

Original Research Article

Serum Iron Status and Haematological Profiles in Adult Nigerian Sickle Cell Anaemia Patients

Abstract

Background: Sickle cell anaemia (SCA) patients are prone to require long-term frequent blood transfusion because of chronic haemolysis and overlapping hyper-haemolysis. Hence, they become vulnerable to iron overload and its complications. On the other hand, iron deficiency has been documented among un-transfused SCA cases. Thus, there is a need to effectively and efficiently determine iron status of SCA patients.

Objective: We investigated whether adult SCA patients in steady state (SS) or those with vaso-occlusive crisis (VOC) have significantly different iron status viz-a-viz Serum Iron (SI), Serum Ferritin (SF), Total Iron Binding Capacity (TIBC) and Transferrin (TRF) and haematological parameters when compared with age- and sex-matched controls.

Materials and Methods: Ninety subjects, comprising 30 SCA patients in SS, 30 SCA patients with VOC and 30 HbAA, ethnicity, age- and sex-matched controls (CC), were consecutively recruited. Serum samples were analysed for SF, TRF and TIBC using 'WATER' HPLC 616 and 626; SI was determined using the Atomic Absorption Spectroscopic (AAS) method. Haematological parameters were determined using a Sysmex Kx21 auto-analyser.

Results: The red cells indices of SCA patients indicated iron deficiency anaemia. The SCA groups (VOC and SS) had significantly lower SI, SF, TRF and TIBC compared to the control group. The VOC group had a significantly lower mean ferritin level but higher SI and TRF levels compared to those of the SS group. The MCV, MCH and MCHC were significantly lower in the SCA groups compared to the CC group.

Conclusions: This study showed that significant differences exist in haematological parameters and the mean SI, SF, TRF, and TIBC status of SCA groups compared to CC groups. These, along with significantly lower MCV, MCH and MCHC, may be more indicative of iron deficiency anaemia in SCA groups rather than of iron overload.

Keywords: Sickle Cell Anaemia, Vaso-occlusive, Steady state, Adult, Ferritin, Serum Iron.

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38 Introduction

39 Sickle cell Anaemia (SCA) is a widespread genetic disorder characterized by red blood cells
40 (RBCs) deformity to abnormal rigid and sickle shaped forms that results in a risk of serious
41 complications. It occurs in high frequency in many tropical countries. In Nigeria, the prevalence
42 of SCA is 2–3% and the trait (HbAS) is 23% (1, 2). The genetic abnormality is a mutation in
43 position 6 of the beta (β) chain of haemoglobin (Hgb) where valine replaces glutamic acid [β s
44 (6V)]. The homozygote state [β s (6V)/ β s (6V)] known as SCA appears more severe than the
45 heterozygote state [β s (6V) plus another abnormal Hgb feature, such as “C”, “E”, thalassemia
46 etc.]. The clinical features of SCA begin in early childhood, deteriorating the quality of life with
47 advancing age and also reducing life expectancy [3, 4]. SCA patients suffer from various
48 complications such as chronic anaemia, frequent infection, hand- foot syndrome, stroke, acute
49 chest pain, vaso-occlusive crisis, splenomegaly/auto-splenectomy, hepatomegaly, priapism, renal
50 failure, heart failure and leg ulcers [5].

51 Physiologically, the human body effectively conserves iron, with loss being restricted to 1-2mg
52 per day. In conditions such as SCA, where long term blood transfusions may be indicated,
53 exogenous iron can accumulate, circulate as non-transferrin bound iron (NTBI), enter tissues,
54 form reactive oxygen species (ROS) and finally result in organ damage [6]. Hence, iron overload
55 is a presumed and unavoidable complication of multiply-transfused SCA patients.

56 Iron deficiency anaemia (IDA), on the other hand, is common amongst Nigerians due to low
57 dietary intake, malabsorption, excessive sweating and undue blood loss [7]. A study documented
58 a prevalence of 12% of IDA among Nigerian blood donors [7]. However, patients with
59 hereditary anaemias like SCA and thalassemia are not expected to develop IDA since recycling
60 of iron from premature destruction of erythrocytes contributes to iron stores [8, 9]. However,
61 contrary to this expectation, a report from USA suggested that iron deficiency was more
62 common than expected in un-transfused SCA cases, and it is presumed that iron deficiency will
63 be prevalent in SCA patients in tropical countries (9). In addition, causal observation in the
64 present authors’ routine care of SCA patients showed frequent lab findings (film review and red
65 cell indices) consistent with IDA, which had necessitated iron therapy.

