Serum Ferritin Levels In Patients with Sickle Cell Anaemia atthe Kenyatta National Hospital

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Abstract: Background: The iron levels in Sickle Cell Anaemia (SCA) is thought to be increased because of the repeated red cell transfusion, haemolysis with subsequent recycling and accumulation of iron. Red cell transfusion is used 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. Thisstudy 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 in patients with sickle cell Anaemia managed 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 study proforma. 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 was 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 revealed high serum ferritin in majority 70.5% of our patients with SCA. None of our patients had low SF. Very high serum ferritin, more than 1000ng/ml was observed in patients who received ≥15 units of blood over a period of five years. There was a significant association between serum ferritin levels and number of blood transfusions. Annual serum ferritin measurements are recommended to determine the iron status to institute prompt therapeutic measure of iron chelation.

Keywords: Serumferritin, sickle cell anaemia

I. Introduction

Significant progress has been made during the past two decades in the treatment of sickle cell disease. Identification of affected persons in their early years of life provides opportunities for early medical interventions that help in reducing morbidity and mortality in patients with SCD. Improved quality of life has been attained through comprehensive care including prophylactic measures and periodic assessment with monitoring for development of chronic organ dysfunction(1)(2).

One of the parameters used in monitoring these patients is status of body iron and 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 we do not have categorical 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. The conventional belief is that with chronic haemolysis and blood transfusions,

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patients with sickle cell anemia develop iron overload.(3) Studies have shown that iron deficiency is also found in patients with SCD and that iron overload is only associated with transfusion(4).

Red blood cell transfusion has been frequently applied for decades to treat acute illness in Sickle cell disease(SCD). Data from The Stroke Prevention Trials in Sickle Cell Anaemia (STOP) study demonstrating the effectiveness of chronic blood transfusion in preventing stroke has led to rapid increase in patients who are receiving chronic blood transfusion and therefore concerns about iron overload and its associated organ injury. The STOP trial demonstrated increase in Serum Ferritin(SF) in the study participants suggesting the development of iron overload in these patients.

Low serum ferritin concentration is indicative of iron deficiency, while variable results have been demonstrated in transfused cases. Some groups found that serum ferritin concentrations correlate well with the number of units of blood transfused, (3, 5, 6) and others did not find this to be the case. Several factors occurring in sickle cell anemia may increase serum ferritin concentration. Liver disease and chronic infection or inflammations are some of the well-recognized associations. Information is available in the literature, however; specific local data would accrue from investigating the local population. Such data may prove to be essential in advancing our understanding of the disease process and may help in management of patients with sickle cell disease locally.

The goals of this study were therefore to determine the status of serum iron among SCA patients in steady state on follow-up at the haematology clinic at Kenyatta National Hospital (KNH)by measurement of SF, to assess if there is any relationship between SF and blood transfusion, red cell indices or demographic characteristics.

II. Patients, Materials and Methods

This was a hospital based study done between 31st March to 20th August 2015 at the haematology outpatient clinic of Kenyatta National Hospital. A minimum sample of 80 patients was required. The subjects were 13 years and older with documented haemoglobin diagnosis of SCA.

The main objective of this study was todetermine the serum iron levels in ambulatory patients attending haematology clinic at Kenyatta National Hospital. After enrolment into the study, a standardised study proforma was used to record brief history and physical examination of the study patients. The transfusion history was based on recall of lifetime transfusion and chart review. Five millilitres of blood was then drawn to measure SF and Full blood count including peripheral blood film and Erythrocyte Sedimentation rate(ESR). Serum Ferritin was assessed using enzyme immunoassay sandwich method with a final fluorescent detection(ELFA) kits range 32-284ng/ml. The full blood count was done using the haematology cell counter (CELL-DYN 1300), the peripheral blood films were stained using May Grunwald Giemsa method while the ESR was determined using the Wintrobe method.

