Serum Ferritin Levels in Patients with Sickle Cell Anaemia at the Kenyatta National Hospital

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF A MASTER OF MEDICINE IN INTERNAL MEDICINE, UNIVERSITY OF NAIROBI.

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Declaration

I certify that this dissertation is my own original work and has not been presented to the best of my knowledge for award of a degree in any other university.

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Dedication

This work is dedicated to my late parents: Mr. Rafael Oyiro Owor and Mrs. Rosbela Awor Oyiro for their invaluable sacrifice to enable me attain this level of education.

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List of Abbreviations

ALT	Alanine Aminotransferase
AR	Autosomal Recessive
AST:	Aspartate Aminotransferase
CAPE:	Cellulose acetate paper electrophoresis
DMT:	Divalent Metal Transporter
DNA:	Deoxyribonucleic acid
ELISA:	Enzyme Linked immunosorbent assay
ESR:	Erythrocyte Sedimentation Rate
FBC:	Full blood Count
Fl:	Femtolitre
GFR:	Glomerular filtration Rate
Hb:	Haemoglobin
HBD:	Hydroxybutyrate dehydrogenase
HbSC	Haemoglobin S and C
HbSF:	Haemoglobin S and F
HbSS:	Homozygous Sickle Haemoglobin
KNH:	Kenyatta National Hospital
MCH:	Mean Corpuscular Haemoglobin
MCHC:	Mean corpuscular haemoglobin concentration
MCV:	Mean Corpuscular Volume
ML:	Millilitre
MRI:	Magnetic Resonance Imaging
ng:	nanogram
NTBI:	Nontransferin bound iron
PBF:	Peripheral Blood film
RBC:	Red blood cell
SCA:	Sickle cell Anaemia
SD	Standard deviation
SCD:	Sickle cell Disease
SF:	Serum Ferritin
TIBC:	Total Iron Binding Capacity
U/L:	Units per Litre
VCAM:	Vascular Cell Adhesion Molecule
WBC:	White Cell count
µmol:	Micromole

Abstract

Background: The iron levels in Sickle Cell Anaemia (SCA) are thought to be increased as a result of the repeated red cell transfusion, haemolysis with subsequent recycling and accumulation of iron. Red cell transfusion is used more frequently to prevent and treat the complications of sickle cell disease. Studies have shown that the changes in iron status that results from such therapy is associated with significant morbidity and mortality. This study examined the serum ferritin as a marker of iron levels in patients with sickle cell disease who receive chronic red blood cell transfusion.

Objective: The aim of this study was to assess the status of serum iron levels by measurement of serum ferritin levels in patients with sickle cell Anaemia at Kenyatta National Hospital.

Setting: The study was carried out at the Kenyatta National hospital Haematology clinic.

Design: Cross-sectional descriptive study

Materials and methods: This study enrolled 80 patients with sickle cell anaemia. History and physical examination was done and recorded on a standard study questionnaire. Samples of blood were then drawn for serum ferritin, full blood count including peripheral blood film. Serum ferritin was assessed using enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) kits. Blood counts were done using the haematology cell counter (CELL-DYN 1300) whiles the peripheral blood films were stained using May Grunewald Giemsa method. Quality control measures were observed in all tests performed by adhering to reagent manufacturers' guidelines and standard specimen handling/laboratory operating procedures, to ensure validity of results.

Results: Eighty clinically stable patients with SCA were studied. Thirty three were males (41.3%) while Forty seven were females (58.7%). The mean age in the study population were 19.7±5.5 years with the youngest being 13years and the oldest 37years. Serum ferritin mean 939.25±668ng/ml , was found to be significantly elevated in 56 (70.5%) of our study subjects while 24 had normal SF levels and none of them had low SF. Twenty five (31.3%) who had very high SF, above 1000ng/ml, had also been transfused a mean of 15±8.2 units of blood. There was a significant association between SF and the number of units of blood transfused, p=<0.001.There was no demonstrable significant association between SF and age, sex or red cell parameters.

Conclusion: This study demonstrated high level of serum ferritin in sickle cell anaemia patients who had been transfused ≥15 units of blood over a period of five years**.** Overall about a third of the study subjects had Serum Ferritin levels that required further evaluation to find out if they had iron overload.

1.0 Introduction

Significant progress has been made in the past two decades in the management of sickle cell disease resulting into increased life span. In addition, identification of the affected people in their early years of life provides opportunities for early medical interventions that further help in reducing morbidity and mortality associated with SCD. These improvements in quality of life have been attained through comprehensive care including prophylactic measures and periodic assessment with monitoring for development of chronic organ dysfunction(1)

One of the parameters used in monitoring these patients is status of body iron. It has been postulated that increased body iron influences prognosis and hence, management of these patients. However, this notion regarding the levels of iron remains controversial and there is paucity of local data to provide physicians with clear guidelines for using this parameter in their clinics in making decisions regarding red cells transfusion, iron supplementation or chelation.

2.0 Literature Review

Sickle cell disease is inherited in an autosomal recessive or autosomal co-dominant pattern with variable expressivity and penetrance resulting into an abnormal Hb designated as HbSS.(6) It was first discovered by a Chicago Physician, JB Herrick in 1910, who observed sickle shaped RBC in a peripheral smear of an anaemic West Indian student who had severe anaemia and a cardiac murmur. (7) In HbSS, the hydrophobic valine is substituted for the normal, more hydrophilic glutamic acid at the 6th residue of the βglobin chain. In 1949, Linus Pauling et al worn the Nobel Prize after discovering that this substitution is as a result of a single nucleotide mutation (GAG/GTG) in the sixth codon of the βglobin gene.(8)6, 9, 10). There are five haplotypes so far described, four of which occur in Africa: Senegal, Benin, Bantu and Cameroon types. The fifth one has been described in India. Other factors influencing the expression include the amount of HbF and the hereditary persistent fetal hemoglobin (HbSPFH).(11)

2.1 Epidemiology

There are more than 200million sickle cell carriers worldwide and about 200,000 to 300,000 children are born annually with hemoglobinopathies. Approximately 0.14% of African-American children are homozygous for the sickle cell gene and 6-10% of African-American newborns have the trait making it the most common inherited disorder among the African Americans. Homozygous S gene is encounterd mostly among the Negroes in Turkey, and parts of Asia.(12)

In Africa, its distributed along the equatorial belt. HbSS is the commonest variant encontered in East Africa. There is marked variation in the distribution of SCA across East Africa's ethnic groups. Aluoch et al in the survey of SCD in Kenya and East Africa estimated the highest prevalence of 45-50% among the Bwamba tribe of Western Uganda residing at the foot hills of the Ruwenzori hills. In Kenya the highest prevalence of sickle cell trait of 35% is observed among the Kambe of the Mijikenda community in the coastal Kenya. One also observes high of 20-30% sickling along the inhabitants of the Lake Victoria region and no sickling among the nomadic Masai and Turkana of the arid and semiarid parts of Kenya . In their survey of 3605 patients with SCA, they also found out that 77% were below 15 years of age with the oldest being 50 years. The Luos and the Luhya ethnic group had the highest prevalence of the HbSS (58.4 vs 23.9%) while the

Kambes of the Mijikenda having the highest prevalence of the carrier state at 35%(13) There is also noted prevalence of 2-11% in Zanzibar and Mainland Tanzania.(14).

Life expectancy among the SCA patients in North America has improved to an average of 42 and 48 years for men and women respectively(15) . This is due to improved supportive care in a comprehensive care setting which is lacking in most African countries.

In Kenya Approximately 50% of SCA patients die before 20 years of age and most do not survive beyond 40 years. (13)Local data on life expectacy is lacking but the situation is likely to be much dire in Kenya and other African countries. Mortality is highest in infancy due to severe infections and recurrent severe crises.(15) Events such as cardiovascular accidents, renal, hepatic, iron overlaod and cardiac failures also contribute significantly. In the tropics, parasitic infections such as malaria frequent causes of morbidity and mortality among SCA patients.(16)

