important, non- invasive and accessible indicator of the body's iron stores which can be used as a monitoring tool for children with sickle cell disease in our setup.(35) A study by Yassin et al concluded that a major utility of SF was in identifying patients who are likely to be iron overloaded. (37). Even without access to MRI facilities, studies have indicated that ferritin levels above 2700ng/ml correspond to significant hepatic iron loading.(21)

Chelation therapy aims to maintain serum ferritin level to <1000ng/ml, and generally chelation treatment should be commenced after serum ferritin levels of 1500 - 3000ng/ml have been recorded. Thus, the serum ferritin assay is also useful in defining when chelation therapy can be instituted. For example , in this study approximately one third of the children in this setting had serum ferritin levels requiring chelation.

This study has determined that blood transfusion should not be an iterative process in the management of sickle cell disease. As iron overload is a complication of transfusion, the impact of minimizing transfusions can be considered as an option in the management of the various crises that present in children with SCD.

It was difficult to ascertain the exact units of blood transfused from the patient's files as well as the blood component given (packed cells or whole blood). Similarly, prior studies have emphasised that the status of body iron load is best established by keeping accurate records of the amount of blood transfused in addition to regular monitoring of serum ferritin levels.(26)

Strengths of the study

This was the first study to provide insight into the problem of iron overload amongst children with sickle cell disease in Kenya.

This study also provided previously uncollated data concerning the sickle cell child population receiving care in KNH, including their general demographics, treatment, and transfusion history as well as clinical information such as their average serum ferritin levels and liver function.

It was able to illuminate a significant problem of missing data as concerns detailed record keeping of blood transfusions received, including volume and frequency.

This study has been able to offer an accurate determination of the study parameters.

Limitations

A notable limitation encountered during this study involved the difficulty to capture accurate figures on cumulative number of blood transfusions. This limitation was mitigated in part by a detailed review of the child medical record, in addition to parent or teenager recall.

To allow assessment of the effect of blood transfusions on serum ferritin levels, a retrospective study period of 3 years was chosen. However, this study period was subject to recall bias, documentation error and missing data as well as the confounding effect of prior transfusions received, evidence of which may not be captured in this study.

Confounding factors such as prior nutritional status of the child, effect of age and the effect of duration of hydroxy urea use were not controlled for. These are individual patient factors which may influence the ferritin level.

As this was a tertiary hospital study population, the findings may not be generalizable to other settings.

Conclusions

1. Iron overload was highly prevalent in this population of children with SCD.
2. Every additional unit of transfused blood increased the risk of iron overload by two-fold.
3. There is a positive association between iron overload and hepatic dysfunction in these children with SCD.

Recommendations

1. Routine serial serum ferritin monitoring for iron overload should be carried out in children with sickle cell disease.
2. Better documentation and tracking of the actual number and volume of transfusions given to children with sickle cell disease should be done.
3. Further studies can be done to study the correlation between elevated serum ferritin levels with hepatic iron concentration using other methods such as MRI studies.

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APPENDIX

Appendix 1: Parental Consent Form

INFORMATION FORM

Study Title: THE PREVALENCE OF IRON OVERLOAD IN CHILDREN WITH SICKLE CELL DISEASE AGED 1-18 YEARS AT THE KENYATTA NATIONAL HOSPITAL.

Patients study identification number: ----------

KNH/UON ERC Protocol number: ----------

Principal investigator: Dr. Eva Wainaina

Paediatric Resident University of Nairobi.

Introduction

The purpose of this form is to give you the information you will need to help you decide whether or not to be a participant in the above listed study. You can ask any questions to clarify the purpose of the research, what happens if your child participates in the study, the possible risks and benefits and on your rights as a volunteer.

This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form.

Your decision is voluntary, it will not affect your access to health services in the hospital and furthermore, you can withdraw from the study any time without having to give a reason.

If your child is above 7 years, he/she will also be required to also agree to participate in the study after being fully informed about it.

What is the purpose of the study?

The investigator is interested in studying children who have been diagnosed with sickle cell disease. The main purpose of the study is to find out the number of blood

transfusions that have been received in the past and the effects they have had on the body.

There will be approximately 138 participants in this who will be chosen on random.

We are requesting for your consent to consider participating in this study.

What will happen if you decide you want your child to be in this research study?