66 Serum ferritin, serum iron and transferrin are known as acute phase reactants that are affected by
67 both inflammation and infection. Hence, interpretations of these serum proteins may be difficult

in SCA patients whose pathology is underlined by chronic inflammatory state and overlapping acute inflammatory events precipitated by infections. However, circulating ferritin has been identified as an indicator of iron status in the body, hence the need for its inclusion.

There is limited published information on the iron profiles in SCA patients in tropical regions where poverty, under-nutrition, hookworm infestation and undue blood loss alter iron status. The findings of Usanga *et al.* of low iron stores among blood donors strongly corroborated this assertion [10]. However, Aken'Ova *et al.* concluded that SCA patients have adequate levels of iron and ferritin in their serum [11]

Therefore, the present study investigated whether adult SCA patients in steady state (SS) or in vaso-occlusive crisis (VOC) have significantly different haematological parameters and iron status (*viz a viz* serum iron, TIBC, Ferritin and Transferrin) when compared with an age- and sex- matched control group (CC). This will serve to confirm the suspicion of iron overload or iron deficiency anaemia in this area of study.

Materials and Method

Ethical approval was obtained from the University of Ibadan/University College Hospital [UI/UCH] ethical board and written informed consent (attached to every questionnaire completed by each participant) was obtained from individual patients prior to recruitment into the study.

Study Design: This was a prospective, case controlled study, carried out in the Departments of Haematology and Chemical Pathology, University of Ibadan. The 90 consecutively recruited subjects were 30 SCA (HbSS) patients in VOC, 30 SCA patients with SS and 30 HbAA, ethnicity-, age- and sex-matched controls [Table 1]. The SCA patients were those being followed up regularly at our Day-care and Outpatient clinics. Record of previous blood transfusion revealed transfusion loads of 1–2 units in 90% and 3–6 units in 10% of the SCA patients.

Blood Sample Collection: Approximately 6mls of venous blood was collected from each participant. 2mls of blood sample from each participant was put into an EDTA bottle, and this was used for the analysis of haematologic parameters using a Sysmex Kx21 autoanalyser. The remaining 4mls of blood sample from each participant was put in iron-free plain bottles. The blood samples in the iron free plain bottles were allowed to clot. The serum sample separated from it was put into an aliquot and stored at -80°C until analysis for iron studies.

Diagnostic Method: Serum iron was determined using the Atomic Absorption Spectroscopic method (AAS): Serum iron was measured by Ferrozine/Magnesium carbonate method using the fully automated biochemistry I-Lab 650i auto-analyzer [12]. Serum Ferritin, Transferrin and

TIBC were analysed using HPLC Water 616/626, which was controlled using working standards from the certified Reference Standards (1000ppm) [13]. Ferritin, transferrin and TIBC were analysed using 249nm, 237nm and 228nm wavelengths, respectively.

Haematological Analysis: The procedure followed was based on the instruction manual of the Haematology Analyzer (Sysmex Kx21). Total red blood cell (RBC) count ($\times 10^6/\mu\text{L}$), haemoglobin content (Hb; g/dL), haematocrit (HCT; %), total number of white blood cells (WBC) or leukocytes ($\times 10^3/\mu\text{L}$), lymphocyte count (LYM; $\times 10^3/\mu\text{L}$), lymphocyte percentage (LYM; %), and platelet (PLT) count ($\times 10^3/\mu\text{L}$) were assessed. Mean corpuscular volume (MCV; fL), mean corpuscular haemoglobin (MCH; pg), mean corpuscular haemoglobin concentration (MCHC; %), red blood cell distribution width (RDW; fL), platelet distribution width (PDW; fL), mean platelet volume (MPV; fL) and platelet larger cell ratio (P-LCR; %) were also calculated.

Statistical Analysis: The data were presented in $\times \pm \text{SD}$. Student t test was used to determine the differences between the means by using SPSS (version 15). $P < 0.05$ was considered significant.

