Original Research Article

Serum ferritin and severity scores in sickle cell disease patients in Nnewi (south east Nigeria)

Abstract

Background: Sickle cell disease (SCD) patients have mechanisms that are thought to protect them more than apparently normal individuals from iron deficiency. However, evidence exists that in SCD, hypoferritinaemia may be more prevalent than hyperferitinaemia, especially in developing countries.

Method: Serum ferritin (SF) levels was measured - using an ELISA based kit (Biocheck, USA) and calculated disease severity in fifty- two asymptomatic steady state (ASS) SCD patients; who were iron chelation naive and correlated both parameters. Erythrocyte morphology and malaria parasitaemia were assessed, patients with parasitaemia were excluded. 64 apparently normal individuals in the same environment and socioeconomic group were also assessed as above and served as controls. Statistical analysis was done using SPSS version 20. Results were expressed as means and standard error of mean. Level of significance was set at p= 0.05.

Results: 30.7% and 7.6% of the test subjects had hypoferritinaemia and hyperferritinaemia respectively compared to controls where 56% had hypoferritinaemia and none had hyperferritinaemia. Erythrocyte morphology showed hypochromia and microcytosis to different degrees in all test subjects assessed: 1+ (10.5%), 2+ (63.2%) and 3+ (26.3%), while only 5% of controls had hypochromia and microcytosis . Blood transfusion and age did not seem to significantly affect SF levels (p= 0.652 and 0.929) respectively. SF levels increased progressively with disease severity but didn't reach statistical significance (p=0.49).

Conclusion: The results suggest that hypoferritinaemia is more prevalent than hyperferitinaemia, and that SF levels may be a useful index for computing an objective severity score in SCD management. Anaemia of chronic inflammation may cause a significant part of the anaemia in SCD.

Key words: serum ferritin, sickle cell disease, severity score, iron deficiency, hypochromic microcytic anemia

Introduction

Sickle cell disease (SCD) patients are known to absorb more iron from the gut than normal individuals because of intravascular hemolysis and increased loss of iron [1]. Also because of extravascular hemolysis, they are thought to recycle iron that should have been lost from hemolysis. The expectation is that the above mechanisms acting together should protect the SCD individual from iron deficiency [2]. This informs the reluctance to give iron to this group of patients. Some lines of evidence have however shown that iron deficiency may be more than expected in SCD patients [3,4]. This potential is increased in developing countries where dietary iron is low(5;6). Hence the need to screen for iron deficiency in asymptomatic steady state (ASS) sickle cell disease (SCD) patients by measuring their serum ferritin (SF) levels as this can have implications for their management. Objective severity scores were also correlated with their SF levels, since increased levels of iron can add to the oxidative stress already present in this disease(7).

Method

Patient selection

A one year study including fifty-two ASS SCD patients comprising thirty males and twenty- two females, -who had never had iron chelation therapy, were randomly selected from the sickle cell clinic and out stations of Nnamdi Azikiwe University Teaching Hospital. ASS was defined as patients who had not experienced crisis or had any febrile illness in the last two weeks and had not been transfused in the last three months. Written and ethical consent were obtained from the patents or their care givers and the hospital ethics committee respectively. Other data obtained by questionnaire were phenotypic and demographic. Data such as age, sex, frequency of crises, time of last crisis and complications such as priapism, ankle ulcers, stroke, avascular necrosis of any bone, especially the femoral head and any other condition complicating the disease. Most of these patients were on routine drugs such as folic acid, antimalarial prophylaxis, and vitamin supplements.

Sixty-three apparently healthy individuals from the same community and social economic group were selected as controls. Those that had any chronic disease, raised C-reactive protein (CRP), had taken iron medications in the last six months, or in the last one month prior to recruitment, had fever of felt unwell were excluded from the study.

Disease severity

Disease severity was determined by calculating an objective score using a modification of the method described by Hedo et al (8). The following characteristics were assigned points:

Hemoglobin concentrations, ≥10g/dl; ≥ 8g/d to < 10g/dl; ≥ 6 to < 8g/dl; ≥ 4 to < 6g/dl and < 4g/dl score 0, 1, 2, 3 and 4 respectively. Complications score 1 for each. White cell count, <11 × 10^3/µl; ≥ 11to <15 × 10^3 /µl; ≥ 15 to <20 × 10^3 /µl; ≥20 × 10^3 /µl score 0, 1, 2, 3 and 4

respectively. Scores of ≤ 3 were deemed mild disease; scores of 3 ≥ 5 were considered moderate disease, while scores > 5 were taken for severe disease.

