SERIAL EVALUATION OF IRON STORES IN PREGNANT NIGERIANS WITH HEMOGLOBIN SS OR SC

Olalekan O. Abudu, MB, BS, MRCOG, FRCS, Kehinde Macaulay, MS,
O.A. Oluboyede, MD, MRC (Path), FMC (Path)
Lagos, Nigeria

Iron status of nonpregnant and pregnant Nigerian patients with hemoglobin SS or SC were assessed using serial hematological parameters, measured by Coulter counter, and serial serum ferritin concentrations measured by radioimmunoassays.

The median value of 393 μg/L (range, 175 to 900 μg/L) for serum ferritin in nonpregnant patients with Hb SS and SC was significantly higher than that found in nonpregnant patients with Hb AA (median, 89.8 μg/L; range, 13 to 250 μg/L). Apart from packed cell volume values, there were no other significant differences between patients with Hb SS or SC and Hb AA in the other parameters assessed: mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

In both the normal pregnant patients (Hb AA) and pregnant patients with Hb SS and SC the serum ferritin values decreased as pregnancy advanced to 28 weeks and rose gradually thereafter. At similar stages of gestation serum ferritin values were significantly higher in patients with Hb SS or SC than in those with Hb AA. Pregnancy seems to have induced a significant rise in mean corpuscular volume and mean corpuscular hemoglobin values in the patients with Hb SS or SC, especially in the third trimester, than in patients with Hb AA. The pattern of change in mean corpuscular hemoglobin concentration values was similar in both groups of patients.

From the data obtained, it seems the iron status in the patients with Hb SS or SC was good, and pregnancy did not push the patients into an iron deficiency state. The use of prophylactic iron supplementation in pregnant patients with Hb SS or SC appears unjustified.

Sickle cell disease comprises patients with hemoglobin genotype SS and SC (Hb SS and SC). The red cells of patients with sickle cell disease hemolyze readily because of a genetic disorder of the red cells. When the red cells are hemolyzed, it is assumed that the iron component of the heme molecule that makes up the hemoglobin is stored and reused since because there is no active hemorrhage taking place. Because of the chronic hemolysis in these patients and consequent iron storage, these patients are not likely to be iron deficient even though they have chronic anemia.[1]

Fleming,[2] using bone marrow studies, observed that pregnant, anemic Nigerians with hemoglobinopathy have adequate iron stores. These women should, therefore, be advised not to take iron supplementation

TABLE 1. COMPARISON OF CLINICAL DATA OF PREGNANT PATIENTS STUDIED

Subjects	Age (yr) Mean ± SEM	Height (cm) Mean ± SEM	Initial Weight (kg) Mean ± SEM	Gestation at Initial Visit Mean ± SEM	Gestation at Delivery (wk) Mean ± SEM	Birthweight (kg) Mean ± SEM
Hb SS or SC n=15	26 ±1.14	166 ±1.63	56.9 ±1.47	13.5 ±1.01	38.07 ±0.55	2.58 ±0.15
Hb AA n=20	23.9 ±1.12	163 ±0.92	58.9 ±1.77	11.1 ±0.87	39.05 ±0.24	3.17 ±0.08
Difference (t)	0.93	1.18	0.61	1.09	1.24	2.57
Significance (P)	>.1	>.1	>.1	>.1	>.1	<.02

TABLE 2. SERIAL MEAN PACKED CELL VOLUME* IN PREGNANT NIGERIANS WITH Hb AA AND Hb SS OR SC

	Nonpregnant (n=20)	Gestational Age in Weeks						8 Weeks Postpartum
		16	20	24	28	32	36	
Hb SS or SC n=15	26.9 ±1.08	26 ±1.03	24.8 ±0.92	23.2 ±0.59	23.8 ±1.11	24.3 ±0.93	24.6 ±1.06	26 ±1.01
Hb AA n=20	36.9 ±0.65	35.3 ±0.39	35.1 ±0.56	33.6 ±0.58	32.9 ±0.58	33.6 ±0.45	34.9 ±0.35	38.8 ±0.76
Difference df=33	P<.001	P<.001	P<.001	P<.001	P<.001	P<.001	P<.001	P<.001