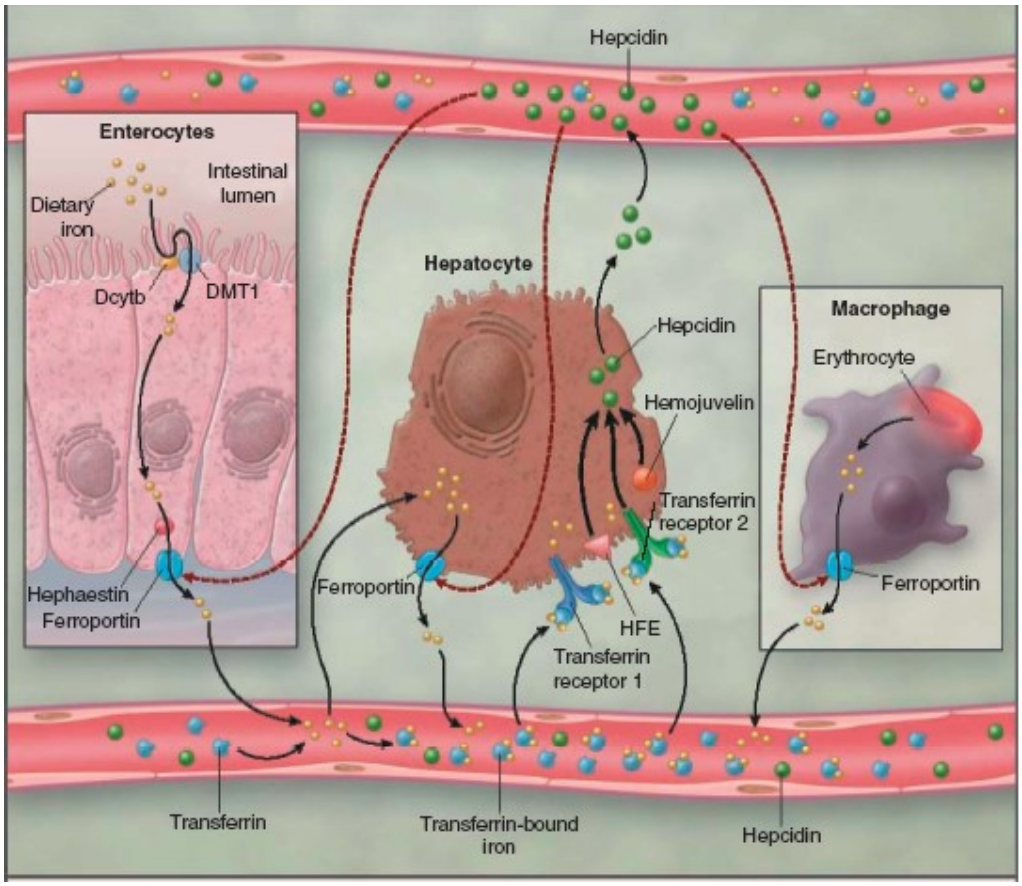
2.2 Pathophysiology of SCA

The hallmark of sickle cell pathophysiology is the intraerythrocytic polymerization of deoxyhemoglobin S. Deoxygenation of HbS results into the normal conformational change of the tetramer hemoglobin exposing on its external surface a hydrophobic β6 valine, instead of the hydrophilic glutamate of HbAA. This leads to decreased solubility hence polymerization.(19). The rate and extent of polymerization are related to the intracellular concentration of HbS, the type and fractional content of other hemoglobin present ; particularly HbF, and percent oxygen saturation. There are factors such as endothelial damage with subsequent activation inflammatory and coagulation pathways may trigger or complicate vaso-occlusion (20). High levels of fetal Hb (HbSF) may substantially reduce symptoms and clinical consequences.(16, 18, 19, 21)

2.3 Iron Metabolism

The total body iron averages 35 to 45 mg/kg mostly in the hemoglobin molecule of the red blood cells, each milliliter of which contains about 1 mg of iron. Men and women have approximately 2 g and 1.5 g of erythrocyte iron, respectively. Iron is stored as

ferritin, predominantly in the macrophages of the spleen, bone marrow, and liver.(22). Significant amount of iron is also found in myoglobin ,cytochromes and other enzymes.(23) Dietary iron usually amounts to 15 to 25 mg daily, of which 5% to 10% is absorbed. This proportion can be increased up to 5-fold in states of iron deficiency and in SCA. The body requires 1 mg of iron daily to compensate for normal obligatory losses through desquamation of cells and trace amounts in urine and bile. (24)Regulation of iron balance relies on the function of a number of key proteins as depicted in figure below.



Fleming RE, Bacon BR. Orchestration of iron homeostasis.(25)N Engl J Med. 2005; 352:1742

Regulators of iron balance

SCA patients are thought to have higher body iron concentration than non-sickle cell anemia cases. This has largely been attributed to frequent transfusions and increased gastrointestinal iron absorption chronic haemolysis(16. 22). The body lacks physiologic pathway for iron excretion.

2.4 Transfusion Iron Overload

Iron overload is an inevitable consequence of recurrent transfusion therapy. Recurrent transfusions in patients with SCA may lead to iron overload with subsequent significantly higher morbidity and mortality.(26)Transfusion of packed RBCs provides 1mg per ml transfused of additional elemental iron. For instance 20 units RBC/year equals to 20units x220ml per unitx1mg per ml which equals to 4400mg of exogenous iron per year.(27). Following recurrent transfusions, there is transferrin saturation. The unbound plasma iron is therefore transported as None transferrin bound iron (NTBI). A subset of NTBI called the Labile Plasma Iron (LPI) may cause end organ damage due to its high oxidative potential. (28)

2.5 Red cell Transfusion in SCA

2.5.1 Indication for Red Cell Transfusion in SCA Patients

Indications for blood transfusion in SCA can either be simple, exchange of chronic. Simple transfusion is indicated in aplastic crisis(29), splenic sequestration(30), hepatic sequestration(31), and perioperative transfusion. Exchange transfusion on the other hand is indicated in cases of acute chest syndrome(32), priapism(33, 34), and acute multiorgan failure syndrome(34). Chronic transfusion or hyper transfusion regime(32, 35), on the other hand, applied mainly in prevention of sickle cell related complications such as recurrence of stroke, stroke, leg ulcers, and renal disease (35)

2.6 Iron Status in SCA

The effect of accumulated iron depends on its distribution in the body. Parenchymal iron overload frequently occurs in idiopathic hemochromatosis and dyserythropoiesis. Iron deposits in the liver, pancreas and heart resulting into functional disturbances and organ damage. Mononuclear phagocyte system iron overload occurs commonly after excessive iron supplementation and multiple blood transfusions. SCA patients are prone to both forms of overload as a consequence of multiple transfusions, drug therapy and enhanced gastrointestinal iron absorption.(1)

2.7 Iron chelation therapy

Chelation Methods

Desferrioxamine is administered by nightly slow subcutaneous or intravenous infusion (up to 40mg/kg) over 8-12 hours. Negative iron balance with prevention of organ damage

can be achieved if initiated early. (41)**.** Vitamin C increases iron excretion by increasing the availability of chelatable iron and can therefore be given 2-3mg/kg during Desferrioxamine therapy. Annual auditory and ocular evaluation is recommended due to potential toxicity of the drug. Poor compliance and cost of Desferrioxamine has diminished its potential therapeutic benefits.

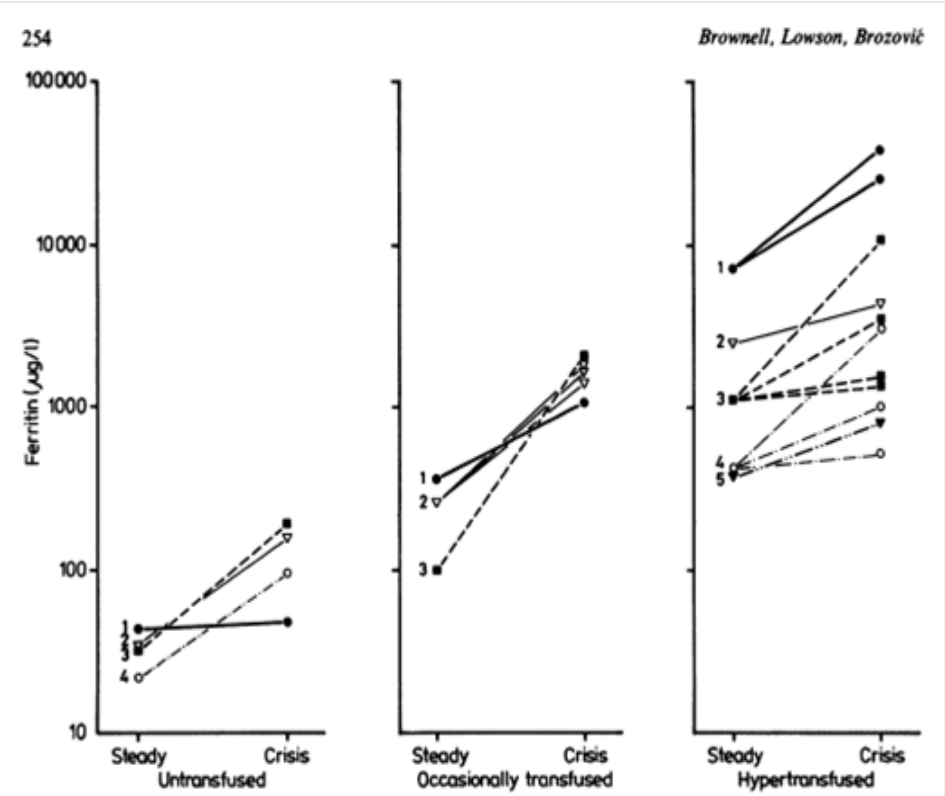
Deferasirox: This is an oral iron chelator approved for treatment of transfusion-related iron overload in adults and children older than 2 years of age. A phase III study by Maria Dominica et al showed that a dose of 20 to 30 mg/kg of Deferasirox daily was sufficient to reduce liver iron concentrations . (42, 43) Annual auditory and ocular evaluation is recommended due to potential toxicity of the drug.