Sample collection and laboratory analysis

Five (5) mls of blood was collected, 2 mls were dispensed into tubes containing Ethylene DiamineTetraacetic Acid (EDTA) for identification of malaria parasite (MP) by microscopy; using thick films stained with Giemsa. The same sample was used to prepare thin films, stained with Leishman, for examination of erythrocyte morphology. The remaining was dispensed into plain tubes for the determination of serum ferritin (SF) and CRP (for control subjects). SF levels were assayed using commercially available kits (Biocheck, USA). This assay was based on Enzyme Linked Immunoabsorbent Assay; the manufacturer suggested 20-250ng/ml, 10-120ng/ml and 7-140ng/ml as normal values for male, female and children (6 months to 15 years) respectively. Hypoferritinaemia for subjects was defined as <30ng/ul while hyperferritinaemia was taken as greater than the higher value for normal in the age and sex bracket. Serum CRP was assayed using CRP latex kits produced by BIOSYSTEMS® Inc according to manufacturer's instructions. All subjects that were MP positive were excluded (9).

Statistical Analysis

Data obtained was analyzed using the Statistical Package for Social Sciences software package version 20 (SPSS Inc., IL, Chicago, USA). Values obtained were tabulated by age and sex and expressed as means and standard error of mean. The chi square or Mann Whitney U tests were used to compare frequencies and generate p values - depending on whether the data was skewed

or not. Pearson's or Spearman's correlation tests were used to determine correlation between variables. P value less than 0.05 were considered significant.

Results

For SCD subjects, the mean±SD and age range were 20.52±10.50 years, and 4-47 years respectively; while for controls they were 24.08±11.19 and 5-47 respectively. The difference between the mean ages for test and control subjects was not statistically significant. Mean±SD SF levels for test and control subjects were 77.22 ±14.16 ng/ml and 22.95 ±4.32 ng/ml respectively. The difference was statistically significant (p = 0.001). The range of SF in the SCD subjects was 8.2-519.2ng/ml, median value was 48.3ng/ml; 30.7% had hypoferritinaemia, while 7.6% had hyperferritinaemia. For controls, range of SF was 0-170ng/ml, median and modal values were 13.5ng/ml and 2.7ng/ml respectively; 56% had hypoferritinaemia, while none had hyperferritinaemia. The mean SF value for different age groups among test and control subjects did not show any statistical difference p=0.93 and0.3 respectively (table 1).

The range, mean and median values of blood pints transfused for SCD subjects were 0- 40, 2.8 and 1 respectively. There was no statistically significant difference in the ferritin levels of SCD subjects that were transfused compared to those who were not transfused p=0.65 (table 2).

Samples of all SCD subjects randomly chosen (nineteen) for assessment of erythrocyte morphology showed hypochromia, and microcytosis to different degrees. These were 1+ (10.5%), 2+ (63.2%) and 3+ (26.3%). Of 56 control samples randomly chosen for assessment of erythrocyte morphology, 5 (9%) showed hypochromia and microcytosis; while 51 (91%) had normochromic, normocytic cells. Mean ferritin levels of SCD subjects, didn't correlate with any of the following variables: severity score, number of blood units transfused, hypochromia, age at which menarche occurred, number of complications or average number of crisis the subject had

per year (p=0.867, 0.286, 0.124, 0.359, 0.124 and 0.456 respectively) table 3. The trend was that mean SF levels increased with degree of disease severity in SCD subjects (figure 1). This trend however didn't reach statistical significance p=0.49

Discussion

The result clearly showed that in the test subjects, hypoferritinaemia was more prevalent than hyperferritinaemia. SF levels didn't correlate with level of erythrocyte hypochromia, number of blood units transfused or disease severity; although the trend was that it seemed to increase as disease severity worsened. Control subjects had a higher level of hypoferritinaemia compared to test subjects and none had hyperferritinaemia.