You will be interviewed, and your child will be examined in the clinic where your privacy will be ensured. You will be asked questions concerning how long your child has had sickle cell disease, the medicine you are taking and how many blood transfusions the child has received. The whole process including the examination of your child will take around 20 minutes.

After the interview, your child's blood will be drawn and sent to the lab. The blood will be tested to see if your child had accumulated any iron as a result of the blood transfusions and if there is an ongoing illness of the child's abdominal organ called the liver.

You will be informed about the results and a copy of the results will be placed in your patients records.

Are there any risks associated with this study?

Your child may feel some pain or discomfort when blood is being drawn from him/her. A small bruise or swelling may also develop at the site which is temporary.

Are there any benefits being in this study?

All the information you provide is useful to help us better understand the course of sickle cell disease and will contribute greatly to future management of the disease.

Your child may also benefit from getting free testing of his/her iron levels and a free assessment of one of the liver enzymes.

It will not cost you anything in terms of financial resources neither is there a reimbursement for participation.

If you have any questions in the future, you can call or send a text message to the number given at the bottom of this page.

For more information, contact Dr. Eva Wainaina, Department of Paediatrics and Child Health, University of Nairobi. From 9-3pm, every Monday to Friday.

STATEMENT OF CONSENT

The person being considered for study is unable to consent for him/herself because he/she is a minor. You are therefore requested to give your permission to include your child in this study.

Parent / guardian statement

I have read this consent form or had the information read to me. I have had the chance to discuss this study and my questions have been answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw any time. I freely agree to participate in this research study. I understand that all efforts will be made to keep information regarding my personal identity confidential. By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I voluntarily agree to my child's participation in this research study YES NO

I agree to have my child undergo blood tests YES NO

I have agreed to provide contact information for follow-up YES NO

Participant printed name----------

Participant signature / Thumb stamp ---------- Date ----------

<u>Researcher's statement</u>

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Principal investigator's Name: ----------Date: ----------

Signature----------

FOMU YA IDHINI

Kichwa cha Utafiti: UCHUNGUZI KWA WATOTO WENYE UGONJWA WA SELA WA MIAKA 1-18 KWENYE HOSPITALI YA TAIFA YA KENYATTA.

(THE PREVALENCE OF IRON OVERLOAD IN CHILDREN WITH SICKLE CELL DISEASE AGED 1-18 YEARS AT THE KENYATTA NATIONAL HOSPITAL.)

Patients study identification number: ----------

Principal investigator: Dr. Eva Wainaina

Paediatric Resident University of Nairobi.

Utangulizi

Madhumuni ya fomu hii ni kukupa habari utakayohitaji kukusaidia kuamua ikiwa utakuwa mshiriki katika somo lililoorodheshwa hapo juu au la. Unaweza kuuliza maswali yoyote kufafanua madhumuni ya utafiti, nini kinatokea ikiwa mtoto wako atashiriki kwenye utafiti, hatari zinazoweza kutokea na faida na haki zako kama kujitolea.

Utaratibu huu unaitwa 'ridhaa inayofahamishwa'. Mara tu utakapoelewa na kukubali kuwa kwenye utafiti, nitakuomba utie sahihi jina lako kwenye fomu hii.

Uamuzi wako ni wa hiari, hautaathiri ufikiaji wako wa huduma za afya hospitalini na zaidi, unaweza kujiondoa kutoka kwa utafiti wakati wowote bila kutoa sababu.

Ikiwa mtoto wako ni zaidi ya miaka 7, atahitajika pia kukubali kushiriki katika utafiti baada ya kufahamishwa kabisa juu yake.

Kusudi la utafiti ni nini?

Mchunguzi anavutiwa kusoma watoto ambao wamegunduliwa na ugonjwa wa seli ya mundu. Kusudi kuu la utafiti huo ni kujua idadi ya uhamisho wa damu ambao umepokelewa zamani na athari ambazo wamepata mwilini.

Kutakuwa na washiriki takriban 138 katika hii ambao watachaguliwa bila mpangilio.

Tunaomba idhini yako kuzingatia kushiriki katika utafiti huu.

Je! Ni nini kitatokea ikiwa utaamua unataka mtoto wako awe katika utafiti huu?