III. Results

In this five-month study carried out between March and August 2015 that targeted SCA patients on regular follow-up at the Haematology clinic at KNH, 86 subjects were consecutively screened and 80 enrolled into the study. One patient was pregnant hence was not recruited into the study, 2 patients had no documented diagnosis of SCA while 3 declined to give consent. **Figure 1.**
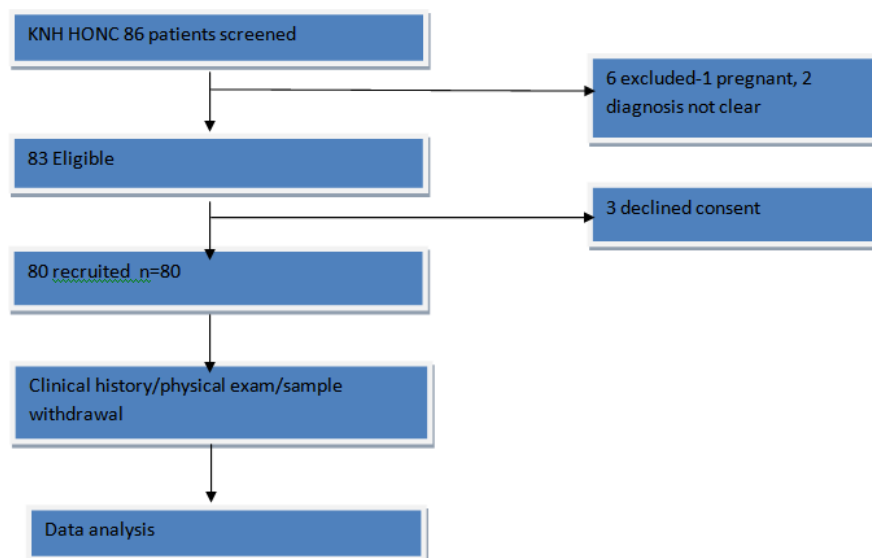
A: Demographic Characteristics of The Study Population

The study included 80 patients with SCA who on follow-up at the haematology clinic. There were 33males (41.3%) and 47females (58.7%). The patients range from 13 to 37 years of age with a mean age of 19.7±5.53 years. Table 1

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%)

B: 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 opioid analgesic while 25(31%) were on Nonsteroidal anti-inflammatory agents(NSAIDS). Only 6(7.5%) were on iron supplementation. Table 2

Seventy-four (92.5%) of the study population had 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%) had 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 though this difference was not statistically significant. The overall mean number of units of blood transfused was 10.5±7.5 ranging from no units transfused to maximum of 28 units transfused. Most patients 45(56.3%) had received between 1-10 units of blood transfusion. It was not possible to calculate the actual volume of blood transfused over the time before this study. This was due to missing data because of poor record keeping and/or poor access to the records especially given the fact that some patients had been admitted in other hospitals

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	32 (97.9%)	46 (97.9%)	78(97.5%)
No	1(2.1%)	1 (2.1%)	02 (2.5%)
Hydroxyurea			
Yes	27 (81.8%)	42(89.4%)	69(86.3%)
No	6 (18.2%)	5(10.6%)	11(13.7%)
Analgesic			
Opioid	10(30%)	22(46.8%)	32 (40%)
NSAIDS	14(42%)	11(23.4%)	25(31.3%)
Transfusion history			
Ever	29(87.8%)	45(95.7%)	74(92.5%)
Never	4(12.2%)	2 (4.3%)	06 (7.5%)
Mean units of blood transfused	9.85(7.01)	10.68(7.99)	10.5 (7.5)

C. Ferritin Levels in The Study Population

The normal reference range for the laboratory method was 32-284ng/ml. The SF levels had a wide range hence a wide standard deviation as well. 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. The SF levels were further categorised as normal range (32-284ng/ml), high (but not meeting the threshold for iron overload(285-1000ng/ml) and very high meeting the threshold for possible iron overload (>1000ng/ml).
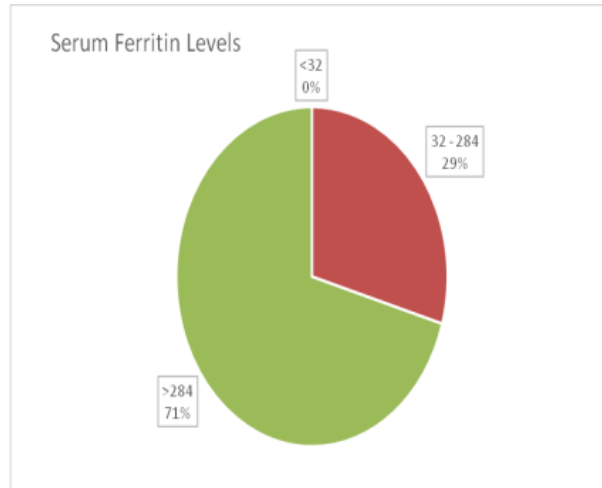
Figure 2

Twenty-five 25(31.3%) patients had SF above 1000ng/ml suggestive of possible iron overload. 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
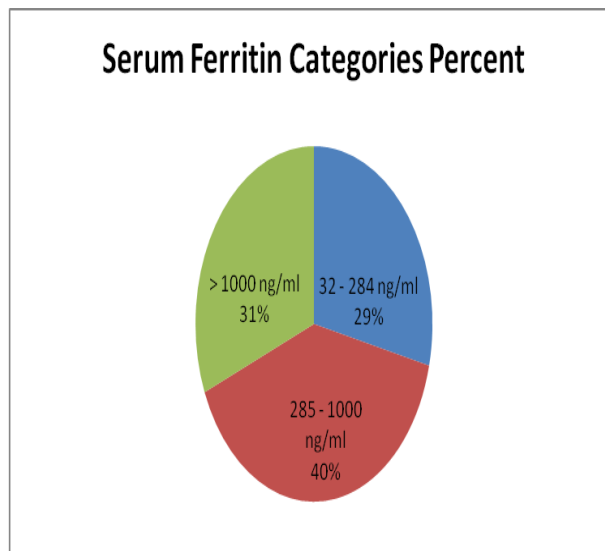

