

# Serum ferritin levels in adults with sickle cell disease in Lagos, Nigeria

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**Background:** Serum ferritin is considered to be one of the most important tools in the measurement of iron balance in steady-state sickle cell disease. Increased gastrointestinal absorption of iron has been reported in sickle cell disease because of the associated chronic hemolysis, and it is also thought that repeated red cell transfusion consequent to chronic hemolysis and anemia causes excessive iron levels. The aim of this study was to determine overall and gender-specific mean ferritin levels in patients with steady-state sickle cell disease in order to establish the prevalence of iron deficiency and overload.

**Methods:** This was a cross-sectional study in homozygous patients with sickle cell disease attending the sickle cell clinic at Lagos State University Teaching Hospital, Ikeja. A 5 mL blood sample was collected in plain bottles from consenting participants during steady-state periods. The serum was separated and analyzed for ferritin by enzyme-linked immunosorbent assay. Another 5 mL sample was collected for a full blood count, done on the same day of collection, to determine red blood cell indices, ie, mean cell volume, mean cell hemoglobin concentration, and mean corpuscular hemoglobin concentration. The Pearson Chi-square test was used for statistical analysis. The differences were considered to be statistically significant when  $P$  was  $<0.05$ .

**Results:** In total, 103 patients were recruited for this study and comprised 58 women (56.40%) and 45 men (43.70%). The overall mean ferritin concentration was  $93.72 \pm 92.24$  ng/mL. The mean ferritin concentration in the women was  $92.00 \pm 88.07$  ng/mL and in men was  $96.41 \pm 99.80$  ng/mL. Only eight (7.76%) of the 103 patients had a serum ferritin level  $< 15$  ng/mL, while two subjects (1.94%) had a serum ferritin level  $> 300$  ng/mL. Ninety-three subjects (90.29%) had serum ferritin within the normal reference range of 15–300 ng/mL.

**Conclusion:** In this study, 90% of subjects with sickle cell disease had normal iron stores; serum ferritin was higher in men than in women, and iron deficiency was more common than overload in the disease.

**Keywords:** serum ferritin levels, sickle cell disease

## Introduction

Sickle cell disease results from an inherited abnormality of hemoglobin structure. The abnormal hemoglobin has valine replacing glutamine at position 6 of the beta-globin chain.<sup>1</sup> The hemoglobin variant produced polymerizes at low oxygen tension, causing the characteristic sickle deformity of red blood cells, increased mechanical fragility, shortened survival, and chronic hemolytic anemia.<sup>2</sup>

The normal iron level in men is 50 mg/kg and 40 mg/kg in women, and the normal serum ferritin range is 15–300  $\mu$ g/L, being higher in men (mean 100  $\mu$ g/L) than in premenopausal women (mean 30  $\mu$ g/L).<sup>3</sup> Ferritin is the primary iron storage protein in

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tissues, and ferritin is also an acute phase reactant, with elevated serum levels in the presence of chronic inflammation, infection, and liver disorders associated with sickle cell disease in crisis. In the absence of deranged iron status, conventional laboratory assessments of iron status, such as mean cell volume, mean cell hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width, may be abnormal in patients with sickle cell disease due to the chronic inflammation and hemolysis associated with the disease.<sup>4,5</sup>

It is also difficult to quantify the contribution of iron deficiency to anemia in chronic inflammatory conditions. This is especially true in conditions such as sickle cell disease where proinflammatory cytokines may induce an anemia of inflammation and alter the biochemical indicators of iron status commonly used to diagnose iron deficiency.<sup>6,7</sup> Despite all these limitations, and in the absence of other pathology, serum ferritin is generally a reliable indicator of the presence of iron overload.<sup>3</sup> Serum ferritin is also considered one of the most important tools in measurement of the state of iron balance in steady-state sickle cell disease.<sup>8</sup> In the event of increased gastrointestinal absorption of iron due to chronic hemolysis associated with sickle cell disease,<sup>9</sup> it is also expected that repeated red cell transfusions consequent to chronic hemolysis and anemia leads to excessive iron levels.<sup>10</sup> Several studies have reported iron overload in sickle cell disease.<sup>11–13</sup> However, other studies have reported iron deficiency in sickle cell disease,<sup>10,14</sup> despite the risk of increased gastrointestinal absorption. This study aimed to determine both overall and gender-specific mean serum ferritin levels in patients with steady-state sickle cell disease in order to establish the prevalence of iron deficiency and overload in steady state.