Utahojiwa, na mtoto wako atachunguzwa katika kliniki ambayo faragha yako itahakikishwa. Utaulizwa maswali juu ya muda gani mtoto wako amepata ugonjwa wa seli ya mundu, dawa unayotumia na jinsi mtoto anavyopewa damu nyingi. Mchakato wote pamoja na uchunguzi wa mtoto wako utachukua karibu dakika 20.

Baada ya mahojiano, damu ya mtoto wako itatolewa na kupelekwa kwenye maabara. Damu itajaribiwa ili kuona ikiwa mtoto wako alikuwa amekusanya chuma chochote kutokana na kuongezewa damu na ikiwa kuna ugonjwa unaoendelea wa kiungo cha tumbo cha mtoto kinachoitwa ini.

Utajulishwa juu ya matokeo na nakala ya matokeo itawekwa kwenye rekodi za wagonjwa wako.

Je! Kuna hatari zozote zinazohusiana na utafiti huu?

Mtoto wako anaweza kuhisi maumivu au usumbufu wakati damu inachotwa kutoka kwake. Mchubuko mdogo au uvimbe pia unaweza kutokea kwenye wavuti ambayo ni ya muda mfupi.

Je! Kuna faida yoyote kuwa katika utafiti huu?

Habari yote unayotoa ni muhimu kutusaidia kuelewa vizuri kozi ya ugonjwa wa seli mundu na itachangia sana katika usimamizi wa ugonjwa hapo baadaye.

Mtoto wako pia anaweza kufaidika kwa kupata upimaji wa bure wa kiwango chake cha chuma na tathmini ya bure ya moja ya enzymes za ini.

Haitagharimu chochote kwa suala la rasilimali za kifedha wala hakuna malipo ya ushiriki.

Ikiwa una maswali yoyote katika siku zijazo, unaweza kupiga simu au kutuma ujumbe mfupi kwa nambari iliyopewa chini ya ukurasa huu.

Eva Wainaina, University of Nairobi, Department of Paeditrics and Child Health,

<u>Fomu ya idhini: Taarifa ya mshiriki</u>

Soma fomu hii idhini au alikuwa taarifa kusoma kwangu. Nimekuwa na maswali yangu yaliyojibiwa katika lugha ambayo mimi kuelewa. Hatari na faida imekuwa alielezea kwangu. Ninaelewa kwamba ushiriki wangu katika utafiti huu ni hiari na kwamba mimi kuchagua kuondoka wakati wowote. Uhuru nakubaliana kushiriki katika utafiti huu. Nafahamu kuwa jitihada zote yatatolewa kwa kuweka maelezo

kuhusu utambulisho wangu binafsi siri. Kwa kutia sahihi fomu idhini, kutokana na juu yoyote wa haki kisheria kwamba nina kama mshiriki katika masomo na utafiti.

Ninakubali kwa hiari ushiriki wa mtoto wangu katika utafiti huu wa utafiti NDIYO HAPANA

Ninakubali mtoto wangu afanyiwe vipimo vya damu NDIYO HAPANA

Nimekubali kutoa habari ya mawasiliano kwa ufuatiliaji NDIYO HAPANA

Mshiriki Piga Chapa na jina lake hapa _____

Saini ya mshiriki / kidole gumba ---------- Tarehe ----------

Taarifa ya mtafiti

Mimi ninahakikisha kwa kikamilifu nime elezea husika ya utafiti huu kwa mshiriki aitwaye juu na kuamini kuwa mshiriki ameelewa na ana hiari na uhuru kupewa ridhaa.

Jina la mtafiti----------

Tarehe: ---------- Saini ----------

Appendix 2 Child Assent Form

Study Title: THE PREVALENCE OF IRON OVERLOAD IN CHILDREN WITH SICKLE CELL DISEASE AGED 1-18 YEARS AT THE KENYATTA NATIONAL HOSPITAL.

Patients' identification number: ----------

KNH/UON ERC protocol number: ----------

Principal investigator: Dr. Eva Wainaina/ Paediatric Resident University of Nairobi.

We are conducting this research to learn more about children who get sick sometimes like you do and what happens to your body when the doctors give you medicine like blood.138 children will be part of the study.

If you agree to be part of this study, we will ask you some questions about the medicine you are taking, and we will also examine you. It wont take long.

We will also need to remove some blood from you which we will send to the lab for testing. You will feel some little pain when the blood is being removed.