Figure 3

Seventy-four patients (92.5%) recalled having at least one unit of blood transfused while only 6(7.5%) reported never having any blood transfusion. More females 45(56.3%) than males 29(36.3%) had 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 though this difference was not statistically significant. The overall mean number of units of blood transfused was 10.5±7.5 ranging from no units transfused to maximum of 28 units transfused. Most patients 45(56.3%) had received between 1-10 units of blood transfusion. It was not possible to calculate the actual volume of blood transfused over the time before this study. This was due to missing data because of poor record keeping and/or poor access to the records especially given the fact that some patients had been admitted in other hospitals.

D. Correlation Of Serum Ferritin And Age
There was a non -linear correlation between SF and age in the study population. SF was not demonstrated to increase with age. There 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

Figure 4: Scatter plot of age and SF levels of the study population

Realtionship between Serum Ferritin & Age
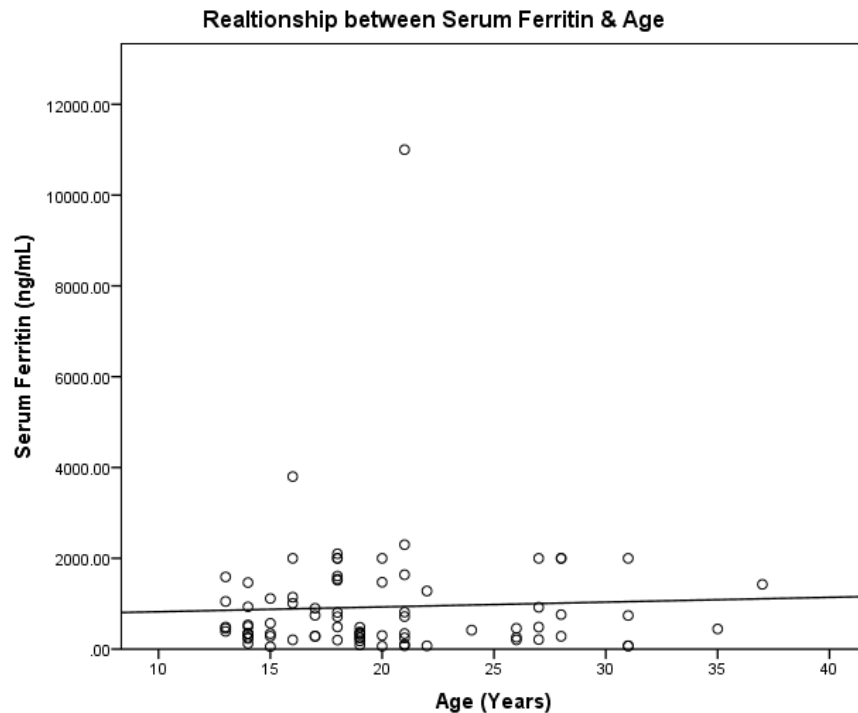
E. Correlation Between Sf and Blood Transfusion

The mean ferritin concentration for the females was 780.4 ± 564.2 ng/mL and for males was 980.35 ± 468.80 ng/mL. The minimum ferritin concentration for women was 53 ng/mL and the maximum was 1690 ng/mL. The minimum for men was 274 ng/mL and the maximum was 11000 ng/mL. The difference in mean ferritin concentration between men and women was not statistically significant using the unpaired $\mathbf{P} = 0.71$,
SF was found to be significantly associated with the number of units of blood transfusion ($p<0.001$). It appears that the more units of blood transfused the higher the serum ferritin and risk of iron overload.
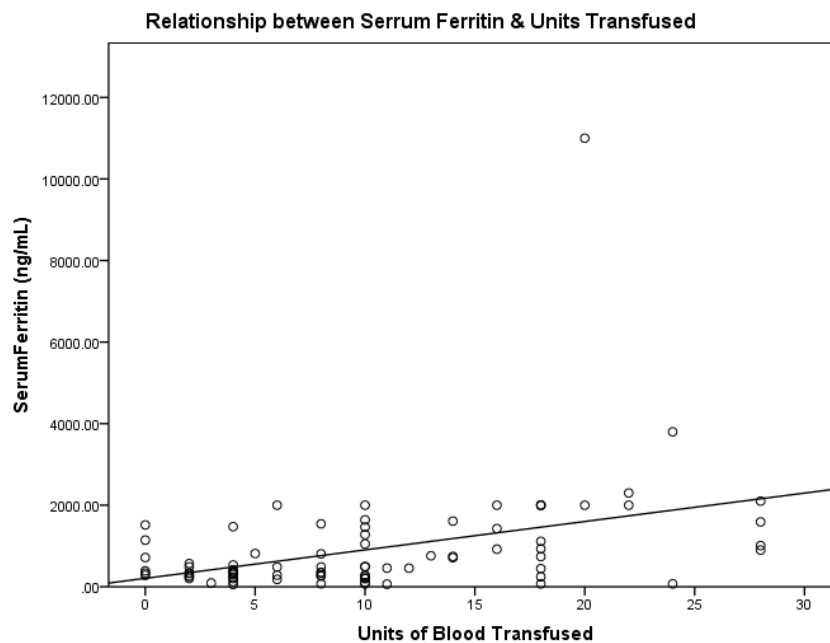
Relationship between Serrum Ferritin & Units Transfused

