PREVALENCE OF IRON DEFICIENCY IN CHILDREN WITH SICKLE CELL ANAEMIA AT MUHIMBILI NATIONAL HOSPITAL.

By

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A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine (Paediatrics and Child Health) of the University of Dar es Salaam.

University of Dar es Salaam

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: *The Prevalence of iron deficiency in children with sickle cell anaemia at Muhimbili National Hospital Dar es Salaam,* in partial fulfillment of the requirements for the degree of Master of Medicine (Paediatrics and Child Health).

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DEDICATION

To my late father Eleuther Mangosongo

Also

To my mother Roselyne, my sisters Pete and Gloria.

To my brothers Gasto, Salutaris and Pascal.

To my nephews Innocent and Austin.

To my nieces Rose, Innocensia and Nancy.

ABSTRACT

Background

Sickle cell anaemia is a genetically inherited haemoglobinopathy and is among the major cause of severe anaemia in children in Tanzania. The anaemia is of haemolytic type but several studies have reported iron deficiency anaemia in these patients. [20,21,22] The possible causes of iron deficiency in these children have been speculated to be similar to non-sickler children. [20]

Objective

To determine the prevalence of iron deficiency and possible contributing factors in sickle cell anaemic patients

Study design

A cross sectional descriptive study

Study setting

The study was conducted at the paediatric out patient clinic for sickle cell anaemia of Muhimbili National Hospital in Dar es Salaam, from August 2002 to February 2003.

Subjects

A total of 100 children aged between 6 months to 10 years inclusive attending outpatient sickle cell clinic, were recruited into the study.

Methodology

All haemoglobin SS patients between 6 months and 10 years inclusive who actually attended the sickle cell clinic, and whose parents/guardians consented for the study were enrolled. Simple random technique was used to select the patients. Five milliliters of blood was taken from all children for Serum ferritin, Serum iron, Total Iron Binding Capacity (TIBC), full blood count and peripheral smear.

Main outcome measure

The main outcome measure was the magnitude of iron deficiency anaemia.

Results

One hundred patients were recruited during the study period. Males were forty-seven (47%) and fifty-three (53%) were females. Forty-five children (45%) were under five years of age. Thirteen (13%) patients had iron deficiency. Females were more Iron deficient than males. The prevalence of iron deficiency was not significantly influenced by sex (p- value=0.208). There were slightly more patients with iron deficiency in children less than 60 months of age (16.2%) compared to those above 60 months of age (10.5%)The difference was not statistically significant (p-value= 0.397). Dietary intake, presence of hookworm and level of hemoglobin concentration did not significantly influence the body iron status (p- values were 0.589, 0.426 and 0.491 respectively).

Conclusions and Recommendation

Iron deficiency occurs in patients with sickle cell anaemia. Age, sex, dietary iron intake, and hookworm infection did not influence body iron status. It is recommended that sickle cell anaemic patients on regular folic acid and chloroquine prophylaxis whose haemoglobin levels are consistently low without the apparent cause of severe anaemia should have blood taken for full blood picture. All patients with a microcytic hypochromic type of anaemia should be investigated for iron deficiency.

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ABBREVIATIONS

SCA-Sickle cell anaemia

MCH-Mean corpuscular Haemoglobin

MCHC-Mean corpuscular haemoglobin concentration

FEP- Free erythrocyte porphyrin

TIBC- Total iron binding capacity

HBSS-Homozygous state for haemoglobin S

HBSC-Haemoglobin SC disease

HBAS-Sickle cell trait

RBC-Red Blood Cell

IDA-Iron Deficiency Anaemia

HB-Haemoglobin

MCV-Mean corpuscular Volume

FBC-Full Blood Count.

RDA-Recommended Daily Allowance

MUCHS- Muhimbili University College Of Health Sciences

DEFINITIONS

- Iron deficiency was defined as the presence of the following on any occasion; serum ferritin <25ng/ml, and one of the following; serum iron < 40ug/dl or TIBC > 400ug/dl.
- Severe anaemia was defined as haemoglobin concentration $\leq$ 5g/dl.

1.0 INTRODUCTION AND LITERATURE REVIEW.

Sickle cell anaemia is a condition resulting from mutant autosomal gene responsible for the synthesis of haemoglobin S (HbS). [1,2] Sickle cell anaemia is among the major cause of severe haemolytic anaemia in children in Tanzania. Clinical features of sickle cell anaemia usually begin around four to six months of age following significant replacement of haemoglobin F by haemoglobin S (HbS). Circulating sickle cells are fragile, non-deformable and are usually rapidly destroyed leading to haemolytic anaemia. [2] Studies have shown that significant folic acid deficiency occurs in sickle cell anaemic patients. [3] Iron deficiency has also been demonstrated in sickle cell patients and this should be considered in sickle cell patients with a hypochromic microcytic blood picture.

1.1 SICKLE CELL DISEASE.

1.1.1 Epidemiology.

The sickle cell gene has a high prevalence in some ethnic groups and geographical areas. The highest gene concentration is in Equatorial Africa where it reaches up to 40%. It is less common in Northern and Southern Africa where the prevalence is 0.2% to 14.7%. [1] The prevalence of sickle cell gene is about 8% in blacks in the United States[2]. In Tanzania, limited studies have been done to identify the magnitude of the problem. In 1972, a study done in some areas reported the prevalence of sickle cell disease to be 16.4% and 31.4% in children aged 1-18months in Dar es Salaam and Musoma respectively. The prevalence was 20%, 38.9%and 18.4% in children aged

more than 12 years in Dar es Salaam, Musoma, and Ulanga district respectively. In Karagwe the prevalence was 12.8% in the age group between 8 days and 20 years. In Dar es Salaam alone, 17% of all children admitted at Muhimbili Medical Center hospital for various reasons are carriers of the sickle cell gene and one in every four has the homozygous state. [4]

At the MNH paediatric out patient clinic, 2000 sickle cell patients were seen from January 2001 to December 2001. These were patients who had been confirmed to be homozygous for the sickle cell gene.

1.1.2 Pathogenesis.

The sickle cell gene is inherited as an autosomal recessive. In the heterozygous state (HbAS) it is referred to as sickle cell trait. The proportion of HbS usually varies between 34%-45%. Sickle cell anaemia results from homozygosity for the HbS gene and thereby designated HbSS. In this state 50% or more of the intraerythrocytic haemoglobin is HbS.

In this condition, valine is replaced by glutamic acid at position 6 of the beta chain. This change produces molecular instability and altered solubility. Homozygous HbSS (sickle cell anaemia), Haemoglobin SC (HbSC), Haemoglobin SD (HbSD), Haemoglobin SO Arab (HbSO Arab) and HbSβ-Thallasaemia are considered as the major HbS diseases. Heterozygosity for HbS (sickle cell trait) and the HbS-HPFH (Hereditary Persistent of Foetal Haemoglobin) syndromes are not considered as HbS diseases. [2,5]

1.1.3 Clinical Presentation of sickle cell anaemia.

The clinical course of patients with sickle cell anaemia is variable. In young patients vasoocclusive and anaemic crises are common. The onset of clinical vasoocclusive symptoms is fairly variable. Symptoms may begin at the age of three months and noted in 50% of cases by one year and virtually in all cases by 5-6 years of life. The development of haemolytic anaemia parallels the declining level of HbF postnatally and becomes obvious by four months of age. During childhood at around 7 years both height and weight are below average with weight being more affected. Chronic ulceration around the ankle is a consequence of chronic anaemia more observed in the tropical than temperate countries. [6] In older patients the effects of chronic anaemia and organ failure secondary to vasoocclusive complications predominate. [7]

1.1.4 Anaemic crisis

Chronic macrocytic haemolytic anaemia characterizes sickle cell anaemia. Three types of anaemic crises can occur:

Hyperhaemolytic crisis.