The good thing about being in this study is you will help us understand a bit more about what happens when you get sick and it may help some other children, maybe even you in the future.

You do not have to agree to be part of this study.

When we are finished, we will write a report of what we learn but we will not put your name or that you were in the study.

If after you agree you want to change your mind and stop being in the study, it is also fine, and you will still continue being treated today in the clinic.

Your parents also know about the study. You can ask me any question you want.

If you agree you want to be in this study, please sign your name.

I ----------want to be in this study.

Signature/ Thumbprint ---------- Date ---------

Appendix 3 Case Record Form

Study Name: THE PREVALENCE OF IRON OVERLOAD IN CHILDREN WITH SICKLE CELL DISEASE

P/I: Dr. Eva Wainaina

ERC Protocol Number:

Subject Initials		**Subject ID**		**Date**			
					Day	Month	Year

Demographics

KNH I.P. Number:

Birthdate

Day	Month	Year

Age	

Gender: (check one)

☐ **Male**

☐ **Female**

Date of Diagnosis

Day	Month	Year

Duration since Diagnosis

Years	Months	Days

Concomitant Medication Log

	Medication	Y/N	Dose/ Schedule/ Frequency	Route of Administration	Start Date	Any other comments
	Hydroxyurea					
	Folate					
	Antibiotic (state)					
	Vit. B12					
	Analgesic (state)					
	Iron					
	Other					

Number of Blood Transfusions (Patient / Parent recall)

Year	Number	
During current year 2021	☐☐	Does not know. ☐
During previous year 2020	☐☐	Does not know. ☐
During 2019	☐☐	Does not know. ☐
Total Number of Blood Transfusions since diagnosis	☐☐	Does not know. ☐

PART 2: ABSTRACTION FROM PATIENT RECORDS

Subject Initials				**Subject ID**						**Date**	Day	Month	Year

Demographics

KNH I.P. Number:

Birthdate

Day	Month	Year

Age	

Gender: (check one)

☐ **Male**

☐ **Female**

Date of Diagnosis

Day	Month	Year

Duration since Diagnosis

Years	Months	Days

Record of Blood Transfusions (In- Patient Records)

Year	Date	Number	Volume of blood transfused (ml)
During current year 2021	JAN		
	FEB		
	MAR		
	APR		
	MAY		
	JUN		
	JUL		
	AUG		
	SEP		
	OCT		
	NOV		
	DEC		
During previous year 2020	JAN		
	FEB		
	MAR		
	APR		
	MAY		
	JUN		
	JUL		
	AUG		
	SEP		
	OCT		
	NOV		
	DEC		
During 2019	JAN		
	FEB		
	MAR		
	APR		
	MAY		
	JUN		
	JUL		
	AUG		
	SEP		
	OCT		
	NOV		
	DEC		
Number of blood			

Year	Date	Number	Volume of blood transfused (ml)
transfusions since contact with KNH			

Additional Notes: _____

_____

PART 2: LABORATORY RESULTS

Subject Initials				**Subject ID**						**Date**			
										Day	Month	Year	

Demographics

KNH I.P. Number:

Birthdate

Day	Month	Year

Age	

_____

Gender: (check one)

☐ **Male**

☐ **Female**

_____

Laboratory Results

Serum Ferritin Assay Measurement:

Result (ng/ml)				**Reference Value (ng/ml)**			

Serum Alanine Aminotransferase (ALT) Measurement:

Result (ng/ml)				**Reference Value (ng/ml)**			

Appendix 4: Laboratory Procedure for Measurement of Serum Ferritin

Liaison [R] Ferritin Assay (REF 313551)

Principle

The method for quantitative determination of ferritin is a sandwich chemiluminescence immunoassay.

Procedure

1. Reagents are removed from the refrigerator and left at room temperature for 30minutes.
2. Reagent integral is prepared before being placed on the machine by gentle and careful side to side mixing to ensure the magnetic particles are completely resuspended.
3. Place the integral into the reagent area of the analyser with the barcode facing left and let it stand for 30 minutes before using. The analyser automatically stirs and completely resuspends the magnetic particles.
4. Load the specimen and start the run.
5. The analyser automatically calculates ferritin concentrations in ng/ml and is able to measure concentrations up to 3000ng/ml.
6. After review and verification of results, the operator will run the print outs.

