# IRON DEFICIENCY IN SICKLE CELL ANAEMIA PATIENTS IN DAR ES SALAAM TANZANIA.

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## Summary

**A cross sectional descriptive study was done to determine the prevalence of iron deficiency and possible contributing factors in sickle cell anaemic patients. One hundred haemoglobin -SS children aged between six months to ten years inclusive were recruited in the study.Patients were selected using a simple random sampling technique. Five milliliter of venous blood was taken from all children for serum Ferritin, serum Iron, Total Iron binding Capacity, Full Blood Count and a peripheral smear for red blood cell morphology. Urine and stool were also taken and examined for the presence of red blood cells, ova and occult blood respectively. A structured questionnaire was used to record social demographic data.**

**The prevalence of iron deficiency was 13%. There were more females (53%) than males (47%) with iron deficiency. The difference was not statistically significant (p=0.208).**

**Children aged less than five years (16.2%) were more iron deficient than those above five years (10.5%). The difference was not statistically significant (p=0.397). Dietary intake, presence of Hookworm and level of haemoglobin concentration did not significantly influence the body Iron status (p- value was 0.589 and 0.491 respectively).**

**Iron deficiency occurs in patients with sickle cell anaemia. Age, sex, the amount of iron ingested in food and the presence of Hookworm infection did not appear to influence the body iron status. Further studies need to be carried out to determine the role of iron therapy in patients whose peripheral smear show Hypochromic microcytic red blood cells.**


**Key words:** Sickle cell anaemia, iron deficiency

## Introduction

Sickle cell Anaemia is a condition resulting from a mutant autosomal gene responsible for the synthesis of HBS. It is among the major cause of Anaemia in Tanzania [(1)].

A study done in 1972 reported a prevalence rate of sickle cell disease of 16.4% and 31.4% in children aged 1-18 months in Dar es Salaam and Musoma respectively. In children aged more than 12 months the prevalence rate was 18-38.9% in Dar es Salaam.[(1)]

Iron deficiency is common in Dar es Salaam. Earlier studies reported prevalence rate ranging between 86-94% in normal children [(2,4,5,6)]

The anaemia of sickle cell haemoglobinopathy is usually normocytic normochromic. The heam iron released following haemolysis is usually recycled for use and or storage. In chronic haemolytic anaemia iron overload is likely to develop due to increased intestinal iron absorption. [(6)] For this reason iron deficiency would be relatively uncommon in sickle cell anaemia. However Iron deficiency have been frequently reported in sickle cell anaemia[(7)] Kimati using bone marrow staining for iron found a prevalence rate of 46.7%[(7)].Nagaraj 1980 found Iron deficiency in all his 25 patients studied [(8)].In Nigeria 47% of the 45 patients studied had iron deficiency [(6)].Among the causes of iron deficiency mentioned were Hookworm, gastrointestinal bleeding, bleeding from renal tract and diet deficient in iron. The difference in prevalent rates could partly be explained by the different methods used to determine iron status. It appears that when bone marrow studies were done higher prevalence rates were obtained. This study was carried out to determine the prevalence of iron deficiency and related causative factors using a bigger sample size han the previous studies carried out in this area.

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## Materials and methods

This was a cross sectional study conducted at Muhimbili National Hospital, Dar es Salaam, Tanzania between August 2002 and February, 2003.The hospital provides both tertiary care and serves as a University teaching Hospital.

A list of all patients aged 6 months to 10 years attending a sickle cell clinic on every Thursday was used. A simple random sampling technique was used to select the patients to be included in the study.

One hundred children aged 6 months to 10 years attending the outpatient sickle cell clinic were enrolled for the study after the following exclusion criteria were met:

Children were excluded from the study if they have received blood transfusion three months prior to recruitment, had an acute illness in last two weeks, had chronic inflammatory diseases, were on iron therapy and those whose parents/guardian not caretakers did not consent for the study.

A structured questionnaire designed for the study was used to collect social demographic data and record the laboratory results. Dietary information was obtained by interviewing the patients/parents using a food frequency questionnaire. Parents were shown common utensils with known volumes and the amount of food fed to their children estimated over a four weeks period.

Five milliliters of venous blood was taken in an empty sterile bottle and serum was frozen at ≤20°C and analyzed within two weeks for TIBC, serum ferritin, serum iron using a Cobas Core automated analyser Blood was also collected in an EDTA bottle for full blood count using an automated Cobas Mira analyzer

### Serum Ferritin

Polystyrene beads coated with monoclonal mouse and ferritin antibody were added to the serum and incubated at 37°C for 15 minutes. A conjugate was added to the mixture and then washed to remove excess conjugate from unbound sample. A Cobas core substrate was added and incubated at 37°C for another 15 minutes. The mixture was then passed in a photometer and serum ferritin determined by colour

density. The concentration of ferritin was directly proportional to the coluor density.