Hyperhaemolytic crisis is characterized by sudden increase in sclera icterus and marked pallor. The haemoglobin falls despite an increase in reticulocyte count. Acute bacterial infections, use of oxidant drugs and the presence or the co-existence of red cell glucose-6- phosphate dehydrogenase deficiency have been implicated. The clinical course is generally self-limiting. [2,7]

Sequestration crisis

Young patients less than 5 years are at high risk of developing sequestration crisis presumably because splenic infarction has not yet occurred. Patients with sequestration crisis present with sudden onset of weakness, pallor and rapidly enlarging spleen. Physical examination reveals rapid pulse, hypotension and massive splenomegaly. The haematocrit may drop to as low as 10% within hours. Hypovolemic shock can occur because of massive pooling of blood within the spleen. [2]

Aplastic crisis

Aplastic crisis occur during or following an infectious illness. The crisis presents as an acute form of transient red cell aplasia. The crisis is characterized by reticulocytopenia and absent red cell precursors in the bone marrow. The short life span of the circulating sickle cell erythrocyte of 20 days compared to the normal erythrocyte life span of 120 days account for the rapid fall in haemoglobin concentration when erythroid production is inhibited by an infection. It is associated with parvovirus infection. [2]

1.1.5 Vasoocclusive crisis

Vasoocclusive crisis is represented by an extremely painful transient episode that result from ischaemic damage to tissues. The crisis can last a few days but sometimes persist for several weeks. Infection, fever, acidosis and dehydration may precipitate vasoocclusive crisis. Other precipitating factors include cold and anxiety. However, in many cases there are no obvious precipitating events. The symptoms appear as early as three months of age. [2,7] Majority of patients with sickle cell anaemia have vasoocclusive

crisis by one year of age. Musculoskeletal pain is the most common complaint. Dactylitis or hand and foot syndrome is the first manifestation of the disease in 30% of patients. Other sites that can be affected are the abdomen, brain, chest and renal, presenting as acute abdomen, stroke or convulsion and acute chest syndrome. [2,7]

2. DIAGNOSIS OF SICKLE CELL DISEASE.

The diagnosis of sickle cell disease can be made as early as the newborn period by electrophoresis techniques using cord blood samples, isoelectric focusing from a dried blood sample and high performance liquid chromatography. The technique separates haemoglobins A, S and C from foetal haemoglobin F. The diagnosis of sickle cell disease is done using sickling test, and haemoglobin electrophoresis.

2.1 SICKLING TEST

A drop of capillary blood from finger-prick is placed on a glass slide and sodium metabisulphate is added to induce sickling. The slide is then covered with a glass slip, sealed to create a hypoxic environment and incubated at 37^0C for 30 minutes, then examined under the microscope. This is a qualitative test that detects the presence of sickled cells.[8]

2.2 HAEMOGLOBIN ELECTROPHORESIS

Haemoglobin electrophoresis is a procedure used to confirm the diagnosis of sickle cell disease by separating haemoglobin A, from other haemoglobins i.e. C and S. This is done using cellulose acetate electrophoresis. A sample of blood is applied on a cellulose acetate paper on which an electric field is applied. Different components of

haemoglobin move depending on the charge of the molecule towards the positive or negative charge.[8]A patient with sickle cell anaemia has HBSS electrophoretic pattern.

3. IRON DEFICIENCY ANAEMIA

Iron deficiency anaemia occurs when the flow of iron to the erythroid marrow is sufficiently impaired to cause a clear decrease in hemoglobin concentration. It is associated with progressive microcytosis and hypochromia. [2]

3.1 MAGNITUDE OF IRON DEFICIENCY ANAEMIA

The magnitude of iron deficiency anaemia varies according to age. The prevalence depends on social economic status, dietary intake, ethnic customs and geographical area. The prevalence is higher during infancy and adolescent periods. In a study done in Dar es Salaam in 1962, which involved children and adults, iron deficiency was found to be the commonest cause of anaemia accounting for 50% of cases. Eighteen patients were children, 17(94.4%) of them were iron deficient but were not categorized into age groups. [9] In another study in 1966, nineteen out of sixty patients with anaemia had bone marrow examination and 94% (18 out of 19 patients) had iron deficiency. Poor iron stores acquired during foetal life was implicated to be the underlying factor.[10] In 1989 a study was conducted to determine bacterial infections in iron deficient anaemic children at Muhimbili Medical Center. Iron deficiency was the commonest causative factor for severe anaemia. The prevalence of iron deficiency was 11% in the first year of life. The contributing factors were low birth weight (67%), low iron containing weaning foods such as maize and undernourished mothers who had poor milk production. [11]

In a study to determine the incidence of severe anaemia during the first year of life in Tanzania, 86% of the patients with severe anaemia had iron deficiency. [12] The overall prevalence of severe anaemia was 10.7% at a haemoglobin concentration cut off point of 6g/dl. In a nutritional survey done in Lindi district south of Tanzania, 59.7% of pre school children (<5years) had iron deficiency anaemia. The prevalence of iron deficiency anaemia in school children (5-14years) was found to be 40.9%. The iron status was assessed using free erythrocyte protoporphyrin test. [13]

3.2 CAUSES OF IRON DEFICIENCY ANAEMIA

Iron deficiency results when the absorption of iron is insufficient to meet requirements. Iron requirements in turn are determined by growth rates and iron losses. The causes of iron deficiency vary with age .It is common between 6-24 months of age. [2]

3.2.1 Aetiologic factors responsible for iron deficiency in different age groups are as follows:

4—6months;

- **Prematurity**: Most of iron is laid down in the foetus during the third trimester. Premature babies are born with poor iron stores leading to iron deficiency anaemia later especially in the second six moths of life.
- **Growth:** Physiological requirements for growth are associated with an increase in iron demand. Iron is required for functional and structural changes in a human body. Functional changes are increase in weight and height while structural changes involve enzymes function.[14]

- **7-24 months;**

- The causes mentioned in the previous age group continue to be responsible for iron deficiency in this age group.

- Unexplained occult blood loss in stool especially in children fed on whole cows milk.

- In addition, dietary deficiency is common. It is suggested that an imbalance occurs between iron intakes and increased iron requirements because of high growth rates that may be responsible for the cause of iron deficiency anaemia. Early infancy is also a period when complementary feeding begins, and if the complementary foods have low amounts of iron available for absorption, depletion of iron stores are likely to develop. Dietary inadequacy as a cause of iron deficiency in the first six months of life is very unusual. [13]

2-5 years:

- Factors mentioned in previous age groups may contribute to the causes of anaemia in this age group, in particular dietary deficiencies.

- **Blood loss:** The commonest cause of blood loss in Africa is intestinal helminthiasis mainly Hookworm, Trichuris trichura and Schistosoma infections. In hookworm infection the red cells undergo digestion and some of the iron is reabsorbed. In schistosoma infection blood loss is discharged from the body via faecal matter or urine. Using radioisotopes it has been

estimated that about 0.03ml and 0.2ml of blood per worm per day may be lost in Necator americanus and Ancylostoma duodenale infections respectively. Total loss may vary from 2-3ml per day in light infection to 100ml per day in a heavy infection.[14] School children were studied in eight countries in Africa and Asia and the report showed a strong association between iron deficiency and hookworm infection. Countries that participated in the study were Tanzania, Kenya, Ghana, Malawi Mali, Mozambique, Vietnam and Indonesia.[15]

5-14 years:

- In this age group the common causes of iron deficiency anaemia are worm infestations and dietary factors.
- Dietary iron insufficiency is a result of low bioavailability of iron from the common food items consumed as reported in a study done in Lindi region [11]. In Kenya, a hospital based study, however, showed that the main cause of anaemia in children is hemolytic anaemia due to Malaria and sickle cell anaemia (30.2%). Iron deficiency of nutrition origin accounted for 26.2%. Hookworm infestation played a significant role in aggravating iron deficiency anaemia. [16]
- Increase in iron requirements following pre-adolescent accelerated growth.