Figure 5

IV. 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.

As iron accumulates, the serum ferritin concentration rises and values above 300 ng/ml signify an increase in iron stores. Increase in iron stores in the multitransfused subjects reflects the amount of ferritin in plasma.(15)The relationship between SF and body iron stores is distorted by important confounders such as vitamin C deficiency, fever, infection, inflammation(61) ,and hepatic dysfunction, which are common occurrence among patients with SCD. These confounding factors were not investigated in our study. One way of improving the utility of SF measurements would be the application of a logarithmic quantization model that uses individual transfused iron values. This model has been shown to yield a median correlation between serum ferritin and transfused iron of 0.92 in patients with sickle cell anaemia (62).

Majority, 92.5% of our study population, recalled having received at least one unit of blood in their lifetime. 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 a mean age of 20.1 years, only 30% of the patients had received blood transfusion in their lifetime (63). In their study, the mean serum ferritin concentration was significantly higher among the SCA subjects who had ever received blood transfusion than those who were never transfused .This difference was found to be statistically significant p=0.004.(63) 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 (55). We studied 80 patients with a mean age of 19.7 ±5.53years and all our studypopulation 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 again found out that the mean SF concentration was higher in the sickle cell anemia patients who had multiple transfusions than in those who were rarely transfused (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. (59).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. They recommended that SF should not be used alone to diagnose iron overload or guide iron chelation therapy, rather it should be interpreted in combination with other parameter for assessing serum iron such as transferrin saturation, serum iron, total iron binding capacity and transfusional iron estimation should be (46). 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, 46, 58, 59, 62). 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. Blood exchange transfusion and use of iron chelator may prevent onset of iron overload and avert organ damage. (59) This study shows a positive association between serum ferritin and number of units of blood transfused, p = 0.001). A linear increase in serum ferritin level is seen in cumulative transfusion cases. Thomas Adamkiewicz and colleagues in Canada studied Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion and found it to nonlinear and are associated with iron load and liver injury. They also demonstrated a rising serum ferritin levels with the increasing number of units of blood transfusions. (43, 62).

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.(38, 45) 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, ferritin is an inflammatory protein and may be elevated in other conditions such a chronic infection, 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. It is also a universal cofactor for mitochondrial energy generation and supports the growth and differentiation of all cell types. 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.(64) 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 serum iron levels in Ninety patients with SCA who also had no history of blood transfusion and found that they had significantly lower serum iron than the controls.(56)
Das et al in India demonstrated as high as 23% prevalence of Iron Deficiency anaemia in children and adolescents cases of HbSS. (47)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 because we selected ambulatory patients clinically in steady state of the disease. Significant thrombocytosis with leucocytosis is common feature in SCA crisis due to chronic inflammation as a contributory pathogenic mechanism in SCA. (15)We did not find any significant association between serum ferritin and any of the red cell parameters.

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.(15, 56). Leukocytosis as observed could be due to subclinical inflammation especially due to chronic inflammatory disease that these patients frequently suffer from.(59)

V.   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 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
3. The serum ferritin levels in these patients do not correlate with either age or gender

VI.   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.

VII.   Study Recommendation

This study revealed that very high level of serum ferritin was observed in sickle cell anaemia patients who received ≥15 units of packed cells over a period of five years, suggesting an increase in iron stores and are at risk of developing iron overload. Serum ferritin should be routinely assayed for patients with HBSS requiring frequent blood transfusions for early detection of iron overload.

Due to the early onset of rising iron stores demonstrated by the elevated SF in most of our study patients and its correlation with the number of units of blood transfused, there is a 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 ferritin and other indicators of body iron status such as liver biopsy, cardiac MRI) with blood transfusion and clinico-pathological parameters.

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