**Serum Iron**

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

**Total Iron Binding Capacity:**

Serum was incubated at 20°C for 20 minutes for the iron to saturate the available binding sites of serum transfferrin. Ferrozin® 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 based on the following criteria. Serum Ferritin ≤25ng/dl and one of the following; Serum iron level ≤40υg/dl, Total Iron Binding Capacity (TIBC) >400υg/dl.

**Peripheral Smear**

A drop of blood from the EDTA container was put on a clean slide and a smear prepared. The smear was fixed and stained with Leishman stain for 2 minutes. A buffer of pH 6.8 was added and air dried before examination under the microscope using oil emulsion at a high power field (100 times).

**Full Blood Count**

One milliliter of blood was fed in a cell dyn 1200 machine (Abbot diagnostic division, Abbot Laboratories USA). The following parameters were obtained: Haemoglobin, Mean Cell Volume(MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC)

**Urine analysis**

Ten to 15 milliliters of fresh mid stream urine was collected in a clean bottle and centrifuged at 1500 revolutions per minute for 5 minutes. The urine was then examined under 40 times high power field for red blood cell counts. Red blood cell count of more than 5cells/HPF was defined as haematuria.

**Stool analysis**

Fresh stool was collected in a covered plastic container and examined at a low power field (10times) objective lens for Hookworm ova.Egg count was done using Keto Katz technique cellophane faecal thick smear.Hookworm infection was classified as follows.

Low infection 1-25 eggs
Moderate infection 26-40 eggs
Heavy infection >40.eggs

Hookworm load (number of eggs per gram)=Number of eggs ×20 as the template used was 50mgs.

**Data analysis:**

The iron content of the diet was calculated manually using charts showing iron content of different types of food.

Daily iron intake of each child was obtained by dividing the total amount of iron consumed in four weeks by 28 days. Using the recommended daily allowance, children were grouped into normal and low dietary intake groups. Data was analyzed using computer Epiinfo version 6 program. Frequency distribution tables were used to determine the prevalence of iron deficiency. Cross tabulation was used to determine the association between various factors (dietary iron intake, hookworm etc.) and iron deficiency.$\chi^2$test was used to compare proportions and p-values of less than 0.05 were considered significant. Fisher exact test was used when the expected frequency was less than 5.

**Results**

One hundred patients were recruited in the study. Forty five percent of patients were below five years. The female to male ratio was 1:0.9 (table 1)

Table 1. Distribution of study subjects by age and sex.

| Age (mo) | Males | Females | Total |
|---|---|---|---|
| ≤60 | 25 | 20 | 45 |
| >60 | 22 | 33 | 55 |
| **Total** | **47** | **53** | **100** |

There were 13 patients with iron deficiency. Out of these 4(8.5%) were males and 9(17%) were females. The difference was not statistically significant (p=0.208) Iron deficiency is much more prevalent in children below five years (16.2%)compared to those above 5 years (10.5%), p=0.397 (Table 2)

Table 2.Iron status by age and sex

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

Thirty (30%) patients were on low dietary intake and 13.3% of them were iron deficient. Twelve point nine (12.9%) of patients on normal diet were iron deficient. About 86.7% of patients with body iron stores were found to be on diet with low iron content. There was no association between dietary iron intake and body iron status (p=0589) Table 3.

Table 3. Iron status by dietary iron intake

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

Eighteen (18%) patients with sickle cell Anaemia had hookworm infection. There were more cases of hookworm infection among non-iron deficient children (83.3%). There were no association between iron status and the presence of hookworm infection (p=0.426). The patients with hookworm were not at an increased risk for developing iron deficiency (RR=1.37 at 95% confidence limits for RR 0.42<RR>4.47). Table 4.

Table 4. Association of Iron status and the presence of Hookworm

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

Fifteen point five percent (15.5%) of iron deficient patients had haemoglobin less than 7g/dl. Only 10,9% of patients with iron deficiency had haemoglobin >7g/dl.