4.0 IRON METABOLISM AND IRON DEFIENCY

4.1 IRON METABOLISM

Iron is an important micronutrient in the synthesis of haemoglobin. The great bulk of iron in the body exists in haemoglobin in circulation. Iron stores at birth are estimated to be 75mg/kg body weight out of which 55mg/kg body weight is in circulating hemoglobin. [2] The total iron content in an adult is approximately 55mg /Kg body weight, of this 67% is involved with oxygen transport (haemoglobin) and 3% with oxygen storage (Myoglobin). Approximately 30% of iron is deposited in stores as ferritin or haemosiderin. Only a trace amount is present in plasma as transferrin (0.07%) and in tissues as heme enzymes (0.2%). There is little exchange of iron in the body and that of the environment, as a result, the body iron is cycled and recycled through several functional pools. [2]

4.2 IRON ABSORPTION.

Iron in ferrous form is absorbed in the jejunum and enters the mucosal cells bound to apoferritin. Iron balance is achieved by control of absorption rather than control of excretion. Iron for absorption is derived from myoglobin, haemoglobin and some from non-animal sources. [2] Iron in the non-heme pool must be converted to the ferrous form for absorption. Ascorbic acid, meat, fish and poultry enhance iron absorption. Tannates (from tea, coffee), egg yolk, phytates, calcium phosphate and drugs like antacids, tetracycline, and cholestyramine inhibit iron absorption. Intraluminal factor that influence absorption is hydrochloric acid of gastric secretions that promotes

solubilization and reduction of ferric iron. In achlorhydria and following gastrectomy, absorption is reduced. Compounds forming soluble ferrous complexes such as fructose and other keto sugars promote absorption. In iron deficiency, absorption is increased and when iron stores are full, absorption is decreased. In children, iron is lost from the body through sweat, urine, desquamated cells and bleeding from various reasons. [2]

4. 3 IRON TRANSPORT.

From the jejunum iron is transported by transferrin a single polypeptide chain protein that is derived from the liver. Transferrin transports iron to the bone marrow where it is incorporated into haemoglobin. The synthesis of transferrin is determined by body iron content. Transferrin is increased in iron deficiency and decreases in states of iron overload. Low levels unrelated to iron status may be seen in malnutrition, inflammation, disseminated malignancies, liver diseases and protein losing disorders. [2]

4. 4. IRON STORAGE

Tissue stores of iron exist in two forms of ferritin and haemosiderin. These provide a buffer for events that upset the balance of iron turnover between the erythron and the macrophages. When the rate of RBC production exceeds the rate of destruction, iron is mobilized from stores. When red cell destruction exceeds production, surplus is diverted to stores for later use. [2]

5. DIAGNOSIS OF IRON DEFIENCY ANAEMIA

There is no single test or combination of tests that reliably document iron deficiency in all clinical situations. The selection of diagnostic studies is governed in part by the

urgency with which a diagnosis must be made. [2] The key diagnostic tests for the evaluation of established anaemia caused by suspected iron deficiency are; transferin saturation, free erythrocyte porphyrin and serum ferritin. These three tests provide quantitative information about three different aspects of iron metabolism that is transport absorption and storage respectively. [2] Bone marrow aspiration can also be done to determine iron stores. Other diagnostic tests include Total Iron Binding Capacity (TIBC), Total Serum Iron (TSI), Full Blood Count (FBC) and peripheral blood smear.

5.1 TRANSFERIN SATURATION

This is calculated from concentrations of the serum iron and the Total Iron Binding capacity (TIBC)

$$\frac{\text{Serum iron}}{\text{TIBC}} \times 100 = \% \text{ Transferrin saturation.}$$

Low serum iron is of little diagnostic significance. It is subjected to diurnal variations and decreases abruptly with a variety of infections and inflammatory disorders. TIBC a functional measure of transferrin varies inversely to iron stores. It is low in both acute and chronic inflammatory states. When transferrin saturation is below 16% in adults, iron supply become inadequate to support basal erythropoiesis. Studies in children in the United State and Finland have set the lower normal range of transferrin saturation after the age of 4 months to be at 7% to 10%. [2]

5.2 FREE ERYTHROCYTE POPHYRIN (FEP)

This is a functional index of the adequacy of iron delivery to the erythroid marrow. In red cell metabolism the porphyrin incorporates iron to form heme. When iron supply is inadequate, porphyrin accumulates within the cell. FEP is not influenced by developmental changes except in the first few weeks of life when high levels unrelated to iron status are observed. Values in excess of 3ug/g haemoglobin are considered abnormal. The availability of simple and rapid techniques for measuring FEP makes this test useful means for demonstrating iron deficiency and erythropoiesis. FEP increases with decreasing transferrin saturation and progressive degrees of iron deficiency anaemia. Increased FEP is also observed with relative iron deficiency that occurs when an increased erythroid iron requirement exceeds the capacity for delivery from normal reserves. This phenomenon is a feature of sickle cell anaemia and severe haemolytic states. FEP is less susceptible to abrupt changes in iron balance than either transferrin or serum ferritin. [2]

5.3 SERUM FERRITIN

Serum ferritin concentration is directly related to iron stores. During infancy and childhood, serum ferritin parallels developmental changes. Serum ferritin is normally low during late infancy and childhood when there is considerable overlap in values obtained in blood from normal and iron deficient subjects. Concentrations less than 10 to 12ug/l are diagnostic of iron depletion at all ages. Conditions that increase serum ferritin levels include acute and chronic inflammations, metastatic tumors, leukemia,

liver disease and other chronic disorders. In these conditions, there is a release of iron from the red cell mass to storage sites. Ferritin synthesis also increases as a nonspecific response to inflammation. [2] In a study that was done to determine the prevalence and optimal method for laboratory diagnosis of iron deficiency anaemia in patients with sickle cell anaemia, it was found that serum ferritin was the most reliable screening test for iron deficiency. Serum ferritin, serum iron and TIBC establish the diagnosis of iron deficiency. [17] Serum ferritin can also be measured by radioimmunoassay. [18]

5.4 BONE MARROW ASPIRATION

Bone marrow aspiration is a useful laboratory aid in the diagnosis of blood disorders. The preferred site for bone marrow aspiration is the posterior iliac crest using a disposable 16 or 18 gauge needle with an obturator. The aspirate is placed onto a clean alcohol washed dry glass slide .The slide is then stained with Prussian blue staining. In iron deficiency, bone marrow hypercellularity and distortion of normoblast nuclei may be seen. This test shows the content of body iron stores. It is a confirmatory test.[2]

5.4 FULL BLOOD COUNT

Mean corpuscular volume (MCV) represent the mean or average volume of a single red cell. The result is expressed in femtoliter (fl) or cubic microns (μ^3). In sickle cell anaemia, MCV is usually elevated. Low values in sickle cell anaemia may be associated with iron deficiency or Thalassemia. The mean corpuscular haemoglobin (MCH) represent average quantity of haemoglobin per individual red cell. The result is expressed in picogram (pg) or micromicrograms. In chronic iron deficiency anaemia

MCV and MCH are reduced. The two parameters are also sensitive for detection of iron deficiency. [2]

5.5 PERIPHERAL BLOOD SMEAR

Peripheral blood smear show sickled cells. The red blood cells appear in various sizes (anisocytosis) and shapes (poikilocytosis) and occasionally show nucleated red blood cells, small red cell fragments and Howell-Jolly bodies. Howell-Jolly bodies are nuclear remnants located near the erythrocyte membrane indicating absence of splenic Function. [2]

6. IRON DEFICIENCY IN SICKLE CELL ANAEMIA

The anaemia of sickle cell haemoglobinopathy is usually normocytic normochromic. The heme iron released following haemolysis is usually recycled for use and storage. For this reason, it would be uncommon to have iron deficiency in sickle cell anaemia. However micronutrients deficiencies including zinc copper and iron have been well documented in several studies in sickle cell anaemic patients. [3,19]Iron deficiency is now increasingly being reported in several studies. In Tanzania few studies have reported iron deficiency in sickle cell anaemia. In a study done at Muhimbili Medical Center in 1979, on phenotypic expression of sickle cell anaemic patients, bone marrow aspiration was performed in 23 patients. The results showed that 26% of patients had absence of iron stores, 39.2% had reduced iron stores, 17.4% had normal iron stores and 17.4% had increased iron stores. The study included 27 children in the age group 8 to 10 years, but the prevalence of iron deficiency in different age group was not reported. [20]