2.8 Assessment of iron in SCD

Currently, SF concentration is widely accepted method of assessing body iron status. (44, 45). The growing indications for red cell transfusions in SCA patients raise the issues of appropriate measurement of body iron burden and optimal timing of iron chelation therapy. Olivieri NF and his colleagues in their study on progression of iron overload in SCD, University of Toronto, Canada studied 42 liver biopsy specimens from 20 patients with SCD (mean age 15.7years) who had received transfusions. They demonstrated that after 1-2years of recurrent transfusions, variable tissue iron concentrations and damage are observed.(46) In patients who received recurrent blood transfusion for the prevention of primary cardiovascular events such as stroke, the serum ferritin rose upto 10-fold after about 42months of followup. (35, 47)(48)

Studies have also shown variable association between SF and liver iron concentration. There some have found poor correlation between SF and quantitative iron on liver biopsy while others have found a correlation though weak. Thomas V. Adamkiewicz and colleagues in Canada studied Serum ferritin alterations in children with sickle cell disease on recurrent blood transfusion. They found that SF levels in their study population was nonlinear but with a significant association with SF levels and hepatotoxicity. The SF level was also seen to rise with the amount of blood transfused.(49)

Alison et al conducted a study (50, 51) on 12 patients with sickle cell disease. They documented 21 vasoocclusive crises with considerable variation in the severity of the episodes.



Rise in serum ferritin concentrations in crises from steady state in each group. Group I: patients who had been untransfused, group II: those who had occasionally been transfused, group III: those who had been hypertransfused.

(51)

The figure above shows the serum ferritin changes during crisis in each patient. The rises in serum ferritin concentrations varied between crises in each patient. The authors noted that the highest ferritin concentrations were associated with the most severe clinical states. This study suggests that serum ferritin measurement may have a role in iron assessment in the steady state and could be quite misleading during periods of crisis or acute events. Its paramount to assess iron balance when transfusion is anticipated hence pretransfusion ferritin value is desirable.(52) Generally, there is consensus that assessment of the severity of iron overload to assess need for iron chelation is an appropriate undertaking (53, 54(55). Indications are that significant correlation in the

degree of haemolysis, liver damage and ferritin concentrations cannot be assumed and requires ferritin determination.

Stettler et al in their study on iron status in patients with SCA in Philadelphia assessed iron status in 104 non-transfused children and adolescents of mean age 17.6 years. They reported normal or high SF in all patients. Other biological or chemical indicators of iron deficiency were also all in the normal range (56). Olaniyi and colleagues in Nigeria in study iron status among adult SCA patients in Nigeria concluded that interpreting iron parameters in SCD patients is complex and highly modified by chronic inflammatory state and chronic hemolytic process. Their study showed that iron status was lower in SCA patients in steady state. The patients who had vaso-occlusive state had higher transferrin saturation, serum iron, lower TIBC and lower ferritin compared to the steady state group. (57)58). Study by Musa et al in Nigeria corroborated the findings of previous researchers of normal or high iron stores in SCA patients. On the other hand the prevalence of iron deficiency in SCA patients in this study was found to be only 7%. Iron deficiency may therefore not be as common as was being reported in some previous studies in SCA. The determination of iron status of SCA patients is better based on measurements of saturation of transferrin receptor/log serum ferritin index with or without hypochromia or microcytosis rather than on SF or hypochromia and microcytosis alone.(59)

In conclusion, there seems to be a strong association between SF levels hepatic and macrophage iron stores, but less with pituitary or cardiac iron. (1) Serum Ferritin is a non-invasive and cost effective method of iron assessment . The plasma levels range from 32-284ng/ml. In patients with regular blood transfusions, is advisable to initiate chelation therapy when SF rises to 1000ng/ml. Serial SF measurement is necessary in order to initiate chelation therapy early. The status of iron stores in adults with SCA is however best determined by keeping accurate records of the amount of blood transfused besides serial determination of SF.(60) Significant number of adults with SCA have iron overload and this is a predisposing factor to disease severity. Most of the studies are in the western population and reveals rising iron stores. The same trend may not be necessarily observed in our population but it's important to document the characteristics of our local population for reference, management and learning purposes.

3.0 Rationale for the study

We intend to help derive data regarding role of ferritin levels in predicting iron status in patients with sickle cell disease. Sickle disease and its complications is a significant cause of morbidity and mortality in Kenya. One of the major contributors of mortality and morbidity in SCA patients is changes in iron status with subsequent complications. Excess body iron in SCA patients is mostly due to recurrent red cell transfusion. There is paucity of local data in regards to iron status in SCA and its management. Furthermore, mortality and morbidity of SCA patients is still high despite application of data and information derived from SCA patients elsewhere.

The evaluation of serum ferritin in a cross section of SCA patients in Kenya will help derive data that would help define the role of ferritin measurement in management of patients with SCA. There is need to rationalize the giving of iron, blood transfusion and or use of iron chelation through the use of simple and non-invasive methods of body iron assessment.

4.0 Objectives

Research Question

What is the burden of Iron status among patients with SCA at KNH?

Broad Objective

1. To evaluate the iron status of ambulatory SCA patients attending KNH haematology clinic.

Primary Objective

1. To determine the levels of serum ferritin in steady state SCA patients attending Haematology clinic at KNH

Specific Objectives

1. To determine the correlation between SF and number of blood transfusions in SCA patients at KNH.
2. To determine the correlation between ferritin levels and demographic characteristics, Red cell indices, white cell count and ESR.

5.0 The Materials and Methodology

5.1 The Study Design:

This was a cross-sectional study that enrolled 80 SCA patients.

5.2 Setting:

Kenyatta National Hospital Haematology Clinic

The study was carried out at Kenyatta National Hospital which is a tertiary teaching and referral hospital located in the capital city of Kenya, Nairobi. It was established in 1900 and is the largest hospital in the Eastern and Central Africa. It has a capacity of 2000 beds. It serves as the teaching hospital for the University of Nairobi, College of Health Sciences, both for the undergraduate and the post graduate programmes. It serves as a referral hospital for Kenya and East Africa. It runs general and specialized clinics and in-patients services in surgical, medical, obstetrics and gynaecology, ophthalmology and paediatrics.

The haematology clinic is carried out every Monday. The venue is usually the Kenyatta National Hospital clinic number 23. About 120 patients are reviewed in the clinic every week out of whom 10 have SCD. The patients' records are available in the health information record office.

KNH haematology and Biochemistry laboratories have the capacity for analysis and evaluation of serum iron levels.

5.3 Study Population

These were patients with SCA aged 13years and above in steady state and on regular follow-up at KNH.

5.4 Case definition

A documented case of SCA at the KNH Haematology clinic. A clinically steady state in SCA was defined as a state of no identifiable active disease process clinically and or crises or any major complications and where these exist, they did not have any direct effect on the study procedures and or the study variables. No laboratory parameters were used in the definition in this study.

5.5 Sampling

All consecutive patients with documented diagnosis of SCA in steady state and on follow-up will be recruited into the study. The sample size was calculated as follows:

Sample size calculation (The Daniel's formula 1999 for finite population (61))

n = Nz2pq/ (E2 (N-1) + z2pq)

Where

- N (population size) = 100, - Z (confidence level) = 1.96
- E (±error) = 0.05, - p (prevalence) = 0.3
- Q (1-p) = 0.5 n = 80

Sample size have 80% power to detect SD of mean diff. on SF level btw a binary variable (age <30 vs. ≥30), using a 2 sided t-test at a 0.05 significance

5.6 Selection of patients

Inclusion Criteria

Clinical diagnosis of sickle cell anaemia with results of haemoglobin electrophoresis showing homozygous HbSS or HbSF in patients 13 years and above who signed consent or assented or legal guardian signed consent. Positive sickling test was subjected to haemoglobin electrophoresis and where HbSS was found they were included. The haemoglobin electrophoresis was not repeated if one had been done before.

Exclusion Criteria

Sickle cell trait (HbAS), acute illness or crisis, Pregnancy and patients on chelation therapy.

6.0 Procedure

The patients were recruited at the haematology clinic. The purpose of the study was explained to the patients and their relatives. The objective of the study being to determine the serum iron status in sickle cell anemia patients attending hematology clinic at the KNH (Appendix I).

The patient or parent/legal guardian was then requested to give written informed consent. Failure to enrol into the study did not jeopardize their care in any way and they could withdraw from the study at any point. Those who were in crisis were attended to.

Following enrolment, the patient's medical history was taken and documented in a standardized data extraction proforma. Sociodemographic data age , sex, education, marital status, county of birth, were documented .Transfusion and chelation history was also documented. Three ml of blood was collected using the standard procedure and then put in sequestrate tube (EDTA) for the Total blood counts (TBC), ESR ,blood film studies at the haematology laboratory.

The TBC was done using the automated CELL-DYN 3700 analyser, PBF after staining with maygrunwald/giemsa stain.

Reticulocyte count was performed by mixing blood with supravital stain in blood slide. ESR was measured using the Wintrobe tube method. Four ml of venous blood was put in a sterile bottle for serum ferritin. The specimens were transported to the respective laboratories within one hour of collection. Specimen for serum ferritin was stored at -20°C till the time of analysis.

Each specimen was accompanied by laboratory request form with name of the patient, hospital file number and specific study number to avoid mix up of samples.

6.1 Specimen Analysis

Laboratory test for serum ferritin were run in batches using enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) kits. The Principal investigator with the assistance of laboratory technical staff did the analysis. Total blood counts were done using

the Haematology cell counter (CELL-DYN 1300) while the PBF were stained using the May Grunewald Giemsa stain by the Principal Investigator with guidance from the laboratory technicians. The PBF slides were first read and reported by the Principal Investigator after which the two Haematologist who were also the supervisors confirmed the report. These reports were then transferred to the standard questionnaire. Copy of the report was relayed to the patients files to help with management and follow-up.