Appendix 5 Laboratory Procedure for Measurement of Serum Aminotransferase

Alanine Aminotransferase Assay using HumaStar 600 (REF 12022600)

Principle

The procedure uses an enzymatic rate method to measure ALT activity in serum or plasma. In the reaction, ALT catalyses transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The rate of change in absorbance at 340nm is measured as it is directly proportional to the ALT activity in the sample.

Procedure

1. Briefly centrifuge vials before opening
2. Use ultrapure water for the preparation of reagents
3. Prepare ALT Assay buffer (and allow buffer to come to room temperature before use), ALT enzyme mix, ALT substrate and ALT positive control
4. Machine requires 20ul of sample which is directly added to the wells
5. Set up the Master Reaction Mix by adding together ALT assay buffer, fluorescent peroxidase substrate, ALT enzyme mix and ALT substrate.
6. 100ul of the Master Reaction Mix is then added to each of the standard, positive control and test wells and mixed together by pipetting.
7. the sample is then run in the panel
8. The operator will review and verify results, place printouts, and ensure any critical results are verified.

Appendix 6 Study Budget

Expense Type	Comments	No.	Cost per item	Total (Kshs)
Proposal development	Computer services/ / data	-	-	5,000
	Printing of Proposals	8 copies	1500	12,000
Clinical assessment	Printing of Case record forms	145	15	2,175
Data Collection	Stationary packs	10	100	1000
	Serum ferritin assay Kit	2	63,500	127,000
	Serum Amino alanine transferase (ALT) testing	145	400	58,000
	Pack of plain vacutainer tubes	150	20	3,000
	Lab Cost of running Serum Ferritin test	145	100	14,500
	Sample transport Cooler box	1		3,000
Analysis	Statistician	1		35,000
Thesis Write up	Computer services			5,000
	Printing of copies of thesis	10 copies	1500	15,000
Contingency Funds	Includes transport, ERC fees, telephone charges etc.			20,000
TOTAL				**298,070**

SOURCE OF FUNDS: Personal Savings

Appendix 7 Proposed Timelines: Ganntz Chart

Activity	**Jan 2021**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan 2022**	**Feb**	**Mar**	**Apr**	**Jun**
Proposal developmentand defence																	
Ethical clearance																	
Data collection																	
Data analysis																	
Thesis manuscript writing/and defence																	

Appendix 8 Research Proposal approval by KNH-UON ERC (P158/03/2021)

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

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

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

Ref: KNH-ERC/A/208

16th June, 2021

Dr. Eva Wangui Wainaina
Reg. No. H58/33015/2019
Dept. of Paediatrics and Child Health
School of Medicine
College of Health Sciences
University of Nairobi

APPROVED
15 JUN 2021
KNH-UoN-ERC

Dear Dr. Wainaina,

RESEARCH PROPOSAL: THE PREVALENCE OF IRON OVERLOAD IN CHILDREN WITH SICKLE CELL DISEASE AGED 1-18 YEARS AT THE KENYATTA NATIONAL HOSPITAL (P158/03/2021)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above research proposal. The approval period is 16th June 2021 – 15th June 2022.

This approval is subject to compliance with the following requirements:

i. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
ii. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
iii. Death and life threatening problems and serious 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.
iv. 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.
v. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
vi. 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).
vii. Submission of an executive summary report within 90 days upon completion of the study.

Protect to discover

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 http://www.erc.uonbi.ac.ke

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director, CS, KNH
The Chair, KNH- UoN ERC
The Assistant Director, Health Information Dept, KNH
The Dean, School of Medicine, UoN
The Chair, Dept. of Paediatrics and Child Health, UoN
Supervisors: Prof. Elizabeth M. Obimbo, Dept. of Paediatrics and Child Health, UoN
Dr. Nyambura Kariuki, Dept. of Paediatrics and Child Health, UoN
Dr. Ahmed Laving, Dept. of Paediatrics and Child Health, UoN

Appendix 9 Turnitin Similarity Index

Prevalence of Iron Overload

by Eva Wainaina

Submission date: 27-Apr-2022 01:21PM (UTC-0400)
Submission ID: 1822034414
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4	Wang, Winfred C, Kerry Dwan, and Winfred C Wang. "Blood transfusion for preventing primary and secondary stroke in people with sickle cell disease", Cochrane Database of Systematic Reviews, 2013. Publication	<1%
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