In another study bone marrow aspirates of 30 patients were examined, nine (30%) were found to have no bone marrow iron and five (16.7%) had small amount of iron stores. Hookworm infestation, gastrointestinal bleeding, bleeding from the renal tract and diets poor in iron content were considered as the possible causes of iron deficiency. [21]

In 1980, Nagaraj investigated 25 patients with sickle cell anaemia for iron deficiency. Eighty percent had never been transfused. The results showed that all patients had low serum iron and transferrin saturation. Three children had no marrow iron and the rest had diminished amount of iron. [22]

A study was done in California USA to determine the prevalence and method for laboratory diagnosis of iron deficiency in seventy patients, 50 with HbSS and 20 with HbSC. The patients were grouped into transfused and non-transfused groups. Serum ferritin, transferrin saturation, MCV and FEP were used to diagnose iron deficiency. It was found that most of the iron deficient patients were from non-transfused group who had low MCV, serum ferritin of <25ng/ml, transferrin saturation <15% and FEP> 90ug/dl. All iron deficiency patients were supplemented with iron and all parameters improved and haemoglobin concentration increased by more than 2g/dl. It was concluded that iron deficiency anaemia, is a potential problem in young non-transfused sickle cell patients. Serum ferritin below 25ng/ml and low MCV are the most useful screening test.[17]

In Nigeria, 45 children aged seven months to fifteen years with HbSS were studied. The investigations done were haemoglobin, reticulocyte count, blood film for cell

morphology, serum iron, bone marrow aspiration and TIBC. Twenty-one (47%) of children showed depletion of iron stores from bone marrow examination. Most children had elevated levels of TIBC and serum iron was below normal levels for age in 14 (31%) of the children. [23]

Rao, in Chicago USA, in 1984 investigated 60 adult patients aged 17 to 62 years. The objective was to define the value of serum ferritin for the diagnosis of iron deficiency in patients with sickle cell anaemia. Serum ferritin and bone marrow aspiration were done, seventeen patients (28.3%) showed absence of stainable iron in the bone marrow, two had decreased stores, 32 had normal stores and 9 had increased iron stores. In iron deficient group, eleven were males and six were females. The speculated causes were gastrointestinal bleeding and urinary iron excretion due to chronic intravascular haemolysis. It was also found that serum ferritin values, <30ng/ml were diagnostic of iron deficiency in patients with sickle cell anaemia with specificity of (98.7%). [24]

In Brazil 34 patients with sickle cell anaemia were compared to 9 siblings with sickle cell trait and 35 patients with normal adult haemoglobin. Levels of zinc, copper and iron were analyzed. It was found that sickle cell anaemic patients had iron deficiency, low plasma zinc levels and were of small stature. [19] There is theoretical evidence that iron deficiency may prevent red cell from sickling on the basis of a decrease in the mean corpuscular haemoglobin concentration. Since iron deficient red cells are less deformable than iron sufficient red cells, iron deficiency could potentially contribute to haemolytic and vasoocclusive crisis.[17] Rao in 1983,reported that reduction in MCHC induced by iron deficiency may ameliorate sickling. It was demonstrated that there is a

decrease in sickling of erythrocytes as a function of oxygen saturation of the blood when patient was iron deficiency. [25]

Iron therapy in sickle cell anaemic (SCA) patients is not advised because of repeated haemolysis in which iron released from destroyed red cells is recycled and retained in the body. It has been observed in different studies that iron deficiency may occur in sickle cell anaemia; therefore iron therapy can be initiated following laboratory investigations. [20, 22]

7.0 RATIONALE AND OBJECTIVES OF THE STUDY.

7.1 Rationale.

Sickle cell anemia is of haemolytic type. [2] The heme iron released following haemolysis is recycled for use and storage. It is not common to have iron deficiency in sickle cell anemic patients. However, several studies have revealed that iron deficiency anaemia occurs in sickle cell anemic patients. The aetiology of iron deficiency anaemia in sickle cell anemic patients has been speculated to be similar to those without sickle cell anaemia.[21, 23] In Tanzania, iron deficiency is a public health problem, but the magnitude of iron deficiency in sickle cell anaemic patients is not known. The previous two reports that used small samples of sickle cell patients gave a prevalence of iron deficiency anaemia ranging from 26% to 30%. [19,20]

The aim of this study was to determine the prevalence of iron deficiency in sickle cell anaemic patients, using a larger sample size than that of previous studies. The information gathered will be used to initiate appropriate intervention measures.

7.2 Hypothesis

There is no iron deficiency among children with sickle cell anaemia.

7.3 Broad objective

To determine the prevalence of iron deficiency and possible contributing factors among children with sickle cell anaemia.

7.4 Specific objectives were to determine:

- The prevalence of iron deficiency among children with sickle cell anaemia by age and sex.
- The association between dietary iron intake and iron deficiency.
- The association between hookworm infestation and iron deficiency.
- The association between haemoglobin concentration and iron deficiency.

8.0 METHODOLOGY

8.1 Study Design

A cross sectional descriptive study was done.

8.2 Study setting:

The study was conducted at Muhimbili National Hospital (MNH) Dar es Salaam, at the paediatrics out patient clinic of sickle cell anaemic patients. Dar es Salaam city had a population of 3 million people in 1999. Nearly 60% of the population was under 19 years of age with an annual growth rate of 7 –10% (Dar es salaam city commission)

MNH is a tertiary referral hospital and a University teaching hospital in the country.

The department of paediatrics and child health runs a sickle cell clinic that operates once every Thursdays except on public holidays. The setting was selected for study convenience.

8.3 Study population

The target population was children aged 6 months to 10 years inclusive attending the sickle cell clinic.

8.4 Inclusion criteria were:

- Children aged 6 months to 10 years inclusive attending the sickle cell clinic.
- Children confirmed to have HbSS

8.5 Exclusion criteria were:

- History of blood transfusion within the last three months prior to recruitment.
- History of acute infection two weeks prior to enrollment.
- Chronic conditions e.g. Tuberculosis, Nephrotic syndrome
- Those who were on iron supplementation.
- Unwillingness of the parents or caretakers to participate in the study.

8.6 Sample size estimation

The sample size was determined using Epiinfo version 6 with an expected prevalence rate of 20%, marginal error of 8% and confidence level of 95%. The sample size was

100 patients. The formula used was

$$\eta = \frac{(1.96)^2 \, p\,(100-p)}{\varepsilon^2}$$

Where η = sample size

p = Expected Prevalence rate = 20%

ε = Marginal error = 8%

8.7 Sampling Technique

A list of all patients aged six months to ten years inclusive attending the clinic on every Thursday was obtained. Simple random sampling technique was used to select the patients to be included in the study. Patients' names were written on small pieces of papers. Papers were then folded, mixed up and spread on the table. The investigator randomly selected ten pieces of papers. Ten patients were recruited once every week.

8.8 Duration of study

The sickle cell clinic attends to about 2000 patients per year with an average of 35 patients per week. Ten patients were recruited weekly. Ten weeks were required to reach a sample size of 100 patients. Two weeks were added to allow for dropouts. So the duration of the study was three months (Twelve weeks).

8.9 Data collection and instruments used.

A structured questionnaire designed for the study was used to collect social demographic data from parents or caretakers. Data collected included name of patient,

date of birth, past medical history and dietary history to obtain food frequency and amount for 4 weeks as indicated in Appendix I. The pilot study done on the amount and frequency of the foods given to the children showed that some foods were given routinely and some were given occasionally. Parents/guardians were asked the type, amount and frequency of foods given routinely on each day in the last one week, and the total amount consumed per month was obtained by multiplying the weekly amount by four. Foods which were given separately had their iron content estimated as a sum of the different foods given over a month period. Parents/guardians were shown a plate, a cup and a glass with known volume capacity to assess the quantities of food fed to their children per day. To exclude any acute or chronic inflammatory conditions, the investigator took history and did a thorough physical examination on every patient.