The pathophysiology of SCD having intravascular and extravascular hemolysis and increased frequency of transfusion as part of it makes them prone to hyperferritinaemia. However, many workers, especially in developing countries, have shown evidence that hypoferritinaemia is more prevalent in this group of patients than hyperferritinaemia (4). This apparent contradiction may be due to the following reasons. Iron excretion in SCD patients have been found to be abnormally high when compared to normal subjects or those with sickle cell trait and this has been linked to extravascular hemolysis in this group of patients (10). In developing countries iron deficiency is wide spread mainly because of dietary lack made worse by a high burden of parasites such as hook worm which are usually common(6) . This is evidenced by the low SF levels found in the control subjects, 56% of them had hypoferritinaemia. The above, combined with the fact that there are high levels of inflammatory cytokines in SCD;- since it is associated with a chronic inflammatory state even in ASS (11) which cause a compartmentalization of iron

in such a way that this element is not as available for erythropoiesis as in normal subjects, may explain the findings of microcytosis and hypochromia even when ferritin levels may be within the normal reference range or higher (12;13). There is evidence that microcytosis is an unreliable indicator of HbS/thallasaemia syndrones in the absence of conclusive family studies and or presence of HbA on electrophoresis (14).

The likely causes of microcytosis and hypochromia in SCD subjects are: HbS/thallasaemia syndrones, iron deficiency (14) and chronic inflammation (as explained above) (15); of all these, findings in this work seem to suggest that the most prevalent mechanism in this data set (Nigerian SCD patients) is through chronic inflammation. So, although, the anaemia of SCD, in the ASS is primarily caused by haemolysis, an important component is the anaemia of chronic inflammation. To the best of our knowledge, we are the first to make this statement and show evidence for it. Since SCD subjects have significantly higher levels of SF compared to controls, yet erythrocyte morphology showed microcytosis and hypochromia for every sample assessed compared to controls where only 9% of samples assessed showed microcytosis and hypochromia. Therefore iron deficiency is unlikely to be the main mechanism here. The prevalence of alpha thalassemia among SCD patients in Nigeria is 0.24 and is the same with the non SCD population (AA, AS, AC genotypes) (16) this therefore as a mechanism is unlikely to cause any difference in prevalence of microcytosis and hypochromia between test and control subjects.

SCD is associated with a chronic inflammatory state and because SF is an acute phase protein, judging hypoferritinaemia using reference values for normal subjects may not be appropriate. Koduri PR in his review suggested a value of <30ng/ul as more likely to be diagnostic for iron

deficiency in SCD patents (12). This is the value used to define hypoferritinaemia for SCD patients in this work.

In this data set, it was also found that number of blood units transfused over the test subjects life time didn't correlate with SF levels neither was there any significant difference in the SF levels of test subjects who were or were not transfused. This agrees with the work of Harmatz et al who showed that SF did not correlate with months of transfusion or tissue iron stores in their cohort of SCD patients, they thus concluded that SF was a poor marker for accurately assessing iron overload in SCD patients.(17); especially with SF levels of < 1500 ng/ml (18). An additional explanation for this finding is the fact that we used the total life time transfusion (TLT) records. Over time, urinary (and other sources) of iron loss in the test subjects may reduce tissue iron levels significantly such that it is unlikely to correlate with TLT levels. The authors also propose that the insignificant difference between transfused and non-transfused subjects may be for this same reason. There is evidence that using transfusion rate (TR) -TLT/ years receiving transfusion- would show a significant correlation between SF and number of pints transfused(19).

SF levels in the subjects increased with increasing disease severity, although this did not reach significant levels.(fig.1). Evidence exists that iron overload seems to be a predisposing factor for disease severity(20). The mechanism that has been proposed for this has to do with increasing levels of non-transferrin bound iron (NTBI) as transferrin gets saturated with increasing transfusions. NTBI and a subset of it, labile plasma iron (LPI) seem to enter the cell in a deregulated fashion and cause organ damage secondary to its high redox potential (7;21).

Interestingly, in disorders of iron metabolism, NTBI can appear in plasma in the absence of transferrin saturation. The mechanism by which this happens is not known (22). The authors propose that SF may then be useful as one of the indices that can contribute to calculating an objective score of SCD severity and should be assessed as part of routine management of these patients.

In conclusion, in this cohort of SCD patients, association with a chronic inflammatory state, which interferes with the release of iron to erythropoietic cells from iron stores seem to be the main mechanism by which microcytosis and hypochromia develop as compared to HbS/ thallasaemia syndrones and iron depletion. Therefore caution should be exercised in giving therapeutic iron to this group of patients. Iron deficiency should be proved by using other methods of evaluation before iron can be safely given. Although SF only, especially at levels < 1500 ng/ml, may not be a good index to monitor iron overload; it may be an important index that needs to be routinely assessed in the management of SCD. Clearly, more work with a larger cohort of patients' needs to be done in our clime to corroborate these findings.

The limitations to this work are that, although the association of SCD with chronic inflammation is well established, markers of inflammation could have been done in the test subjects to see how well their levels correlate with degree of microcytosis and hypochromia in them.