## Materials and methods

This was a cross-sectional study in homozygous patients with sickle cell disease attending the sickle cell clinic of Lagos State University Teaching Hospital, Ikeja, between October and December 2011. Approval for the study was obtained from the research and ethics committee at the hospital. Consenting participants were helped to complete a structured questionnaire, consisting of demographic information, history of smoking, consumption of alcohol, drug use apart from the usual routine medications for sickle cell disease, history of blood transfusion, and frequency of bone pain crises. Inclusion criteria were hemoglobin phenotype SS, no history of crises in the previous three months as established by a careful history and complete physical examination, no previous history of surgery, and no history of blood transfusion in the previous

three months. Exclusion criteria were a history of blood transfusion in the past three months, hemoglobin phenotype SC, previous history of surgery, and human immunodeficiency virus infection. Treatment with hydroxyurea is not a routine and standard practice at the center where the study was carried out. Participants were routinely treated with folic acid, vitamin B complex, and paludrine.

## Collection of samples

A 5 mL blood sample was collected from each subject into a nonanticoagulated bottle during a period of steady state. The serum was separated and stored at  $-20^{\circ}\text{C}$  on the day of collection until analysis for ferritin. Another 5 mL sample was collected from each subject for a full blood count, done on the same day of collection, to determine mean cell volume, mean cell hemoglobin, and mean corpuscular hemoglobin concentration.

## Testing

An enzyme-linked immunosorbent assay kit (Biotec Laboratories Ltd, Ipswich, UK) was used for determination of ferritin. After incubation, bound/free separation was performed by simple solid-phase washing. The ferritin concentration in the sample was calculated based on a standard curve. The kit has a control and six standards ( $S_1$ – $S_6$ ) with concentrations of 0, 5, 20, 100, 400, and 1000 ng/mL, respectively. The standards were calibrated against the World Health Organization Human Liver Ferritin International Standard 80/602. Assay of the control, standards, samples, and blanks was performed in duplicate, using the mean value for each parameter. Values of the absorbance of the standards against concentration were plotted to obtain a standard curve using the best-fit points. Absorbance values for the samples were interpolated on the standard curve to obtain the corresponding concentrations expressed in ng/mL. The mean reference values and ranges quoted by the manufacturer are 53 ng/mL; 6–180 ng/mL and 175; 20–400 ng/mL for both premenopausal women, and men, respectively.

## Analysis of data

The descriptive data are shown as the mean  $\pm$  standard deviation. The Pearson chi-square test and *t*-test were used for analytical assessment. The differences were considered to be statistically significant at  $P < 0.05$ .

## Results

In total, 103 patients were recruited for the study, comprising 58 (56.40%) women and 45 (43.70%) men. The mean age for

the entire group was  $24.08 \pm 7.84$  (range 14–144) years. The mean age for the women was  $25.13 \pm 8.47$  years and for the men was  $22.82 \pm 6.89$  years. The majority (77, 74.8%) had previously received blood transfusions, while 26 (25.2%) gave no history of blood transfusion (Table 1).

Overall mean hemoglobin, cell volume, cell hemoglobin, and corpuscular hemoglobin concentrations were  $7.92 \pm 1.49$  g/dL,  $80.32 \pm 11.30$  fl,  $26.40 \pm 3.19$  pg, and  $32.42 \pm 1.05$  g/dL, respectively. The minimum and maximum hemoglobin concentrations were 4.6 g/dL and 11.8 g/dL, respectively; the minimum mean cell volume, mean cell hemoglobin, and mean corpuscular hemoglobin concentration values were 62.9 fl, 16.5 pg, and 29.9 g/dL, respectively, and the maximum values were 103.0 fl, 34.30 pg, and 34.40 g/dL, respectively. Mean hemoglobin, cell volume, cell hemoglobin, and corpuscular hemoglobin concentration values for men were  $8.11 \pm 1.53$  g/dL,  $81.80 \pm 14.12$  fl,  $26.56 \pm 3.70$  pg, and  $32.44 \pm 1.01$  g/dL, respectively; and for women were  $7.78 \pm 1.42$  g/dL,  $79.09 \pm 6.39$  fl,  $26.27 \pm 2.48$  pg, and  $32.41 \pm 1.10$  g/dL, respectively (Table 1).

The overall mean ferritin concentration was  $93.72 \pm 92.24$  ng/mL, the minimum was 5 ng/mL, and the maximum was 480 ng/mL. The mean ferritin concentration for women was  $92.00 \pm 88.07$  ng/mL and for men was  $96.41 \pm 99.80$  ng/mL. The minimum ferritin concentration for women was 5 ng/mL and the maximum was 430 ng/mL. The minimum for men was 10 ng/mL and the maximum was 480 ng/mL. The difference in mean ferritin concentration between men and women was not statistically significant

using the unpaired *t*-test ( $0.24, P = 0.81$ , Table 2). The mean ferritin concentration of subjects who had a history of blood transfusion was  $91.72 \pm 98.21$  ng/mL and for those who had not been previously transfused was  $100.83 \pm 68.75$  ng/mL. This was also not statistically significant ( $P = 0.662, t\text{-test} = 0.44$ , Table 2). Only eight (7.76%) of the 103 patients had serum ferritin  $< 15$  ng/mL. While the majority (95, 92.23%) had serum ferritin  $> 15$  ng/mL, only two (1.94%) had serum ferritin  $> 300$  ng/mL. In total, 93 (90.29%) had serum ferritin within the normal reference range of 15–300 ng/mL (Table 1).