6.2 Quality Assurance

The standard operating procedures was adhered to**.** In the pre-analytical phase, we ensured request/order forms were written for the tests required with proper patient identification before drawing the samples. The patient unique identification number was written on the request form and the sample collection container. Sufficient amount of sample i.e. 3mls for TBC, ESR and PBF and 4mls for serum ferritin, LDH were drawn in the appropriate container in proper order using standard procedures and delivered to the laboratory within the 3-4 hours. The machines used for analysis were calibrated according to manufactures recommendations.

The KNH/UON haematology and biochemistry laboratories run daily internal and external quality control on all tests. Their machines/equipment are calibrated daily using commercially available kits.

7.0 Data Management

The data acquisition instrument was the study questionnaire. Patients files were reviewed to obtain information on the disease and medication used and if possible transfusion history. A brief targeted history and physical examination was undertaken after recruitment. Data collected was verified then coded. The samples were also given the corresponding unique code/number. The forms were then kept under lock and key till "n" was achieved. Data was gathered, pooled, screened then entered into the pre-programmed format in the Statistical Package for Social Science (SPSS) version 21.0 and cleaning done before analysis. Back up for the data was done in an external hard drive and written on a compact disk (CD), and versatile compact disk (DVD). Access to these back-ups was limited to the PI only.

7.1 Data Analysis

80 SCA patients were enrolled in this study. Our sample size had at least 80% power to detect standard deviations of mean differences on serum ferritin level between a binary variable using a two sided t-test at a 0.05 significance level. Descriptive statistics were used to summarize basic demographic variables, including frequencies, mean, median and SD for level of ferritin. Chi-square testing was used for binary variables and t-test for continuous variables. Multiple logistic regression analysis was performed to assess any associations between elevated ferritin levels and other patient parameters, including demographics, clinical and laboratory data.

8.0 Ethical Consideration

Informed consent/assent form was signed by the patients or parent in case of patients below 18 years of age, as established and consistent with the policies of research of clinical type for government of Kenya ministry of health/research science and technology and university of Nairobi/KNH ethics and Research Committee. The venepanctures were done by the PI using the standard procedure to minimize harm. Confidentiality was maintained throughout the study. The finding of this study was communicated to the clinicians for timely and continued management of the involved subjects and those seen in future. Those who did not sign informed consent or assent were not victimized.

9.0 Results

The study was conducted between March 2015 to Aug 2015. The characteristics of the study population is presented below:

9.1 Demographic characteristics of the study population

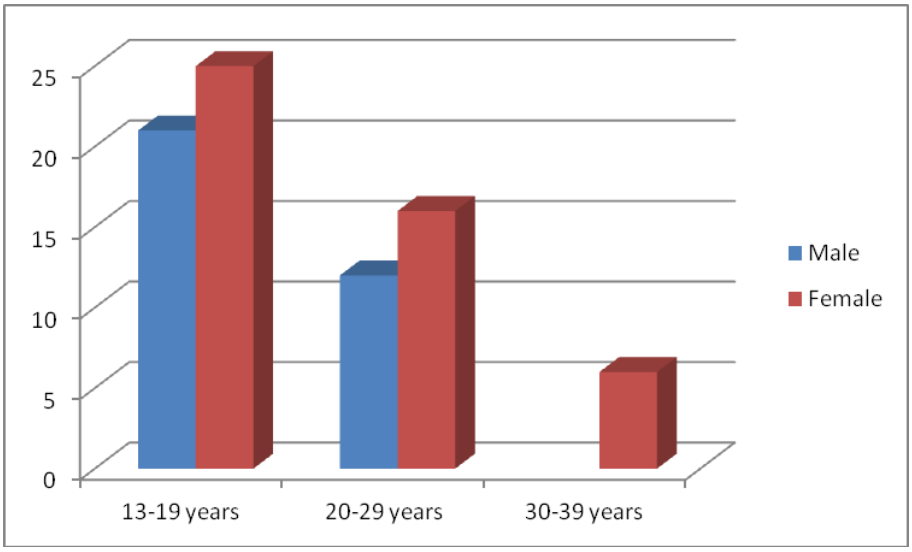
Eighty six (86) patients with SCA aged 13 years and above were screened and subsequently, 80 were enrolled into the study. There were 33 (41.3%) males while the females were 47 (58.7%) of the study population. The mean age of the population was 19.7 ±5.53years with a range of 13-37years. The average age of the males was 18.5±3.4 years while that of the females was 20.5±6.5 years. Majority of the patients were in the 13-19 age group 45 (58.8%) while only 6(7.5%) were above 30 years of age. Figure 1

The majority of the patients (85%) were from Western and the Lake region of Kenya mainly inhabited by the Luo and Luhya ethnic groups. Most of them were current residents of Nairobi. Minority cases from the Kamba and Kisii/Kuria ethnic groups were also noted. Only 8.9% of the patients were married with about 28.2 % employed and majority (45%) having attained only secondary level of education. Table 1

Table 1: The demographic characteristics of the study subjects

Parameter	Male: N=33(41.3%)	Female N=47(58.7%)	Overall n=80
Mean Age(SD)	18.56(3.4)	20.59(6.5)	19.76±5.53
Formal Education Level			
Primary	08(24.2%)	13 (27.7%)	21(26.3%)
Secondary	18 (54.5%)	18 (38.3%)	36(45.0%)
College	07 (21.2%)	16 (34.0%)	23(28.7%)
Occupation			
Employed	03 (9.1%)	11(23.4%)	14(17.5%)
Self-employed	03(9.1%)	06(12.8%)	09(11.3%)
Unemployed	27(81.8%)	30(63.8%)	57(71.2%)
Marital Status			
Married	01 (3%)	07(14.9%)	08(10.0%)
Single	32 (97.0%)	40(85.1%)	72(90.0%)

Figure 1:The Age ,sex distribution of the study population



9.2 Treatment History of the study population

Nearly all patients 97.5%, were on folate therapy. More females 46 (58%) than males 27(34%) were on Hydroxyurea. Thirty two (40%) of the study population were on an opiod analgesics (Tramadol and dihydrocodein) while 25(31%) were on Nonsteroidal anti-inflammatory agents (Ibuprofen, diclofenac, aceclofenac). Six (7.5%) were on iron supplementation. Table 2

Transfusion History

Seventy four (92.5%) of the study population recalled having received at least one unit of blood transfusion in their lifetime and only 6 (7.5%) could not recall having received any blood transfusion. More females 45(56.3%) than males 29(36.3%) reported having ever received blood transfusion. The mean units of blood transfused was also higher among the females (10.68±7.99 than the males 9.85±7.01. The average number of units of blood transfused was 10.5±7.5 ranging from no unit transfused to maximum of 28 units transfused. Most patients 45(56.3%) had received between 1-10 units of blood transfused. Table 2, Figure 2

Table 2:Treatment History of the Study population

Parameter	Male	Female	Overall
Mean age years (SD)	18.56(3.4)	20.59(6.5)	19.76±5.53
Folate Yes No	 32 (97.9%) 1(2.1%)	 46 (97.9%) 1 (2.1%)	 78(97.5%) 02 (2.5%)
Hydroxyurea Yes No	 27 (81.8%) 6 (18.2%)	 42(89.4%) 5(10.6%)	 69(86.3%) 11(13.7%)
Analgesic Opiod NSAIDS	 10(30%) 14(42%)	 22(46.8%) 11(23.4%)	 32 (40%) 25(31.3%)
Transfusion history YES NO	 29(87.8%) 4(12.2%)	 45(95.7%) 2 (4.3%)	 74(92.5%) 06 (7.5%)
Mean units of blood transfused	9.85(7.01)	10.68(7.99)	10.5 (7.5)

Figure 2:The units of blood transfusions in the study subjects

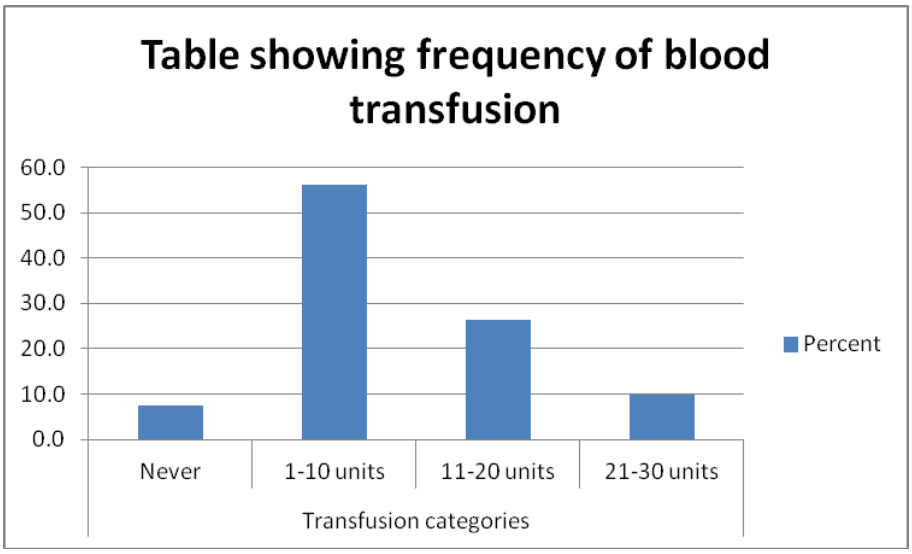


Table 3: Mean Serum Ferritin (SF) in study subjects

SF(ng/ml / Blood	Mean	SD	N	Min	Max
YES	943.7	510	74	282.30	11000.00
NO	798.1	530.2	6	53.00	1016.00

Seventy four (92.5%) of the study patients had at least one blood transfusion in their lifetime, while six (7.5%) patients had never been transfused with blood. The mean number units of blood transfused were 10.46±7.5. The median units transfused were 10 ranging from 0-28 units. The mean SF was 943.7ng/ml in ever-transfused group compared to 798.1ng/ml in the never-transfused group. This was statistically significant at p=0.04. Table 3

9.3 Laboratory results of the study population

The red cell indices were analysed according to the reference ranges of the laboratory used. The mean haemoglobin was slightly higher in the males 8.6±1.6 g/dl than in the females 8.5±1.1g/dl. The mean MCV, MCH and MCHC were found to be within the reference ranges for both sexes.