8.10 Laboratory diagnosis of iron deficiency

Five milliliters of blood were taken from the anterior cubital fossa of each child, using a disposable syringe and needle after a thorough cleaning of the venepucture site with a swab soaked in 70% alcohol. Blood was put in an empty sterile bottle for Total Iron Binding Capacity (TIBC), serum ferritin, serum iron, and in an EDTA bottle for full blood picture.

Serum iron, Serum Ferritin and TIBC were determined using Cobas Mira automated analyser. The blood samples were centrifuged for ten minutes to separate blood cells from serum at a speed of 3000 revolutions per minute. The serum was frozen at a temperature of -20 ^{0}C and was analysed within two weeks after collection.

Determination of Serum ferritin, Serum Iron and Total Iron Binding Capacity.

Serum ferritin:

- Polystyren beads coated with monoclonal mouse anti ferritin antibody were added to the serum and then incubated at 37^0C for 15mins.
- A conjugate was added to the mixture and then washed to remove excess conjugate from unbound sample.
- A cobas core substrate was added and then incubated at 37^0C for 15 minutes
- The mixture was then passed in a photometry. The concentration of serum ferritin was determined by colour intensity. (i.e. concentration is directly proportional to colour intensity).

Serum iron:

A ferrozin^R reagent (Roche product) was added to the serum and formed a complex. The complex was measured photometrically. The serum iron concentration was directly proportional to colour intensity.

Total Iron Binding Capacity:

Serum was incubated at 37^0C for 20 minutes with iron to saturate the available binding sites of serum transferin. Ferrozin^R reagent was added. Unbound excess iron formed the ferrozin red coloured chelate complex that was measured photometrically to determine the concentration of unbound iron. Total Iron Binding Capacity was then determined by the following formula.

TIBC =Serum iron concentration + Unbound iron concentration

Iron deficiency was diagnosed when the serum ferritin was <25ng/dl and one of the following serum iron levels <40ug/dl or TIBC above>400ug/dl (Normal values: Appendix V.) Bone marrow aspiration being an invasive procedure was not done.

Peripheral blood smear

A drop of whole blood from the haematological container (EDTA) was taken and put at the edge of a glass slide for thin smear preparation. This was then fixed by Leishman stain for two minutes. A buffer of PH 6.8 was added and dried in air before examination under microscope using oil emulsion by a high power field (100x).

Full blood picture

This was determined using a Cell - dyn 1200 machine. (Abbot diagnostic division, abbot laboratories, USA). This is a cell count machine that gives the following parameters: HB, MCV, MCH, and MCHC.A blood sample from EDTA bottle was mixed up using a mixing machine. 1ml of blood sample was then fed in a Cell – dyn machine to determine the above parameters. (Normal values: Appendix V).

Cut off points for anaemia

Anaemia is a reduction of haemoglobin to at least two standard deviation below the mean, adjusted for age, sex and altitude. The haemoglobin levels for most of sickle cell patients are below two standard deviation. However, for the purpose of this study, the severity of anaemia was classified as for the normal population:

Severe anaemia Hb ≤5 g/dl

Moderate anaemia Hb 5 - < 10 g/dl

Mild anaemia Hb 10-<11 g/dl

Normal Hb ≥ 11g/dl.

Urine analysis

Ten to fifteen millimetres of fresh mid stream urine collected in clean bottle was taken and centrifuged at 1500 revolutions per minutes for 5 minutes. The urine was then examined at a high power field (40x) objective for RBC. Red blood cell counts of more than 5 cells per high power field (5/HPF) were defined as haematuria.

Stool analysis

Fresh stool was collected in a covered plastic container and examined at low power field (10x) objective for hookworm ova. Egg count was done to determine hookworm load, using Kato Katz Technique – Cellophane faecal thick smear. The procedure for determining hookworm count and load was as follows: -

- Small amount of faecal material was placed on a scrap paper and pressed by the small screen on top of faecal material in order to sieve through.
- Sieved faeces were then scraped using a flat side of the spatula.
- A template with a hole was placed on the centre of a microscope slide and faeces were then added to fill the hole. Excess faeces were removed by passing the spatula over the template.

- The template was removed.
- Faecal material was then covered with pre- soaked cellophane strip.
- Microscope slide was inverted and faecal sample was pressed firmly against the wet cellophane strip on another microscope slide. With this pressure, faecal material was spread evenly between the microscope slide and the cellophane paper.
- The slide was removed gently to avoid separating the cellophane strip or lifting it off.
- After 30 minutes the slide was put under the microscope for egg count. Hookworm infection was classified as follows:
    - Low infection 1-25eggs.
    - Moderate infection 26-40eggs
    - Heavy infection > 40eggs

Hookworm load (i.e. number of eggs per gram) was obtained by multiplying the number of eggs by 20 as the template used was of 50mg.

8.11 Ethical issues/consideration:

Parents/guardians were explained about the research and gave their informed written consent. Children were managed according to the management protocol of the concerned unit i.e. along routine lines of patient care at the center. The results of each child in the study were communicated to the paediatrician /physician taking care of the

child. Those with iron deficiency and/or hookworm infection were treated according to the management protocol of each condition.

8.12 Ethical clearance

Ethical clearance and permission to conduct the study was obtained from the MUCHS high degree ethical committee of the research and publication committee.

8.13 Data processing and Analysis

The iron content was calculated manually using charts showing iron composition on different types of foods as indicated in Appendix II. Daily iron intake for each child was obtained by dividing the total amount of iron obtained by twenty-eight days. Using iron recommended daily allowance as indicated in Appendix IV children were grouped into normal or low dietary iron intake. The investigator and computer programmer who is familiar with research did data entry and analysis using computer Epiinfo 6 program. Frequency distribution tables were used to determine the prevalence of iron deficiency. Cross tabulation was used to determine the association between the contributing factors (i.e. dietary and hookworms infection) and iron deficiency. χ^2 test was used to compare proportions and p-values of less than 0.05 were considered significant. Fisher exact test was used where the expected frequency was less than 5.

8.14 Quality control

Each questionnaire was checked for accuracy and completeness on daily basis using a master sheet by the principal investigator to ensure that the data collected was free of error.

8.15 Limitation of the study.

- Some parents /guardians refused to consent for the study.
- There could have been incorrect dietary recall.

RESULTS

One hundred patients were recruited during the study period. A total of 13/100 (13%) patients had iron deficiency.

Table 1:Distribution of study group by age and *sex*

Age (Mo)	Males	Female	Total
≤ 60	25	20	45
>60	22	33	55
Total	47	53	100

- There were 53 females and 47 males.
- The female to male ratio was 1: 0.9
- There were 45 patients in age group less than 60 months and 55 patients in age group of more than 60 months.

Table 2:Iron status by sex and age

	Iron deficient		Non-iron deficient			
	n=13	(%)	n=87	(%)	Total=100	P-value
Sex						
Male	4	(8.5)	43	(91.5)	47	
Female	9	(17.0)	44	(83.0)	53	0.208
Age (mo)						
≤ 60	7	(16.2)	36	(83.8)	43	
> 60	6	(10.5)	51	(89.5)	57	0.397

- Thirteen (13%) patients were iron deficient.
- Four (8.5%) males and nine (17%) females were iron deficient.
- The difference was not statistically significant (p value= 0.208)
- Seven (16.2 %) patients in age ≤ 60months had iron deficiency.
- The prevalence decreases to 10.5 % in the age group of more than 60 months.
- The difference was not statistically significant. (P-value= 0.397)

Table 3: Iron status by dietary iron intake

Dietary Iron intake	Iron deficient n (%)	Non-iron deficient n (%)	Total
Normal	9 (12.9)	61 (87.1)	70
Low	4 (13.3)	26 (86.7)	30
Total	13	87	100

Fischer exact test P-value: 0.589

- Thirty (30%) patients were on low dietary iron intake
- Four (13.3%) patients on low dietary iron intake were iron deficient.
- There was no association between iron status and dietary intake. (P-value= 0.589)