No statistically significant relationship was found between hemoglobin and corresponding ferritin concentration ( $P = 0.877$ ), nor between patient age and ferritin concentration ( $P = 0.187$ , Table 1).

## Discussion

Blood transfusion in patients with sickle cell anemia serves two major functions, ie, increasing the oxygen-carrying capacity of the blood<sup>15</sup> and replacing abnormal red cells with normal ones, thereby alleviating symptoms and preventing complications.<sup>16,17</sup> Over 70% of the patients recruited for this study had a previous history of blood transfusion. This highlights the fact that the practice of blood transfusion is common in the sickle cell anemia population, and almost all adult patients may have had multiple transfusions. However, this study did not show a statistically significant correlation between previous history of blood transfusion and ferritin concentration.

**Table 1** Demographic distribution, history of blood transfusion, red blood cell indices, and ferritin levels

	Gender		
	Male	Female	Total
Mean age (years)	58 (56.40%)	45 (43.70%)	103 (100%)
Previous history of blood transfusion	Yes	No	
	77 (74.8%)	26 (25.2%)	
Red blood cell indices	Minimum	Maximum	Mean
	Male	Female	
Hemoglobin concentration	4.6	11.8	$8.11 \pm 1.53$
Mean cell volume (femtoliter)	62.9	103.0	$81.80 \pm 14.12$
Mean cell hemoglobin (picogram)	16.5	34.30	$26.56 \pm 3.70$
Mean cell hemoglobin concentration (g/dL)	29.9	34.40	$32.44 \pm 1.01$
Ferritin concentration levels	Male	Female	
Mean $\pm$ standard deviation (ng/mL)	$96.41 \pm 99.80$	$92.00 \pm 88.07$	
15–300 ng/mL	93 (90.29%)		
<15 ng/mL	8 (7.76%)		
>300 ng/mL	2 (1.94%)		
Hemoglobin versus ferritin levels	$P = 0.877$		
Age versus ferritin levels	$P = 0.187$		

**Table 2** Correlation between mean ferritin concentration, gender, and history of blood transfusion

Parameters	n	Mean ferritin	P value	t-test
Male	45	96.41 ± 99.80	0.812	0.24
Female	58	92.00 ± 88.07		
	<b>Yes</b>	<b>No</b>	<b>P value</b>	<b>t-test</b>
Blood transfusion	77	22	0.662	0.44
Mean ferritin	91.72 ± 98.21	100.83 ± 68.75		

Mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration are all reduced in anemia of chronic disease. Expectedly, in this study, mean hemoglobin concentration, mean cell volume, and mean cell hemoglobin were higher in men than in women. Increased erythropoiesis due to androgens in males, and iron loss or blood loss in females during menstruation may be responsible for higher levels of the red cell indices in males. However, reference ranges for erythropoietin are not different between the men and women.<sup>18</sup>

The mean ferritin concentration obtained for women was much higher than the nonsickle cell value quoted by the manufacturer of the kit used for the study. However, a lower value was obtained in the men. As expected, the mean ferritin value obtained in this study was higher in men than in women.<sup>3</sup>

The majority (90%) of the patients had serum ferritin within the normal reference range of 15–300 ng/mL. This was an unexpected result, given that over 70% of the patients recruited for this study had a previous history of blood transfusion, and also considering that, given the high rate of chronic hemolysis seen in patients with sickle cell disease, increased gastrointestinal absorption of iron due to chronic hemolysis would have been expected.<sup>9</sup> It has been shown that repeated red blood cell transfusion consequent to chronic hemolysis and anemia provides excessive iron levels and ferritin values above the normal reference range.<sup>10</sup> This study reported only about 2% of patients with a ferritin concentration above the normal reference range of 300 ng/mL, while 7.76% had ferritin levels below the lower limit of the normal reference range (15 ng/mL). A similar result was obtained by Aken'ova et al who reported a prevalence of 6% for iron deficiency in adult patients with sickle cell disease in Ibadan, Nigeria.<sup>19</sup> Iron deficiency may be more common in sickle cell anemia than is widely believed.<sup>14,20</sup> Iron deficiency is thus more common than iron overload in patients with sickle cell disease, which is not consistent with the numerous reports of iron overload in patients with sickle cell disease.<sup>11–13</sup> Major confounders in our study were the other causes of anemia

not evaluated, including malaria, hookworm infestation, and folate/vitamin B12 deficiency, but unless associated with loss of iron they would not impact on the results.

## Conclusion

The majority of patients with sickle cell disease have normal iron stores, mean serum ferritin is higher in men than in women, and iron deficiency is more common than iron overload in sickle cell anemia.

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## Disclosure

The authors report no conflicts of interest in this work.

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