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118 Results

119 The demographic pattern of the subjects is as shown in table 1.

120 Table 1: Mean age, sex and BMI of study groups

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Group	Age(years)	Male	Female	BMI
Control (n=30)	26.1 $\pm$ 4.8	14	16	22.5 $\pm$ 2.6
HbS (SS) (n=30)	27.1 $\pm$ 6.3	18	12	20.8 $\pm$ 3.1
HbS (VOC) (n=30)	24.9 $\pm$ 4.9	13	17	22.3 $\pm$ 8.9

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140 Table 2: Shows the comparison of the mean values of SI, SF, TIBC and TRF in the study groups

HbSS	VOC (×±SD) N=30	SS (×±SD) N=30	CC (×±SD) N=30	P,valuevoc vs SS	P,valuevoc vs CC	P,valueSS vsCC
TIBC(umol/L)	2064.42±135.03	2011.04±157.87	3184.41±254.70	0.165	0.000*	0.000*
Ferritin (ng/ml)	73.67±7.00	108.40±50.40	216.12±14.55	0.000*	0.000*	0.000*
Transferrin	224.38±11.12	216.50±15.00	301.34±31.99	0.024*	0.000*	0.000*
Serum Iron (umol/L)	110.44±19.56	76.52±4.88	155.19±9.7	0-000*	0.000*	0.000*

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According to Table 2, the SCA groups (VOC and SS) had significantly ($p = 0.000$) lower serum Iron, Ferritin, TIBC and transferrin than the control group, while the SCA in the VOC group had comparable TIBC but significantly lower Ferritin ($p = 0.000$) and significantly higher serum iron and Transferrin ($p = 0.000$ and $p = 0.024$, respectively) compared with SCA in the SS group.

Table 3: Mean values of Haematological parameters in SCA patients (SS vs VOC) and Controls (CC).

HbSS	VOC	SS	CC	VOCvs SS	VOCvsCC	SSvsCC
	×±SD	×±SD	×±SD	p-value	p-value	p-value
PCV (%)	24.2±5.8	21.7±4.5	40.1±3.5	0.085	0.000*	0.000*
WBCx10⁹/m m³	14.0±4.7	10.7±3.9	5.3±1.5	0.000*	0.000*	0.000*
PLT x10⁹/mm³	266.8±137.3	359.7±141.6	204.5±48.1	0.001*	0.000*	0.001*

Hgb (g/dl)	8.3±1.5	7.7±1.7	12.9±1.2	0.165	0.000*	0.000*
MCV(FL)	80.8±10.8	74±10-3	86.7±12.4	0.001*	0.000*	0.05*
MCH (pg/dl)	29.8±7.9	26.8±4.2	28.9±3.3	0.04	0.031*	0.88
MCHC (g/dl)	35.5±3.2	35.8±2.1	31.9±1.6	0.82	0.000*	0.000*

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161 According to Table 3, the PCV, Hgb, WBC, Plt count and red cell indices viz a viz MCV, MCH,
162 and MCHC were significantly lower ($p = 0.000$) in SCA patients (SS and VOC) compared to
163 control group. While the Hct and Hgb were significantly lower ($p = 0.000$), the Plt and the WBC
164 were significantly elevated ($p = 0.000$) in SCA compared to the CC group. The MCV and
165 MCHC were significantly reduced [$p = 0.05$ and $p = 0.000$, respectively] in SCA. Although the
166 MCH was elevated in the VOC group compared to the CC group, it was not significantly
167 different in the SCA (SS) group compared to the CC group. In the SCA (VOC) and SCA (SS)
168 groups, WBC and MCV were significantly higher ($p = 0.000$ and $p = 0.001$, respectively), while
169 the platelet count was significantly higher ($p = 0.001$) in the SS group compared to the CC
170 group.

171 172 Discussion

173 Iron is a very important element in human metabolism. It plays a central role in erythropoiesis
174 and it is also involved in many other intracellular processes in all the tissues of the body [14].
175 Iron is a universal cofactor for mitochondrial energy generation and supports the growth and
176 differentiation of all cell types.

177 A SCA patient in the tropics is prone to iron overload as an inevitable complication of episodic
178 and/or chronic blood transfusion, but at the same time the patient is not immune from inherent
179 and environmental factors that precipitate IDA in the tropics. Such factors include poor nutrition,
180 parasitic infestations like hookworm and schistosomiasis as well as varying bacterial infections
181 that may disturb iron metabolism. In addition, poor absorption and metabolism of iron resultant
182 from multiple mucosal/sub-mucosal infarcts and multiple organ failure, to which SCA patients
183 are particularly highly susceptible to, are other factors that can precipitate iron deficiency
184 anaemia.

185 In SCA, it has been hypothesized that iron deficiency might be beneficial by reducing the
186 percentage of sickle cells and hence reducing the prevalence of painful crises [15]. However,
187 iron deficiency may be deleterious to SCA patients since iron will not be available for effective
188 erythropoiesis and other physiological activities requiring iron. Therefore, sufficient measures
189 are required to maintain iron homeostasis in SCA patients.