Table 4: Association of iron status and the presence of hookworm

Hookworm	Iron deficient n (%)	Non-iron deficient n (%)	Total
Present	3 (16.7)	15 (83.3)	18
Absent	10 (12.2)	72 (87.8)	82
Total	13	87	100

RR 1.37 95%CI 0.42<RR<4.47 Fischer exact test P-value 0.426

- Eighteen (18%) sickle cell children had hookworm.
- There were more cases of hookworm infestation among the non- iron deficient patients (83.3%).
- There was no association between iron status and the presence of hookworm (p value=0.426).
- The risk of presence of hookworm and development of iron deficiency is no significant (RR=1.37 95% confidence limits for RR 0.42 < RR < 4.47)

Table 5: Iron status by Haemoglobin Concentration

Haemoglobin (g/dl)	Iron deficient n (%)	Non-iron deficient n (%)	Total
≤ 7	7 (15.5)	38 (84.5)	45
> 7	6 (10.9)	49 (89.1)	55
Total	13	87	100

Chi-Square = 0.47 P-value 0.491

- Forty-five (45%) patients had haemoglobin concentration less or equal to 7g/dl.
- Fifty-five (55%) patients had haemoglobin concentration of more than 7g/dl.
- Seven (15.5%) patients with haemoglobin concentration less or equal to 7g/dl had iron deficiency.
- Six (10.9%) patients with haemoglobin concentration more than 7g/dl had iron deficiency.
- The levels of haemoglobin were not significantly associated with iron status (P value =0.491).

DISCUSSION

In this study it has been found that iron deficiency exist and it's prevalence in sickle cell anaemic patients was 13%. This is not unusual because previous studies done in the same setting showed a much higher rate of 26% and 30% respectively. [20,21] The prevalence of iron deficiency can vary significantly depending on the methodology used. In this study biochemical tests serum iron, Total Iron Binding Capacity (TIBC) and serum ferritin were used to establish the diagnosis of iron deficiency while bone marrow examination was used in the other two studies.[20, 21]

In a Nigerian study using bone marrow aspiration, the prevalence rate of 47% was obtained. [23] Another study reported a much lower prevalence of 9% by taking patients who were diagnosed to have iron deficiency biochemically using transferrin saturation, serum ferritin and TIBC following response to oral iron therapy.[17] The prevalence of iron deficiency state could have been underestimated as not all patients with iron deficiency are expected to respond with a rise of haemoglobin concentration.

The study done to determine the impact of weekly iron supplementation on iron status showed no significant difference in the rise of the levels of haemoglobin concentration between treated and the placebo group. [26]

It appears that when bone marrow examination is used to establish the diagnosis of iron deficiency higher prevalence rates are realized. Bone marrow examination is a reliable method that is not affected by other factors in the body, while the biochemical markers seem to be influenced by a variety of factors like infections. The difference in the magnitude of iron deficiency in the studies done could partly be explained by the

differences in the age groups of the studied population and the method used to establish the diagnosis of iron deficiency. [21, 23] These studies included pre-adolescent children. In study done in non-sickler that included pre-adolescent children, the prevalence of iron deficiency was 53.6%. Free Erythrocyte Pophyrin (FEP) was used to determine iron deficiency. [13] In the current study there was slightly more females than males. The female to male ratio was1:0.9. However there was no significant association between sex and iron status (p value=0.208). There were 17% females compared to 8.5% males with iron deficiency. One study reported male preponderance, however the sample size was too small for any meaningful statistical analysis. [21] In another study that looked at normal children in a Tanzanian community, sex did not seem to influence the iron status of children up to 15 years of age.[13]

The prevalence of iron deficiency was found to be higher in children less than 60 months of age (16.2%) than in children more than 60 months (10.9%). However the difference was not statistically significant (p value=0.397). The study patients were pooled into two age clusters to booster up the numbers in each category for a meaningful statistical analysis. Five of 13 (45.5%) patients with iron deficiency were below the age of thirty-six months. Iron deficiency is seen to decrease slightly after 36 months and then rises up after 97 months. In a study done in normal children in a Tanzanian community similar findings were obtained. The prevalence of iron deficiency was 67.8% and 53.6% in under five and above five years of age respectively.[13] Other studies carried out in sickle cell population did not give the prevalence according to age.[20, 21]

Iron deficiency has been associated with age and is fairly common between the ages of six to twelve months. This is due to rapid growth rate, which increases the iron demand. The problem is further aggravated by low iron containing complementary foods introduced during the weaning period. The foods are cereal based and have little amount of iron to meet the recommended daily allowance. These cereals also have phytates that are known to inhibit iron absorption. [2]

Dietary iron intake was not significantly associated with iron deficiency in this study (P value =0.589). The prevalence of iron deficiency was almost similar in normal and low dietary iron intake groups, 12.9% and 13.3% respectively. This suggests that other factors like bioavailability of iron and its absorption may contribute to iron deficiency. Daily iron intake in iron deficient and iron sufficient groups with a normal dietary iron content were actually above the recommended daily allowance (RDA). The recall bias might have been there but long-term dietary exposures are more relevant to chronic diseases. In this context it was better to rely on monthly estimates of dietary intake rather than short-term dietary intake that may not adequately reflect exposure.[27] Iron deficiency could be due to increased demand for growth and /or malabsorption. [2, 14] In a study to determine the iron bioavailability, it was reported that iron deficiency was associated with low iron bioavailability from the food consumed in their study area. The food was predominantly cereal and vegetable based that had high content of phytate. [13] Among 30 patients who were on low iron diet only 4 (13.3%)were iron deficiency and 86.7% were iron sufficient. It appears that dietary iron do not seem to influence significantly the body iron status in patients with sickle cell anaemia (P value= 0.589).

Children who were iron sufficient inspite of low dietary iron were of much older age group. The older the children the more is the chronicity of the haemolytic crisis, which is associated with recycled iron. [21]

Iron deficiency could also result from abnormal blood losses caused by hookworm infection, occult blood loss and haematuria.

In this study the presence of hookworm was relatively low among the study group. Eighteen (18%) of the children had hookworm ova. Hookworm infection is a known cause of iron deficiency. Eighty five percent of children in a study group received antihelminthes three months prior to enrollment. This is a result of the introduction of Integrated Management of Childhood Illnesses (IMCI) in the region that recommends deworming every six months and at school age this is taken care of by school health programmes.

Kimati et al reported three of 20(15%) sickle cell anaemic patients had hookworm infection. [21] Okeahialam et al found 8 among 45(18%) sickle cell anaemic patients had hookworm infection. [23] These rates were not very much different from the rate reported in this study.

A study done in a general population found the prevalence of hookworm infection to be 3.8% and 13% in pre-school (<5years) and school children (>5years) respectively. There was no significant association between hookworm and iron deficiency. [13] These studies indicate that the prevalence of hookworm infection in sickle cell patients is similar to the prevalence in the non-sickler population. In this study three of 13 patients (23%) with iron deficiency had hookworm. There was no increased risk or association

of developing iron deficiency in the presence of hookworm infection among the study group (RR1.37). Blackman reported a high prevalence rate of hookworm in children who had iron deficiency. Among seventeen children with iron deficiency 12 (71%) had hookworm ova, patients were not categorized into age groups. [9]

Hookworm infections were much commoner above 3 years of age. This agrees with a hospital-based study done by Kalokola, who found that hookworm was rare below the age of 3 years. [28]

In this study among 18 sickle cell patients with hookworm, 15 had low, 2 had moderate and one had heavy hookworm load. This implies that most patients had low hookworm load. The patient with a heavy hookworm load was iron sufficient and had six blood transfusion episodes. The last one was six months prior to enrollment. The patient had not received antihelminthes six months prior to enrollment. The iron sufficiency could be explained by multiple blood transfusions. Other studies done on sickle cell patients did not report on hookworm load. [21,23] In view of small numbers of patients in each category, Fischer exact test was used to determine the association between variables. A meaningful comparison could be obtained using a much larger sample size than the one used in this study.