The mean serum ferritin was higher in the males 980±487ng/ml than in the females 780±456ng/ml but this difference was not statistically significant, p=0.71. Mean LDH levels were also found to be higher in the males 581±225IU/L as compared to the females 509±217U/L. This difference was also not statistically significant. Table 4

Table 4: Laboratory Results of the Study population

Parameter(mean)	Overall n=80	Male n=33	Female n=47
Hb/dL(SD)	8.72(1.2)	8.6(1.6)	8.5(1.1)
WBC(X109)	10.80(3.3)	11(3.7)	10.9(2.9)
MCV(Fl)	89.7(8.3)	90(9.3)	88(7.8)
MCH(g/dl0	25.3(3.7)	31(4.1)	30.1(3.4)
MCHC(g/dl)	30.7(3.8)	35(1.8)	34.5(1.6)
PLT	436.1(151.4)	442(128.4)	389(136.8)
SF (ng/ml)	939.25(668)	980.9(487)	780.4(456)
LDH(u/l)	539.25(222.4)	581(225)	509(217)

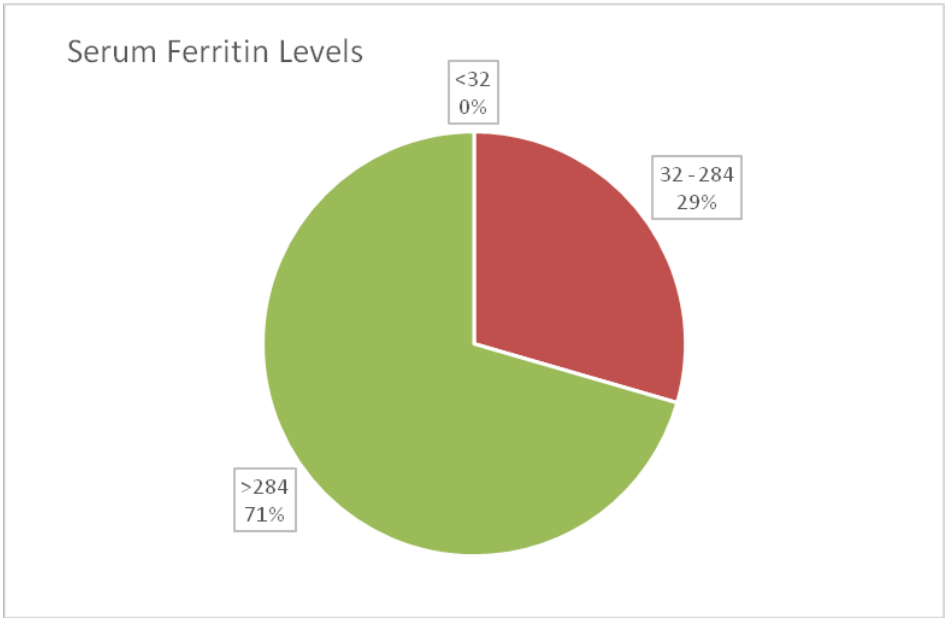
9.4 Peripheral Blood Film

The red cells showed moderate to marked anisopoikilocytosis with macrocytes 61.2%, microcytes 10%, target cells 61.2% and Sickle cells 72.5%. Other poikilocytes of note seen included pencil forms and RBC fragments seen in 16% of the films. Polychromasia was seen in 90% of films with hypochromasia being seen in 3% while 10% of the films were normochromic. Also noted were nucleated red cells in about 70% of the films with Howell-Jolly body inclusions seen in 2% of the films. Leukocytosis was only noted in 21.2% of the films in which also reactive lymphocytes were observed. Thrombocytosis was a common finding in 52% of the films but the platelets were normal in morphology. There were no parasites in any of the films.

Table 5: Serum ferritin levels in the Study subjects

		N	%
Serum Ferritin categories	<32	0	0.0%
	32- 284	24	29.5%
	>284	56	70.5%

Figure 3: Serum ferritin levels



The normal reference range for serum ferritin for the laboratory method used was 32-284ng/ml. The SF levels had a wide range hence a wide standard deviation. The mean SF level was 939.25±668ng/ml. The lowest SF level observed was 53ng/ml while the highest was 11,000ng/ml. Twenty three 24 (29.5%) had SF within normal reference range while the majority 56 patients (70.5%) had high SF. None of the study patients were found to have serum ferritin below reference range (Figure 3, Table 5).

The SF levels were further categorised as normal range (32-284ng/ml), high (285-1000ng/ml) and very high (>1000ng/ml).

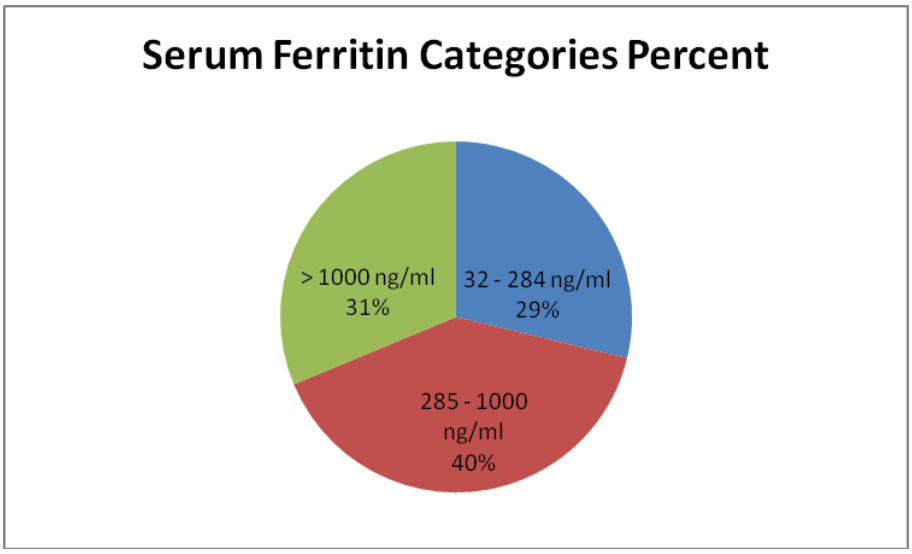
Twenty five 25(31.3%) patients had SF above 1000ng/ml. The prevalence of iron overload in this patient population could therefore be estimated at **31.3%.** Thirty two (40%) had SF of between 285-1000ng/ml which is high but not meeting the threshold for iron overload.

These patients would require serial SF monitoring as they are at risk of overload (table 7, figure 4 and 5)

Table 6: Normal/high SF Categories of the study subjects

Serum Ferritin categories	Frequency	Percent
32-284 ng/ml	23	28.8
285-1000 ng/ml	32	40.0
> 1000 ng/ml	25	31.3
Total	80	100.0

Figure 4: Distribution of SF among the Study patients



9.5 Relationship between SF and Age

There was a non -linear correlation between SF and age in the study population. SF was not demonstrated to increase with age. Figure 6There was also no significant association between SF and gender. The mean SF was 980ng/ml and 780ng/ml in Males and females respectively p=0.71 (table 7)