190 The SCA patients involved in this study were those of ‘low blood transfusion burden’ because
 191 the patients were consecutively recruited without taking into account the number of previously
 192 received blood transfusions. The information on the number of previous blood transfusion
 193 received was obtained via the questionnaire. The analysis of the questionnaire eventually
 194 revealed that 90% of the SCA patients had received 1–2 units while 10% received 3–6 units in
 195 the past.

196 Therefore, the findings of significantly lower ($p = 0.000$) mean serum Iron, ferritin, TIBC and
 197 transferrin in SCA groups compared to the control group may not be out of place. Although
 198 TIBC was comparable in the two SCA groups, serum iron and transferrin were found to be
 199 significantly higher ($p = 0.000$ and $p = 0.024$, respectively) in the VOC group. This finding may
 200 not be unconnected with the fact that transferrin is an acute phase reactant. However, serum
 201 ferritin, which is also an acute phase reactant, was found to be significantly lower ($p = 0.000$) in
 202 the VOC group compared to the SS group.

203 It appears somewhat difficult to explain the finding of significantly lower mean levels of serum
 204 Iron, ferritin, TIBC, and transferrin in SCA compared to the control group. Therefore, it is
 205 possible that the baseline iron status of these patients could be below normal or borderline, or
 206 they might in fact have undetected IDA. The cause of such IDA may not be different from that of
 207 the general population. The finding of significantly lower MCV, MCH and MCHC in this index
 208 study equally strongly suggests the existence of IDA in the SCA group compared to the control
 209 group. Another possible explanation is multi-organ failure inherent in SCA patients, which in
 210 turn causes deranged iron metabolism [15]. Previous studies have shown that repeated attacks of
 211 crisis, transfusions, and underlying chronic inflammatory state, culminate in multi-organ failure,
 212 which may ultimately cause death [16, 17].

213 Olubuyide *et al.* in 1983 [18] documented a mean Ferritin level of $296.3 \pm 61.9\text{ng/ml}$ in a
 214 Nigerian SCA group. The mean serum ferritin levels determined in the present study, of
 215 $73.67 \pm 7.00\text{ng/ml}$ in SCA (in VOC) and $108.40 \pm 50.4\text{ng/ml}$ in SCA (in SS), were considerably
 216 lower than the one determined over three decades ago. The low transfusion burden of 1–2 units
 217 in 90% and 3–6 in 10% may partly explain the difference in mean levels. In addition, the
 218 analytical methods employed may account for the difference. In this case, HPLC was employed
 219 in carrying out the analysis.

220 The literature has divergent and multiple reports as regards iron status in SCA patients both in
 221 respect of multiply transfused cases and un-transfused cases. In the study of Patria *et al.* [16],
 222 15% of SCA patients who received blood transfusion and 16% of patients who did not receive
 223 blood transfusion were found to have increased serum ferritin ($p < 0.0001$). However Davis *et al.*
 224 [19] did not find a statistically significant difference in ferritin levels of SCA patients who
 225 received <5 units of blood compared to those who had never undergone transfusion. However,
 226 Porter *et al* [20] found good correlation coefficient between serum ferritin and the number of
 227 units transfused ($r = 0.86$).

The finding of a low iron status in SCA patients compared to the control group in this study may be advantageous to the patients, according to Ballaset *al.* [21], who showed that patients with low value of serum ferritin and percentage saturation have lower incidence of acute painful episodes.

As regards haematological parameters, the significantly reduced PCV and Hgb in the SCA group compared to the control group in this study confirmed the chronic haemolytic process on-going in SCA patients. In addition, significantly reduced mean MCV and MCHC values in SCA compared to control groups strongly support the finding of significantly reduced serum iron, serum ferritin, demonstrated in SCA groups compared to the control group.

Significantly elevated ($p = 0.001$) platelet count and white cell count in SCA patients compared to the control group also confirmed chronic inflammation as a contributory pathogenic mechanism in SCA. However, this study showed that platelet and white cells are significantly higher in the SCA (SS) group compared to the SCA (VOC) group.

The finding of low iron status in SCA groups compared to the control may be connected to the fact that the transfusion burden in this group of SCA was low; the traditional avoidance of iron-containing supplement by SCA patients as a means of preventing iron overload; the possibility of poor absorption and metabolism as a result of multiple organ failure and, finally, poor nutrition as a result of low socio-economic status compounded by the high financial burden of managing the disease.

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