* % ± SEM
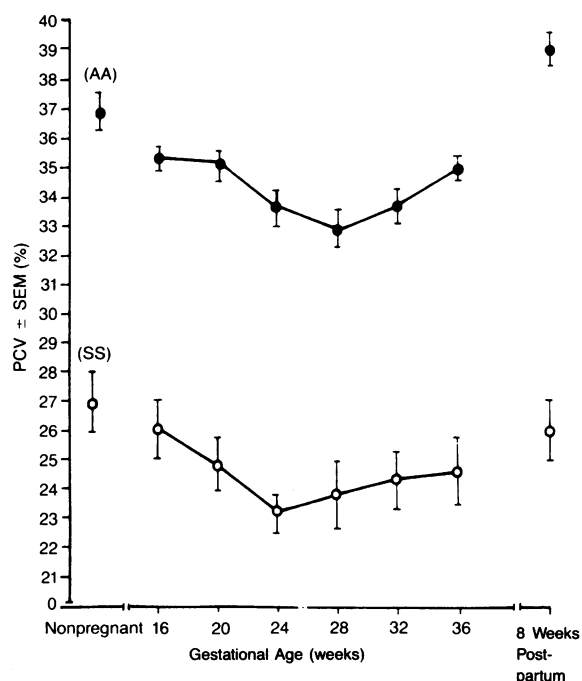
Figure 1. The effect of pregnancy on packed cell volume (PCV) in patients with Hb AA and sickle cell disease (Hb SS or SC).

in pregnancy. However, Anderson[3] reported a high incidence of iron deficiency in Jamaican mothers with hemoglobinopathies. Roopnarinesingh,[4] in another study of 22 pregnant Jamaicans with anemia and hemoglobinopathy, found that 14 women had no stainable iron and four had scanty intracellular iron. Only five of these patients had homozygous sickle cell disease (SS) and two of them had stainable iron in bone marrow (three had hypercellular marrow and two had normocellular marrow). The mean gestational age in that study was 29.6 weeks (range 25 to 36 weeks), and the mean packed cell volume was 21.5% (range 19.8% to 23.7%). Oluboyede,[5] in a study of iron stores in pregnant women in Ibadan (Nigeria) with Hb SS or SC, found that 63% had scanty iron or no iron in the bone marrow. The mean gestational age in his study, which compared Hb SS with Hb SC and not with normal hemoglobin genotype (Hb AA), was 23.3 weeks. All these studies were cross sectional.

Hematological indices, like many other physiological parameters, change during pregnancy and are affected by the hemodilution of pregnancy[6,7]; therefore, a serial study is more informative in pregnancy than a cross-sectional study. Recently, Puolakka et al[8] and Taylor et al[7] have shown that serum ferritin concentration (measured by the radioimmunoassay

method) in pregnant women reflects the iron store accurately. Earlier, Jacobs and Worwood[9] have shown that serum ferritin concentration is directly related to available storage iron in the body in healthy individuals. Serum ferritin concentration measurement is a quantitative evaluation, unlike a bone marrow study, which is not only subjective and semiquantitative, but also invasive and time consuming, resulting in the study of a relatively small number of samples.

Because iron stores in pregnant patients with sickle cell disease remain controversial and because of the shortcomings of the design of previous studies alluded to already, we carried out a longitudinal study of iron stores in clinically stable, healthy, pregnant and nonpregnant Nigerians with Hb SS or SC. We used healthy nonpregnant and pregnant nonanemic Nigerians with Hb AA for comparison.

SUBJECTS AND METHODS

Seventy-five Nigerian women, 40 with normal hemoglobin genotype AA and 35 with hemoglobin genotype SS and SC were studied. None of the women had donated or received blood or iron therapy in the 12 months prior to the commencement of the study. All were healthy and had no hepatosplenomegaly. They all had a normal, regular menstrual history. All women with Hb SS or SC were in a "clinical steady state."

Nonpregnant Women

Twenty nonpregnant women had hemoglobin genotype AA, with a mean age of 24.8 ± 0.65 years, a mean height of 159 ± 0.97 cm, and a mean weight of 56.5 ± 2.12 kg. The women were not on any medications at the time of the study.