Figure 5: Scatter plot of age and SF levels of the study population

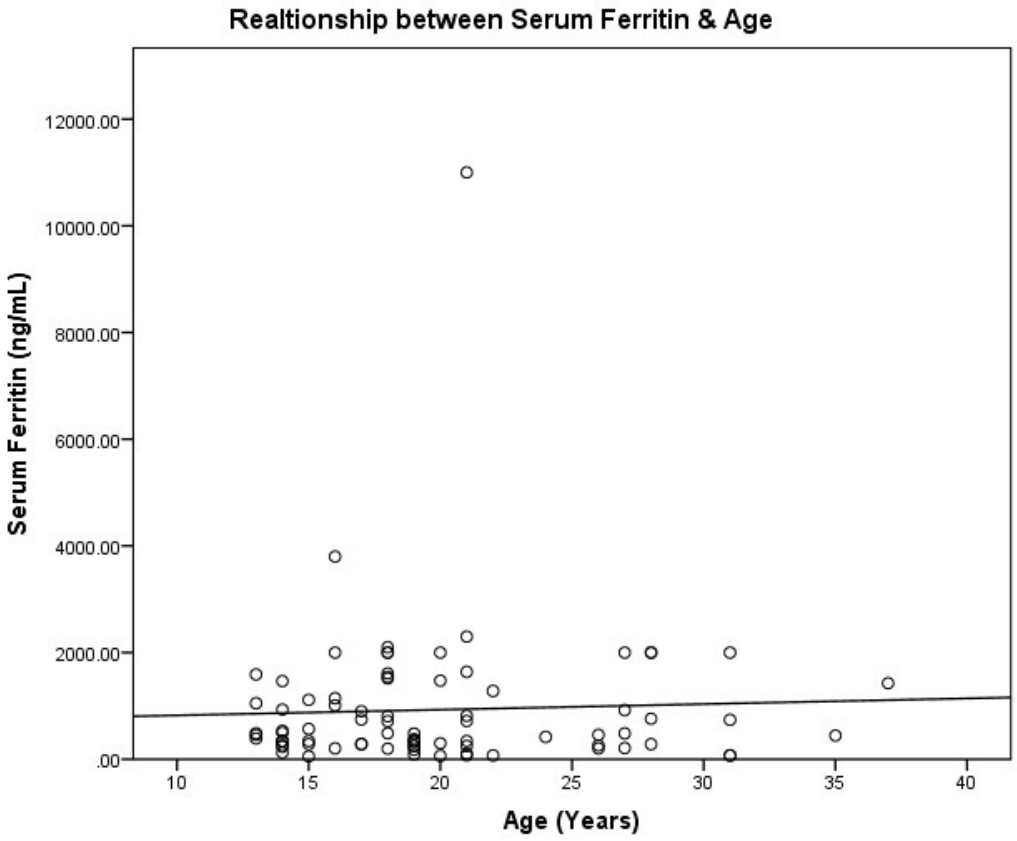


Table 7: Relationship between SF and gender

Parameters	**n**	**Mean ferritin**	***P*** **value**
Male	33	980 ± 456.8	0.71
Female	47	780.5 ± 420.2	

Table 8: Serum ferritin levels and mean number of units of blood transfused in study subjects

Parameter	Ferritin	N	Mean	Std. Deviation	P value
Mean number of blood units transfused	32-284	23	8.0870	5.85362	<0.001
	285 - 1000	32	8.4839	6.51087	
	>1000	25	15.1200	8.16660	
	Total	80			

The mean SF concentration in this study was 939.25 ±768 ng/mL, ranging from 53-11,000 ng/mL. Among the females the average SF was 780.4 ± 564.2 ng/mL while in males it was 980.35 ± 468.80 ng/mL. The SF ranged from 53-1690 ng/mL in the females and from 274-11000 ng/mL in the males. There was no significance difference in SF levels between males and females; unpaired ***P*** = 0.71. There was however a strong association between SF and the number of units of blood transfusion (p <0.001). (Figure 7).

Figure 6:

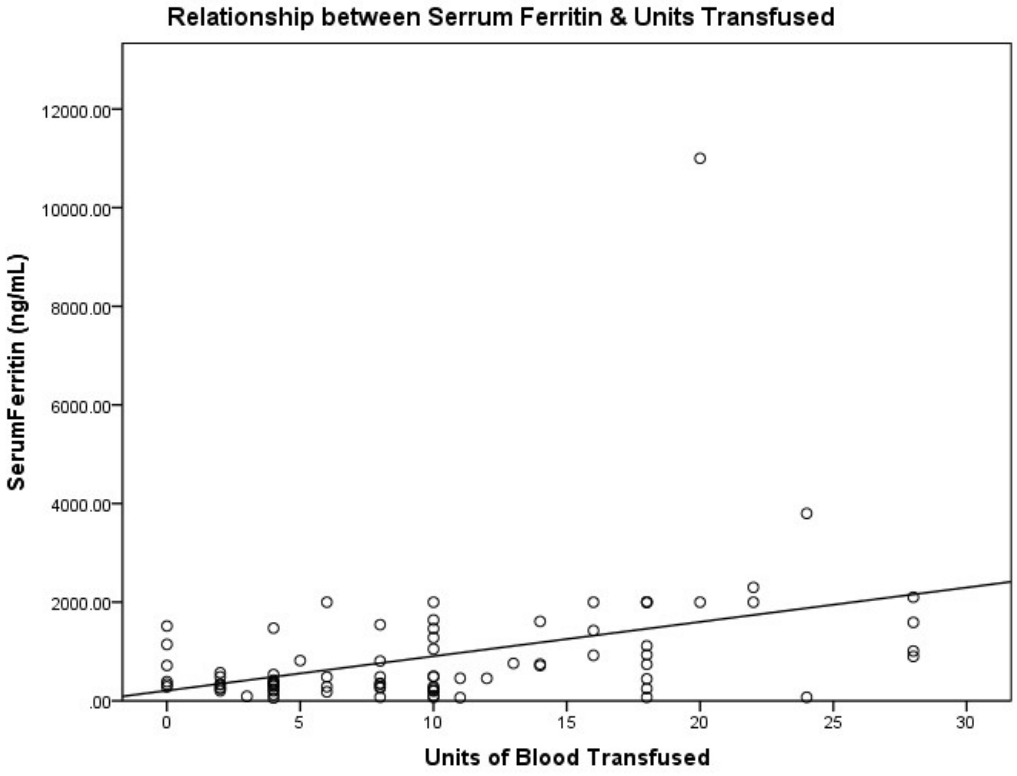


Table 9: Serum ferritin and Red cell indices, WBC and ESR

Parameter	SF categories	N	Mean	SD	P value
Hb(g/dl)	32-284	23	8.4	1.2	0.133
	285 - 1000	32	8.8	1.2	
	>1000	25	9.0	1.1	
WBC	32-284	23	11.5	3.2	0.615
	285 - 1000	32	10.2	3.3	
	>1000	25	9.4	3.2	
Mean corpuscular volume (MCV) fl	32-284	23	87	8.2	0.114
	285 - 1000	32	90.7	8.7	
	>1000	25	90.0	7.8	
Mean Corpuscular Haemoglobin(MCH)g/dl	32-284	23	29	3.4	0.593
	285 - 1000	32	31.1	3.9	
	>1000	25	31.0	3.9	
Mean Corpuscular Haemoglobin Concentration(MCHC)g/dl	32-284	23	35	1.8	0.865
	285 - 1500	32	34	1.7	
	>1500	25	35	1.5	
Reticulocyte %	32-1000	21	3.8	1.5	0.528
	285 - 1000	32	3.3	1.3	
	>1000	24	4.2	1.6	
ESR Mm/hr	32-284	23	9.4	4.9	0.545
	285-1000	32	10.7	7.9	
	>1000	25	13.6	8.2	

We did not find any statistically significant association between SF and any of the red cell indices or WBC and ESR (Table 9)

10.0 Discussion

The findings of high ferritin levels indicating increase in body iron stores are in keeping with the expectations of others who have examined for levels of serum ferritin levels of SCA on hospital managements. Indeed currently none invasive methods of assessing serum iron status is by measuring serum ferritin**.**

This index study showed that majority of SCA patients had high serum ferritin levels. Fifty six (70.5%) of SCA patients in this study population had elevated serum ferritin with twenty five (31.3%) having very high SF more than 1000ng/ml. Twenty three (28.8%) of our study population had SF within the normal range and none had low SF. Serum Ferritin values beyond 300ng/ml signifies increased iron stores while those beyond 1000ng/ml is predictive of iron overload. Serum ferritin of 1000ng/ml is the validated threshold for considering iron chelation therapy when used with transfusional iron overload of more than or equal to 20 units of transfused blood. This was an expected finding in SCA anemia, which is characterized by chronic hemolysis punctuated by hyper hemolytic states with subsequent recycling of iron and anemia leading to recurrent blood transfusion resulting into increased iron stores.

Increase in iron stores in the multitransfused subjects reflects the amount of ferritin in plasma.(16) The association between SF and body iron stores is affected by confounders such as vitamin C deficiency, fever, infection, inflammation(62) ,and hepatic dysfunction, which were not investigated in our study. (63).

Majority, 92.5% of our study population, recalled having been transfused at least one unit of blood in the preceding five years. The mean units of blood transfused were 10.46±7.5, ranging from no units transfused to maximum of 28 units of blood transfused.

Our findings of high ferritin levels indicating increase in body iron stores are in keeping with other studies on iron status among SCA patients. Diop et al in a study of adolescent and adults with SCA in Senegal found out that at an average age of about 20 years, only 30% of the study patients had received blood transfusion in their lifetime.(64) Our study also demonstrated a strong association between SF and units of blood transfused, p=0.001.

Stettler et al in their study on iron status in patients with SCA in Philadelphia assessed iron status in 104 non-transfused children and adolescents of mean age 17.6 years. They also found normal to high SF in all their patient population (56). We studied 80 patients with a mean age of 19.7 ±5.53years and all our study population had normal to high SF levels. We also demonstrated high serum ferritin even in those who reported never having been transfused at all. Ikusemoro and his coworkers evaluated serum iron status in a study of 86 multiply transfused patients against controls in Benin City. They found out that the mean SF levels was higher in the patients who had multiple blood transfusions ($p < 0.001$). Their study also demonstrated a strong positive correlation between the serum ferritin and the number of units of blood transfused ($r = 0.719$, $p = 0.0001$. (60).This is in keeping with the results from our study which have also shown a strong association between SF and units of blood transfused $p=0.001$. They also did not find any association between SF with age or sex. Hamartz et al at The University of California however found SF as a poor marker for accurately assessing serum iron overload. (47). This is in line with our findings that suggests that SF should be interpreted alongside transfusional iron overload.