In this study, tests for occult blood in stools and red blood cells in urine were negative. One study reported presence of occult blood in stools and urine, and this could have contributed to the cause of iron deficiency in sickle cell patients and hence the high prevalence rate reported. [21]In this study, the mean haemoglobin was 7.4g/dl. Seven patients (15.5%) with iron deficiency had haemoglobin less than 7g/dl and 6 (10.5%)

patients had haemoglobin more than 7g/dl. The levels of haemoglobin did not appear to influence significantly the body iron status (p value=0.491). The distribution of haemoglobins was pooled into two categorical groups because of the smaller numbers of patients seen. It was observed that three (23%) iron deficient patients had haemoglobin less than five. The mean haemoglobin in iron deficient group was 6.5g/dl. This is almost similar to the report of Nigerian study where all 21 patients who had iron deficiency had mean haemoglobin of 6.2g/dl. [23]

In this study fifty-seven patients had blood transfusion and all had been transfused more than six months prior to enrollment. Thirteen of the patients had more than three blood transfusions. Among the thirteen-iron deficient sickle cell patients, seven patients did not receive blood transfusion, five had one blood transfusion and one had two-blood transfusions. In another study, it was found that the prevalence of iron deficiency was more in those who had no blood transfusion than those who had received blood transfusion. [17] This is in agreement with the current study that iron deficient most of patients had no blood transfusion. Iron overload in sickle cell anaemia occurs because of repeated blood transfusions and repeated haemolysis in which iron released from destroyed red cells is retained in the body. [21] None of the patients studied had evidence of iron overload since serum ferritin levels were within normal reference ranges. Patients in iron deficient group had MCV levels below normal for their age in this study. This agrees with the study, which found that the age dependent MCV values were useful screening test for iron deficiency. [17] Eighteen patients in iron sufficient group had MCV levels below normal in this study.

Serum ferritin below 25 ng/ml has been previously reported to be a useful screening test for iron deficiency. [17, 25] In this study, it has been observed that all patients who had iron deficiency had serum ferritin below 25ng/ml. Serum ferritin is an acute phase reactant protein that rises during acute inflammatory conditions. As from the findings of Allison et al, serum ferritin was found to be elevated during vaso-occlusive crisis compared to the patients who were well and asymptomatic.[29] In this study, this fact was dealt with by excluding all patients with acute infections or who were in crisis by thorough history and physical examination.

The treatment of iron deficiency with iron in patients with sickle cell anaemia is rather controversial, taking into consideration of the already existing risk of iron overload following repeated haemolysis. Some studies have shown that a reduction of the MCHC induced by iron deficiency may ameliorate sickling. [25] Vichinsky et al reported response following treatment of iron deficiency with iron in patients with sickle cell anaemia.[17]

Conclusions

- Iron deficiency is common in sickle cell anaemic patients at Muhimbili National Hospital.
- Age, sex and dietary iron intake did not seem to influence the body iron status.
- Hookworm infections did not influence the body iron status. Lack of association in this study between iron deficiency and hookworm could be due to low hookworm load as a result of recent derworming.

Recommendations

- Sickle cell anaemic patients on regular folic acid and chloroquine prophylaxis whose haemoglobin levels are consistently low without apparent cause of the severe anaemia; should have blood taken for a full blood picture. All patients with hypochromic microcytic type of anaemia should be investigated for iron deficiency.
- Iron therapy should be initiated in sickle cell patients when there is evidence of iron deficiency by haematological investigations.

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APPENDIX I QUESTIONNAIRE –ENGLISH VERSION

1.Study number..... File Number.....

2. Name of patient.....

3. Date of Birth..... Birth weight .....(kg)

4. Age in months.....

5. Sex: MALE

FEMALE

PARENT/GUARDIAN Father Mother Guardian

Occupation: ---------- ---------- ----------

Education

No formal education ---------- ---------- ----------

Primary School ---------- ---------- ----------

Secondary School (I-IV/V-VI)---------- ---------- ----------

Higher Education (specify) ---------- ---------- ----------

6. **PAST MEDICAL HISTORY**

History of blood transfusion 1 2 if 1 number of transfusions.....

Uvulectomy 1 2

7.HISTORY OF DERWORMING 1 2

WHEN DERWORMED ……---------- Months---------- Weeks

8. HISTORY OF HAEMATURIA 1 2

9. HISTORY OF MALAENA 1 2

1= Yes 2= No

LABORATORY INVESTIGATIONS

1.HB _____

2. RBC Count _____

3.HC _____

4. MCV _____

5. MCH _____

6.MCHC _____

7 .WBC _____

8.Count.PLATELET Count_____

9. PERIPHERAL SMEAR _____

10. TSI _____

11. TIBC _____

12. SERUM FERITTIN _____

13. STOOL

Occult blood 1 2 Ova (Hookworm) 1 2

14.URINE

Rbcs 1 2

Ova 1 2

FOODS

1. Fill the questionnaire by writing the number from the bottom response selected by patient.

   Example:Q: How often has your child eaten rice during the last one month?

   A: 2-4 times per week (Then write"4" as an answer for rice, the number shown at the bottom of the Card for 2-4 times per week).

Never 0 times in a month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	1 time per day	2-3 times per day	4-5 times per day	6+ times per day
1	2	3	4	5	6	7	8	9

5.FOOD EATEN:

5.1 How often has the child eaten_____during the last month (from__/__/__to__/__/__)

Vegetable and Legumes	Frequency	Utensil	Amount
Green mungs			
Pigeon Pea			
Green Peas			
Bambara nuts			
Beans			
Sweet Potatoes leaves			
Amaranth			
Cassava leaves			
Pumkin leaves			
Cabbage			
Carrots			
Okra			
Bitter tomato			
Tomato (fresh)			
Others			

5.2 How often has the child eaten_____during the last month (from__/__/__to__/__/__)

Fish / Meat	Frequency	Utensil	Amount
Goat meat			
Pork			
Chicken			
Beef			
Liver			
Fish			

5.3 How often has the child eaten_____during the last month

(from__/__/__to__/__/__)

Fruits	Frequency	Utensil	Amount
Ripe bananas			
Mango			
Oranges			
Tangarines			
Pawpaw			
Plums			
Avocardo			
Jack fruit			
Cucumber			
Water melon			
Pineapple			
Pears			
Lemon			
Guava			

5.3 How often has the child eaten_____during the last month
(from__/__/__to__/__/__)

Grains	Frequency	Utensil	Amount
Wali			
Rice (			
Maize(boiled)			
Maize(roasted)			
Stiff Porridge			
Irish Potatoes			
Sweet Potatoes			
plantain			
Taro			
Cassava			
Others			

5.4 How often has the child drunk_____during the last month (from__/__/__to__/__/__)

	Frequency	Utensil	Amount
Milk tea			
Black tea			
Soya			
Porridge			
Juice(fresh)			
Juice(			
Others			

5.6. Which grains do you usually use for child's porridge? (Do not read options to the mother/interviewer

	Yes	No
Maize		
Ulezi		
Soya		
Cassava		
Wheat		
Rice		
Mixed grains		

5.7. What ingredient is usually added to child's porridge? (Do not read options to the mother/interviewer).

	Yes	No
Milk		
Sugar		
Groundnuts		
Dried fish		
Beans		
Eggs		
Margarine		
Cooking oil		

5.8. Which milk does the child usually drink?

	Yes	No
Cow's milk		
Powder milk (in packets)		
Milk in cans/tins		

5.9. How much of these foods are usually added to the family pot per day?

Food	Utensil	Fraction
Tomato		
Onion		
Green Pepper		
Carrot		
Coconut milk		
Vegetable oil		
Margarine		
Sugar		
Salt		
Other (specify)		

APPENDIX II: FOOD COMPOSITION TABLE IRON COMPOSITION IN FOODS COMMONLY EATEN IN EAST AFRICA

The symbols after iron value provide an indication of the contribution that a food can make in supplying that nutrient