Table 1. Mean of Serum Ferritin levels in different age groups among SCD patients and controls

Groups		Age categories	N	Mean	Std. Error Mean	P-value
Control	Ferritin	<15 years	14	14.04	3.30	0.295
		>15 years	49	25.30	5.35	
SCD	Ferritin	<15 years	12	81.83	30.27	0.870
		>15 years	40	76.15	16.63	

Table 2. Mean Serum Ferritin values in transfused and non-transfused SCD subjects

Groups	N	Ferritin (ng/ul) (Mean± SEM)	Mann-Whitney Test
Transfused	29	91.76 ± 24.58	0.652
Not transfused	23	58.89 ± 17.31	

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227 **Table 3. Correlation of severity score and other parameters with ferritin in SCD subjects**

Parameters	Pearson's correlation	P-value
Ferritin vs number of unit transfused	0.024	0.867
Ferritin vs average number of crisis/yr	0.106	0.456
Ferritin vs age of Menarche	-0.230	0.359
Ferritin vs level of Hypochromia	0.309	0.124
Ferritin vs number of SCD complication	-0.069	0.124
Severity score vs Serum ferritin	0.151	0.286

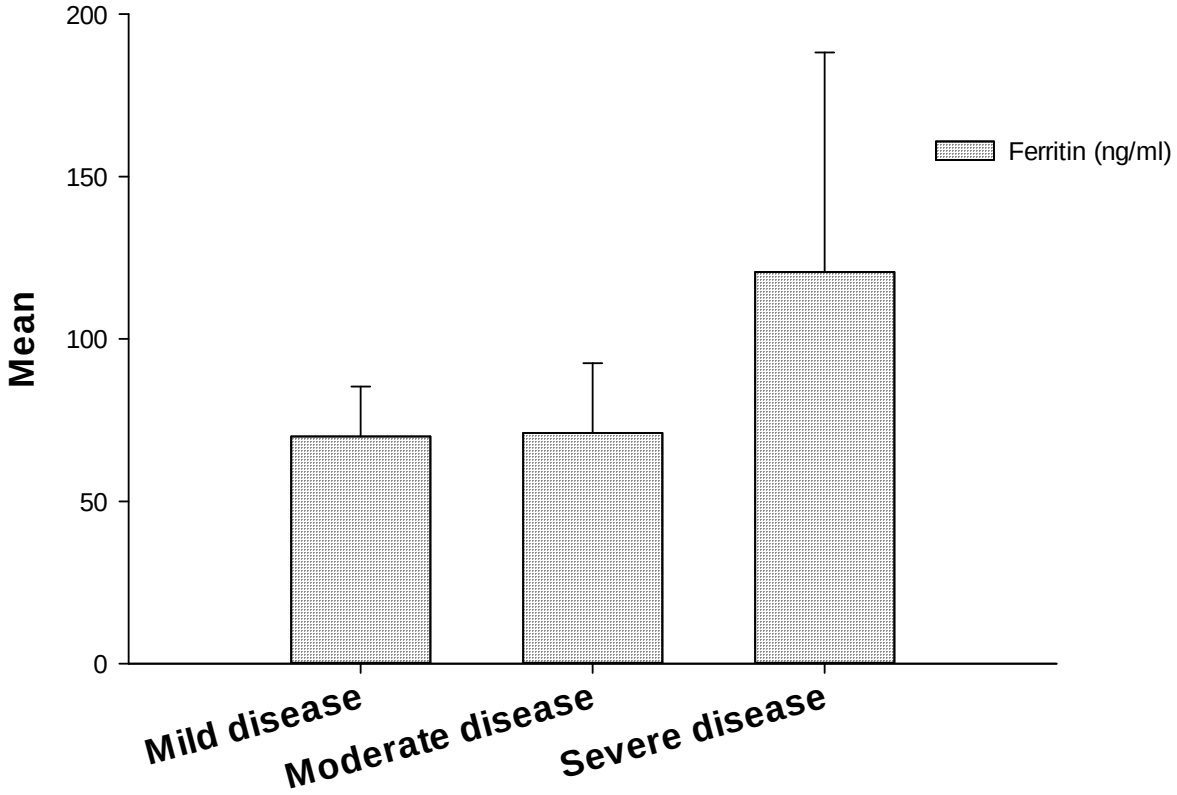

Fig.1 bar chart showing degrees of disease severity against mean serum ferritin levels in SCD subjects

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