We found that 31.3% of the study population had very high SF more than 1000ng/ml and had received an average of 15 units of blood. Studies in thalassaemia and myelodysplastic syndrome have demonstrated benefit of initiating chelation therapy at SF more than 1000ng/ml and blood transfusion history of more than or equal to 20 units of blood. (1, 47, 60, 63, 65). Blood transfusion is a lifesaving treatment in SCA and is also often used as prophylactic treatment of complications such as prevention of primary and second stroke. Most patients require transfusion from early childhood. Recurrent transfusions invariable lead to iron overload. (60)

This study demonstrated an association between serum ferritin and number of units of blood transfused , $p = 0.001$). Thomas Adamkiewicz and colleagues in Canada studied Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion and also found a significant association with iron load and liver damage. (44, 63).

Serum ferritin levels should therefore be interpreted with caution when used to measure body iron status and as a guide to long term chelation therapy. Its value is improved when used together with transfusion iron overload calculation. Direct iron store determination is

necessary and the new approaches of body iron assessment such as Super-conductance quantum interference devise (SQUID) and the special MRI should be fully supported and validated.

Iron overload is associated with poor clinical course of SCA.(39, 46) Majority of our patients had high ferritin levels with 31.3% having SF of 1000ng/ml signifying concern about iron overload. Iron overload also predisposes to disease severity. The contribution of iron overload to the poor clinical course with increased morbidity and mortality among our SCA patient population is yet to be determined and this study therefore has set the hypothesis that need to be explored further. However, serum ferritin is also inflammatory protein and may be elevated in other conditions such a chronic infections, subclinical illness, dietary iron, alcohol intake, hemochromatosis, hepatocellular disease and megaloblastic anaemia which we did not investigate in this study. Further studies are therefore needed to address these confounders.

None of our study patients had low serum ferritin. Low SF has a high specificity and sensitivity for iron deficiency anaemia. Iron deficiency, complicating SCD, is likely to worsen the clinical state of the disease since iron plays a central role in erythropoiesis and many other intracellular processes in all the tissues of the body. The chronic hemolysis with the subsequent recycling of iron makes iron deficiency a rare complication among SCA patients. Mohany et al in India observed low SF in a significant number of Indian children and adolescents with SCA.(66) Most of these patients had no history of blood transfusion. He however did not investigate for other causes of iron deficiency in his study. His conclusion was that the children were iron deficient.

Persons with SCA could suffer iron loss through other means such as bleeding chronic leg ulcers, gastrointestinal loss due to chronic NSAID use and worm infestation among others. Olaniyi et al. at the University of Ibadan, Nigeria studied SF levels in 90 patients with SCA who had not received blood transfusion and revealed that they had lower serum iron than the controls.(57)

Das et al in India demonstrated as high as 23% prevalence of low serum ferritin in children and adolescents cases of HbSS. (48)They also detected in the same study high SF in 15.4% of the cases which correlated well with number of blood transfusions. Most other studies have not demonstrated iron deficiency. These variations could be explained

by environmental causes common to a particular geographical location of study such as tropical setting with heavy infectious diseasee diseasee burden and parasitic infestations.

As regards haematological parameters, the significantly reduced Hgb with normal MCV in this study confirmed the chronic haemolytic process on-going in SCA patients. All our study subjects had haematological evidence of anaemia of varying degree with a mean Hb of 8.72±1.2g/dl with mean MCV of 89.7±8.3fl. Low MCV and MCH in some patients fell below the reference range and may be suggestive of developing iron deficiency or coexistent of alpha thalassaemia with SCA. However the thalassaemia status of these SCA patients was not pre-determined. We found platelet and white cell counts within normal range. This could be due to the fact that we selected ambulatory patients clinically in steady state of the disease. (16).

Our study demonstrated mean MCV within normal range. However there was significant polychromasia with reticulocytosis witnessed in this study could be explained in various ways. Some patients could be recovering from an acute haemolytic episode, since some had reported a crisis in the days preceding the tests. The nature of the disease could also independently give rise to the macrocytosis as seen in the PBF samples or from chronic haemolysis that does results into increased folate utilization leading to folate deficiency that commonly manifests as macrocytosis even though almost all patients were on folate supplementation and reported proper adherence. The chronic anaemia also leads to increased erythropoiesis, and peripheral macrocytosis occurs due to the presence of reticulocytosis. Majority of these patients were also on Hydroxyurea which can also cause macrocytosis. Studies have also reported low vitamin B12 among SCA patients. The nucleated RBC (56%) observed in this study is not unusual in SCA. It could be due to hyposplenism and autosplenectomy or increased compensatory and medullary erythropoiesis as evidence in SCA. The accompanying Howell-Jolly inclusion bodies suggest presence of autosplenectomy in minority of patients. Splenic infarction and fibrosis results from repeated episodes of splenic vascular occlusions during sickling which leads to autosplenectomy. The presence of Howell-Jolly bodies is associated with severe disease.(16, 57)

Leukocytosis as observed could be due to subclinical inflammation especially due to chronic inflammatory disease that these patients frequently suffer from.(60)

11.0 Conclusion

From the results of this study, it can be concluded that:

1. Most (70.5%) of the SCA patients have raised serum ferritin and would require serial measurement of SF in order to diagnose iron overload early for prompt chelation therapy.

2. Thirty one (31.3%) percent of our study population had SF more than or equal to 1000ng/ml and should therefore be investigated further for iron overload

12.0 Study Limitations

The lack of locally generated data on reference ranges of most parameters analyzed. It would have been preferable to compare the values in this study with those generated from the local population but this was not possible due to limited resources.

Poor record keeping interfered with collection of information on blood transfusion details; where this information was available, documentation of the actual volume of blood transfused was not available. The information on blood transfusion from other hospitals was also challenging to obtain.

Inadequate resources to investigate for other confounders for raised serum ferritin levels among SCA patients.

13.0 Study recommendation

Due to the early onset of rising iron stores demonstrated by the elevated SF in the majority of our study patients and its correlation with the amount of blood transfused, there is need to properly document the amount of blood transfused to a patient with SCA to enable proper calculation of transfusional iron overload.

Further studies are required to evaluate the impact of iron overload in SCA patients in terms of morbidity, mortality and quality of life, variation in serum SF and other indicators of body iron status.

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15.0 APPENDICES

Appendix I: Informed Consent Information

Title of the study "*Serum Iron Status in Sickle Cell Anaemia Patients at Kenyatta National Hospital"*

Principal Investigator: Dr. Oyiro Peter

Introduction: We are interested in finding out the body iron status in patients with SCA in Kenyatta National Hospital. The results from this study will help us manage these patients better since we know that excess of little iron in the body is not desired and hence needs to corrected.

Procedure to be followed: Upon accepting to participate in this study your parents or guardians will be requested to sign a consent form. Once in the study you will be required to answer questions relating to your socio-demographics, present and past illnesses and current treatments.

Risk: There will be minimal risk associated with participation in this study. You may experience pain during blood sample withdrawal for the various tests that we shall conduct.

Benefits: The study will help in the development of policy and treatment guidelines that will improve the quality of care for future generations.

Confidentiality: Strict confidentiality will be maintained and all data obtained will be securely stored and used for purposes of this study only.

Who can participate in this study? Any person aged 13 years and above on treatment and follow-up for SCA at the Hematology-Oncology Clinic at KNH.

Participation: This study is voluntary. Refusal or withdrawal will not be penalized. You may withdraw from the study without any penalty.

Appendix II: Swahili Consenting/Assenting Information

TITLE OF STUDY: "Serum iron status in sickle cell anaemia patients at Kenyatta national hospital"

Maelezo ya idhini

Jina langu ni Dkt Oyiro Peter, mwanafunzi katika kitengo Clinical Medicine and Therapeutics, School of Medicine, chuo kikuu cha Nairobi. Ninafanya utafiti kuhusu sickle cell anaemia. Utafiti huu utatusaidia kuchunguza huu ugonjwa ili tuweze kuufahamu vizuri Zaidi na kutuwezesha kuwahudumia hawa kwa hali ya juu zaidi.Wewe/mtoto wako mumechaguliwa sababu mnao huu ugonjwa. Sampuli ya damu itachukuliwa kutoka kwako/mtoto wako na mimi ama daktari mwingine kwa kutumia sindano. Utafiti utafanywa katika chuo kikuu cha Nairobi, idara ya Human Pathology. Sampuli itachukuliwa kwa njia isio tofauti na kama ilivyo kawaida.

Hatari/Faida

Wakati wa kuchukuwa sampuli mtoto wako atahisi uchungu kiasi. Hakutakuwa na malipo yoyote kwa kuhusika katika utafiti huu. Hutaulizwa kulipa chochote juu ya malipo ya kawaida katika hospitali. Daktari wa mtoto wako atajulishwa majibu ya utafiti huu na natumai yataboresha matibabu yako.Majibu yatawekwa kwenye rekodi za mtoto wako.

Utaratibu wa kuchukua sampuli

Sehemu yamkono ulio na mshipa wa damu ulio mzuri itasafishwa. Kwa kutumia sindano sampuli ya damu ya kutosha itachukuliwa kwa kutumia utaratibu usio tofauti na ilivyo kawaida katika hospitali hii. Kama kutakuwa na uchungu, haitakuwa zaidi ya ile mtu anayohisi akindungwa sindano.