The level of haemoglobin was not significantly associated with the body iron status (p= 0.491). Table5

Table 5. Iron status by Haemoglobin concentration

| | **Haemoglobin** | | **Iron deficient** | | **Non Iron deficient** | |
|---|---|---|---|---|---|---|
| | **Gm/dl** | **N** | **(%)** | **N** | **(%)** | **Total** |
| ≤7 | | 7 | (15.5) | 38 | (84.5) | 45 |
| >7 | | 6 | 10.9) | 49 | (89.1) | 55 |
| **Total** | | **13** | | **17** | | **100** |

## Discussion

In the current study the prevalence rate of iron deficiency was 16.2% and 10.9% in children below and above five years respectively. In chronic haemolytic anaemia and following repeated blood transfusions iron overload is likely to develop due to increased intestinal iron absorption [6]. On the contrary, iron deficiency has been reported to occur in patients with sickle cell anaemia. Previous studies done in a similar setting gave prevalence rates of 26% and 30% respectively [7].

In Nigeria Okeahialam reported a prevalence rate of 47% using bone marrow iron studies [9]. The current study using a sample size double the sample size used in these previous studies reports an overall prevalence rate of 13%. The lower prevalence rate reported in this study is probably a reflection of the differences in the techniques used to determine iron deficiency and the age of the study patients. The difficulties in assessing the state of iron balance in sickle cell anaemia have been elaborated before. The biochemical makers appear to be influenced by a variety of factors such as inflammation, acute haemolysis, a number of blood transfusions and hepatic abnormalities [6]. In this study a detailed history and physical examination was done to exclude these conditions.

However, it appears that when bone marrow studies were used higher prevalence rates were obtained. The magnitude of iron deficiency was not significantly influenced by sex. The female: male ratio was 1:0.9 and the p-value was 0.208 (Table2). Earlier in Kimati's study slight, male preponderance was noted, although the sample size was rather small [7].

Our study agrees with the results of a community-based study done in normal children where no sex preponderance was reported. The prevalence of iron deficiency in normal children was found to be 67.8% and 53.6% in children below and above 5 years respectively [10].

Since the community based study and our study used biochemical parameters to assess the iron status one may safely conclude that iron deficiency is not as frequent in children with Sickle cell anaemia as it is in normal children.

In normal children the differences noted in the two age groups could be partly explained by the rapid growth rate in the former but also by the low iron containing weaning/complementary foods [10].

In sickle cell patients the growth rate in earlier years is fairly slow so that there might be other factors to account for this observation.

The prevalence of iron deficiency was similar in both patients taking normal diet and those on low iron containing foods. Dietary iron intake was therefore not significantly associated with body iron status (p=0.589 Table3).

Earlier reports suggested that in general food and nutrient intake by sickle cell patients meets or exceeds recommendations and not significantly different from healthy controls. The absence of dietary influence on iron deficiency might be attributed to the dietary recall bias. However, taking monthly estimates of dietary intake minimized this problem. Long -term dietary intake assessment has been previously found to adequately reflect the exposure [11]. Children who were iron sufficient in spite of low dietary intake were of the older age group. The older the child, the more the chronicity of heamolytic episodes that are associated with recycled iron and greater intestinal iron absorption. [7]. Since our study gave no indication for abnormal losses through haemorrhage in gastrointestinal tract or urine, the differences observed might be at the level

of absorption. It is also possible that the iron requirements in sickle cell patients were much higher than in non sickle cell patients such that the recirculated iron is not sufficient enough to meet the demand.

The prevalence of hookworm infection was 18% similar to prevalence of 20% and 18% reported by Kimati[7] and Okeahialam[9] respectively. There were more cases of hookworm infection among non-iron deficient children (83.3%) however, there were no association between iron status and hookworm infection (p=0.426). Patients with hookworm infection were not at increased risk for developing iron deficiency (RR=1.37 at 95% confidence limits for RR 0.42<RR>4.47) (Table 4). Although the prevalence of hookworm infection was fairly high, the hookworm load was low in both sickle cell and non sickle cell patients. Kalokola had earlier shown that hookworm was also not a common cause of iron deficiency anaemia in non sickle cell patients in under fives.[1]

The Haemoglobin level was not significantly associated with body iron status (p=0.491). This is not supprizing, since anaemia might be of multiple aetiological factors.Although iron deficiency is fairly common, no clear causative factors have been identified. Since iron deficiency has significant influence on growth, mental and psychomotor development, clear guidelines are needed on how to deal with the problem. This is especially so when taking into consideration the controversial benefits of iron therapy and the benefits of the state of iron deficiency in suppressing the symptoms of sickle cell anaemia [12].

## Conclusion

Iron deficiency is common in patients with sickle cell anaemia. Where facilities are available iron deficiency should be fully investigated and treated.

When iron deficiency is suspected on account of the presence of microcytic hypochromic red blood cells after examining a peripheral blood smear or a full blood count, a therapeutic trial of iron could be given. This strategy needs to be studied further before recommending it for a wider usage.

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