COMPOSITION PR 100 GRAMS	IRON (MILLIGRAMS)
CEREALS AND GRAIN PRODUCTS	
1. Maize, yellow immature on cob, fresh	1.8
2. Maize, white whole kernel, dried	3.6
3. Maize, yellow whole kernel, dried	4.9
4. Maize, white on cob, toasted	3.0
5. Maize, flour, 60-80% extraction	1.1
6. Maize, meal	4.2
7. Maize, meal (Dona)	2.5
8. Millet, finger, whole grain	17.0
9. Millet, finger, flour	54.0
10. Millet, bulrush, whole grain	21.0
11. Rice lightly milled, parboiled	1.7
12. Rice, milled, polished	1.7
13. Sorghum, whole grain	11.0
14. Sorghum, flour	10.0
15. Wheat, whole, parboiled	6.1
16. Wheat, flour, 85%	3.6
17. Wheat, flour, 70% extraction	2.2

Wheat products:

18. White bread	1.7
19. Brown bread	2.2
20. Cakes	0.3

STARCHY ROOTS, TUBER AND FRUIT

1. Breadfruit, pulp, raw	2.0
2. Cassava, bitter, fresh	1.9
3. Cassava, meal	3.6
4. Plantain, ripe, raw	1.3
5. Potato, raw	1.1
6. Sweet potato, yellow, raw	2.0
7. Sweet potato, pale raw	2.0
8. Taro.cocoyam, raw	1.2
9. Turnip/Swede, root, raw	0.5
10. Yam, Fresh	0.8
11. Yam, flour	1.1

GRAIN LEGUMES AND LEGUME PRODUCTS

1. Beans/peas, fresh, shelled	1.8
2. Beans, dried	8.2
3. Beans, green in pod, raw	1.4
4. Bonavist/Hyacinth bean, dried	9.0
5. Chickpea, whole seeds, raw, dried	11.0
6. Cowpea, mature pods, dried	5.0
7. Cowpea, young green pods, raw	1.4
8. Kidney been, red, dried	8.2
9. Lentil, dried	7.0

10. Mung bean, green, dried	7.0
11. Mung bean, black, dried	8.9
12. Pea, dried	18.0

COMPOSITION PR 100 GRAMS EDIBLEPORTION (-= not analysed)	IRON MILLIGRAMS
13. Pigeon pea, dried	5.0
14. Soya bean, dried	6.1
1.5 Velvet bean, dried	-
NUTS AND SEEDS	
1. Bambara groundnut, fresh	12.0
2. Cashew nut, dried	18.0
3. Coconut, immature kernel, fresh	1.8
4. Coconut, mature kernel, fresh	2.5
5. Groundnut, dry	3.8
6. Melon seeds, without coat	7.4
7. Pumpkin seeds, without coat	2.8
8. Sunflower seeds, without coat	7.6
VEGETABLES AND VEGETABLE PRODUCTS	
1. Amaranth, leaves, raw	8.9
2. Amaranth, leaves, cooked	7.7
3. Bamboo shoots, raw	0.9

5. Bean sprouts, raw	8.2
6. Carrots, raw	0.7
7. Cassava, leaves, raw	7.6
8. Cauliflower, raw	1.2
9. Cucumber, raw	0.5
10. Cowpea, leaves, raw	5.7
11. Egg plant, raw	1.3
12. Hare's lettuce, raw	3.1
13. Leaves, pale green	0.7
14. Leaves, medium green	1.8
15. Leaves, dark green	7.2
16. Lettuce, raw	0.7

COMPOSITION PR 100 GRAMS EDIBLE PORTION (-= not analysed)	IRON (MILLIGRAMS)
17. Mushrooms, fresh	1.5
18. Okra, pods, raw	1.2
19. Okra, leaves, raw	0.7
20. Onion, shallot, raw	0.8
21. Peppers, capsules sweet green, raw	2.6

22. Peppers, capsules, sweet red, raw	2.6
23. Pepper, leaves, raw	10.0
24. Pumpkin, squash, raw	1.4
25. Pumpkin, leaves, raw	0.8
26. Sweet potato, leaves, raw	6.2
27. Taro, leaves, raw	2.0
28. Tomatoes, raw	0.6
29. Turnip, leaves, raw	1.9

FRUIT

1. Avocade, raw	1.4
2. Baobab, ripe, raw	7.4
3. Banana, ripe raw	1.4
4. Citrus, orange/tangerine, raw	0.1
5. Citrus, grapefruit raw	0.6
6. Citrus, lemon/lime	0.7
7. Dates, dried	9.4
8. Ground cherry/Cape gooseberry, raw	0.9
9. Guava, raw	1.3
10. Mango, ripe, without skin	1.2
11. Mango, unripe, without skin	1.4
12. Papaya, raw	0.6
13. Pineapple, fresh	0.4

14. Pomegranate, fresh	1.0
15. Tamarind, dried	2.2

COMPOSITION PR 100 GRAMS EDIBLE PORTION (-= not analysed)	IRON (MILLIGRAMS)
16. Tree tomato, raw	0.7
17. Watermelon	0.3
SUGAR AND SYRUPS	
1. Soft drinks, commercial	-
2. Sugar cane	1.4
3. Sugar	0
MEAT, POULTRY AND EGGS	
1. Bacon, fat, whole side	1.3
2. Beef, moderately fat	3.6
3. Egg, hen	2.0
4. Goat, moderately fat	2.3
5. Heart, beef	4.8
6. Kidney, beef	4.6
7. Liver, beef	10.0

8. Mutton, moderately fat	2.0
9. Pork, moderately fat	1.8
10. Poultry, for example chicken	1.1
11. Turtle	1.0

FISH AND FISH PRODUCTS

1. Crustaceans, crab, lobster, prawn	1.6
2. Fish, dried	4.9
3. Fish, average filet	1.7
4. Small dried fish, dagaa	8.6

MILK AND MILD PRODUCTS

1. Milk, cow, whole	0
2. Milk, cow, skimmed	0.1
3. Milk powder, cow whole	0.5

COMPOSITION PR 100 GRAMS EDIBLE PORTION (-= not analysed)	IRON (MILLIGRAMS)
4. Milk powder, cow, skimmed (DSM) vitamin A enriched	1.0
5. Milk goat	0.1
6. Buttermilk	0.1
7. Special baby food milk powders, average	5.8

OILS AND FATS

1. Beef suet	0
2. Butter, from cow's milk	0
3. Coconut oil	0
4. Fish liver oil	- -
5. Ghee, clarified butter	0.4
6. Lard/animal fats	0.1
7. Margarine, fortified	0
8. Red palm oil, fresh	0
9. Red palm oil, stale	0
10. Salad oil	0
11. Sunflower oil	0

OTHER

1. Beer, local	0.3
2. Caterpillars, dried	2.3
3. Coconut milk	0.3
4. Termites, fresh	1.0
5. Yeast, bakers dry	5

APPENDIX III

Consent form for participation in the study of prevalence of Iron Deficiency in children with sickle cell anaemia.

My name is Dr. Bona Mangosongo

I'm a Senior Resident in Paediatrics & Child Health MUCHS. I'm doing research study in order to determine the prevalence of Iron deficiency and its contributing factors among children with sickle cell anaemia.

I would like to ask you for your permission that your child participates in this study. First I will explain to you about the study and answer any question you have.

Study aims:

This study will determine the prevalence of Iron deficiency and its contributing factor among children with sickle cell anaemia.

How to participate in the study.

A small amount of blood 5mls will be taken using a sterile needle from your child's vein after you have agreed to participate in the study. Stool and urine will be taken as well for investigation if your child will be found to have iron deficiency, I would like to perform a bone marrow aspiration. Participation in this study is completely voluntary. Even if you will not participate in this study your child will continue to receive the same service from this center just like other children.

Confidentiality

Your child's name and other particulars will not be made public in any way and so your participation will be anonymous. Only the chief investigator and dedicated laboratory staff will handle the specimens and information obtained.

Benefits

Parents and guardians whose children will participate in this study will benefit by knowing their iron status and treatment will be initiated to those who will be found to have iron deficiency and worm infestation.