Haki za mshiriki

Kushiriki kwako katika utafiti huu ni kwa kujitolea na ukiamua kutoshiriki, mtoto wako hatanyimwa huduma ambazo angepata kwa kawaida katika hospitali hii. Ukiamua kutoshiriki katika utafiti huu hakutakuwa na adhabu yoyote.

Muda wa ushiriki

Ni sampuli moja tu itakayochukuliwa kutoka kwa mtoto wako kwenye utafiti huu. Utayapata majibu ya mtoto wako kama bado utakuwa kwenye hospitali ama utarudi baadaye ukimpleta mtoto wako kwenye kliniki.

Hakikisho ya siri ya utambulisho wa mshiriki

Rekodi kuhusu ushiriki wa mtoto wako kwenye utafiti huu zitabaki siri na zinaweza tu kujulishwa daktari anayemtibu mtoto wako. Utapewa fomu ya idhini utie sahihi na unaweza weka nakala ya majibu ya mtoto wako.

Appendix III: Consent Forms

Adult-English

I ---------- ----------have been explained to about this study entitled **"***Serum iron status in sickle cell anaemia patients at Kenyatta national hospital"* , by Dr. Oyiro Peter, do understand its purpose, and do voluntarily agree for an examination and blood samples to be taken from me /child. I understand that I/my child will not suffer any extra discomfort and that l will not pay any extra cost. The records and results relating to my participation in this study will remain confidential and will be communicated only to me.

Sign---------- Date ---------- Mobile No ----------

Fomu ya Idhini ya mtu mzima

STUDY NUMBER: _____

Mimi _____ nakubali kushiriki katika utafiti unaofanywa na Dkt Oyiro Peter. Nakubali sampuli itolewe kwangu kama nilivyoelezwa. Naelewa sitapata maumivu zaidi ya ilivyo kawaida. Sitalipa malipo yoyote kwa utafiti huu. Nitaelezwa majibu yoyote yanayoweza kuboresha matibabu yangu. Pia naelewa kuwa naweza kuzitisha ushiriki wangu kwenye utafiti huu wakati wowote bila hofu ya adhabu yoyote.

Jina la mshiriki _____

Nambari ya simu _____

Sahihi ya mshiriki _____

Tarehe _____

Jina la shahidi _____

Sahihi ya shahidi _____

Tarehe _____

Sahihi ya mtafiti mkuu(Ama mwakilishi wake)_____

Mawasiliano

Ukiwa na swali lolote wakati huu ama baadaye kuhusu haki zako kama mshiriki, kuhusu utafiti huu ama madhara yoyote yatakayotokea tafadhali wasiliana na Mtafiti mkuu, Dkt Peter Oyiro, nambari ya simu 0700934072 ama mwenyekiti kamati ya maadili ya utafiti ya Hospitali kuu ya Kenyatta/ Chuo kikuu cha Nairobi kupitia S.L.P 20723-00200 Nairobi ama piga simu 020-726300-44102.

Consent form (Minor)

I have read this information and had it explained to me .I have had my questions answered and I know that I can ask questions later if I have them.

I therefore consent to take part in the research.

Name: ----------

Signature/Mark ----------

Date ----------

I have accurately read out the information sheet to the potential participant who is a minor and to the best of my ability made sure the minor understood. I answered all the questions asked by the minor. I confirm the minor has given assent freely and understands that the parents/guardians still have to sign a consent form. I confirm that the individual has not been coerced into giving consent.

Name of researcher ----------

Signature of researcher----------

Date----------

Appendix IV: Participant's Statement *(to be signed by parent/guardian):*

I…… do hereby give consent/permission to Dr. Oyiro Peter to include the above minor in this study entitled 'SERUM IRON STATUS IN SICKLE CELL ANAEMIA PATIENTS AT KENYATTA NATIONAL HOSPITAL '. I have read and understood the contents of this form, and have been accorded the opportunity to ask questions. I am also aware that the minor can withdraw from this study without any penalties

NAME……. SIGNED……

WITNESS…… DATE……

Fomu ya Idhini ya mzazi wa motto

STUDY NUMBER: _____

Mimi_____nakubali mtoto wangu _____ kushiriki katika utafiti unaofanywa na Dkt Oyiro Peter. Nakubali sampuli itolewe kwa mtoto wangu kama nilivyoelezwa. Naelewa mtoto wangu hatapata maumivu zaidi ya ilivyo kawaida. Sitalipa malipo yoyote kwa utafiti huu. Nitaelezwa majibu yoyote yanayoweza kuboresha matibabu ya mtoto wangu. Pia naelewa kuwa naweza kuzitisha ushiriki wa mtoto wangu kwenye utafiti huu wakati wowote bila hofu ya adhabu yoyote.

Jina la mshiriki _____ Nambari ya simu _____

Sahihi ya mshiriki _____ Tarehe _____

Jina la shahidi _____

Sahihi ya shahidi _____ Tarehe _____

Sahihi ya mtafiti mkuu(Ama mwakilishi wake)_____

Mawasiliano

Ukiwa na swali lolote wakati huu ama baadaye kuhusu haki zako kama mshiriki, kuhusu utafiti huu ama madhara yoyote yatakayotokea tafadhali wasiliana na Mtafiti mkuu, Dkt Oyiro Peter, nambari ya simu 0700934072 ama mwenyekiti kamati ya maadili ya utafiti ya Hospitali kuu ya Kenyatta/ Chuo kikuu cha Nairobi kupitia S.L.P 20723-00200 Nairobi ama piga simu 020-726300-44102.

Appendix V: Proforma Questionnaire

Study No_____ Date _____ Institution Collector's Name_____

PATIENT

Name_____ Age_____ Sex_____ County of Birth_____

Occupation ☐ 1. Employed 2. Unemployed 3. Self-employed 4. Other

Education level ☐ 1. Primary 2. Secondary 3. College 5. None

Marital Status: Married ☐ Single ☐ Divorced ☐ Separated ☐ NA ☐

Current Treatment: _____ Duration since prescribed_____

Folate_____<5yrs 5-10yrs >10yrs_

Vitamin B12_____

FeSO4_____

Analgesic (state)_____

Antibiotic (state) _____

Paludrine_____

Hydroxyurea_____<5yrs 5-10yrs >10yrs_

Number of blood units transfused since diagnosis _0-10 10-20 >20_____

Laboratory Investigations

Parameter	Results	Reference
Hemoglobin		Male 14-17 g/dl Female 12-16 g/dl
White blood cell count and differentials		4.0 – 10.0 X 109/L Segmented Neutrophils 2 – 7 X 1099/L (45 – 75%) Lymphocytes 1.5 – 3.4 X 109/L (16 – 46%) Monocytes 0.14 – 0.86 X 109/L (4 – 11%) Band neutrophils < 0.7 X 109/L (0 – 5%) Eosinophil's < 0.45 X 109/L (0 – 8%) Basophils < 0.10 X 109/L 0-3%
Red blood cells(RBC)		Female 4.0 – 5.2 X1012/L Male 4.4 – 5.7 X 10 12/L
Mean corpuscular volume (MCV)		80-100 fL
Haematocrit		Female 37– 46% Male 42 –52%
Mean Corpuscular Haemoglobin (MCH)		28-32 g/dL
Mean Corpuscular Haemoglobin Concentration(MCHC)		32-36 g/dL
Platelet		130 – 400 X 109/L
Reticulocyte count		20-84 X 109/L 0.2-2%
Peripheral Blood film report		
Erythrocyte Sedimentation rate (ESR)		Female < 20 mm/h Male < 6 mm/h
Serum ferritin		32-284 ng/mL
Lactate Dehydrogenase (LDH)		95 – 195 U/L
Hemoglobin electrophoresis		

Appendix VI: May Grunwald Giemsa Stain

Prepare smears and air-dry and fix by immersing in methanol for 5-10 minutes

Transfer the fixed slide to a jar containing May Grunwald stain that has been freshly diluted with an equal volume of buffered water for 15 minutes.

Transfer films (without washing) to the jar containing Giemsa stain, freshly diluted with 9 volumes of buffered water at PH 6.8 for 10-15 minutes

Transfer the smears into container with buffered water at ph 6.8

Rapidly wash in 3-4 changes of water and finally allow to stand undisturbed in water for 2-5minutes, for differentiation to take place

Stand the slide upright to air dry and examine thereafter

Appendix VII: Serum Ferritin Assay

To follow the Kit manufactures instruction and procedures

Principle

The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. The reaction medium is cycled in and out of the SPR several times.

Procedure

1. Remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes
2. Use one FER strip and one FER SPR for each sample, control or calibrator
3. Type or select FER on the instrument to enter the test code
4. Mix the calibrator, control and sample using a vortex type mixer
5. Pipette 100µl of calibrator, control or sample into the sample well
6. Insert the SPRs and the strips into the instrument. Check to ensure that the colour labels with the assay code on the SPRs and the reagent strips match
7. Initiate the assay as directed in the Operator's manual. The assay will be complete within approximately 30 minutes
8. After the assay is completed, remove the SPRs and strips from the instrument
9. Dispose off the used SPRs and reagent strips in an appropriate recipient.