Twenty nonpregnant women had sickle cell hemoglobin disease; 19 had Hb SS and 1 had Hb SC. The mean age was 22.8 ± 0.74 years, mean height was 161 ± 1.75 cm, and mean weight was 54.7 ± 1.94 kg. These nonpregnant women with sickle cell disease were on routine folic acid (5 mg daily) and proguanil (100 mg daily).

Pregnant Women

Twenty pregnant women had hemoglobin genotype AA and 15 had sickle cell disease (13 with Hb SS and 2 with Hb SC). The pregnant women were all registered patients with documented histories of their last menstrual periods. Table 1 shows their clinical data. The women with normal hemoglobin genotype AA were on prophylactic oral ferrous gluconate (200 mg three times daily), folic acid (5 mg daily), and

pyrimethamine (25 mg weekly) all through pregnancy, and were advised to continue until their babies were weaned. The pregnant women with sickle cell disease received no iron but continued with their routine folic acid (5 mg daily) and proguanil (100 mg daily).

Blood specimens were obtained with minimal stasis in the basal state between 9 AM and 11 AM. The pregnant women had blood specimens collected at intake, 16 weeks of gestation, and thereafter at four weekly intervals, and again at delivery 8 weeks postpartum. Cord blood specimens also were obtained at delivery. Sera were stored at -20°C until assayed in batches using ferritin radioimmunoassay kits supplied by Radiochemical Centre. The assay used I 125 labelled ferritin, double antibody technique, and human spleen ferritin as standard. Intra-assay variabilities were 10% and inter-assay variabilities were 15%.

Hematological indices were determined on a Coulter counter, model S plus (Coulter Electronics, Ltd), that was calibrated with "4C."

Statistical Analysis

The distribution of ferritin concentration is skewed[9,10]; therefore, logarithmic transformation of individual ferritin values was done before differences among means were determined using the student t test. Median values of ferritin concentrations are given also. Means and standard error of means of all other measurements are given. Significance was at the 5% level.

RESULTS
Packed Cell Volume

In both groups of women, the packed cell volume (PCV) fell progressively during pregnancy and rose thereafter (Table 2). In the pregnant women with Hb AA, the mean PCV levels were significantly lower at weeks 24 ($P<.02$), 28 ($P<.01$), 32 ($P<.05$), and 36 than the value for nonpregnant women of 36.9%. In the pregnant women with Hb SS or SC, the PCV was only significantly lower ($P<.05$) at 24 weeks compared with the value for nonpregnant women of 26.9%. Compared with the 8 weeks postpartum PCV values in each group, the maximum fall of 5.9% in PCV in women with Hb AA was greater than the maximum fall of 2.8% in women with Hb SS or SC. This may be evidence of less hemodilution in the pregnant women with sickle cell disease. Figure 1 shows the effect of pregnancy on PCV in both groups of women.
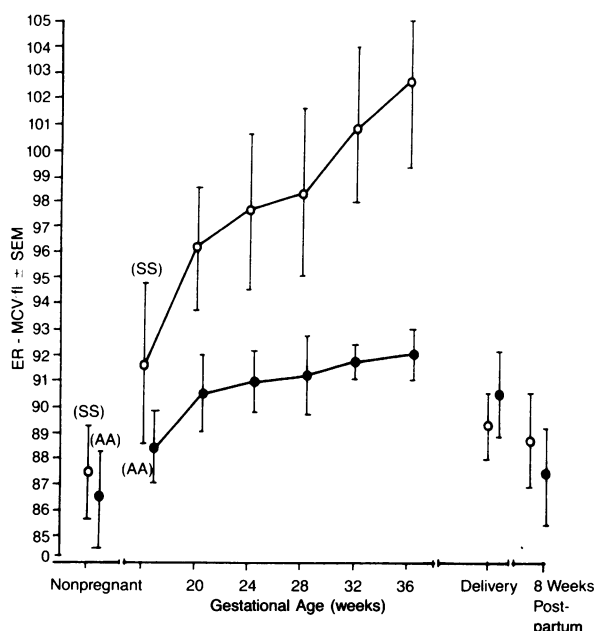
Mean Corpuscular Volume

The mean corpuscular volume (MCV) values rose

TABLE 3. SERIAL MEAN CORPUSCULAR VOLUME*
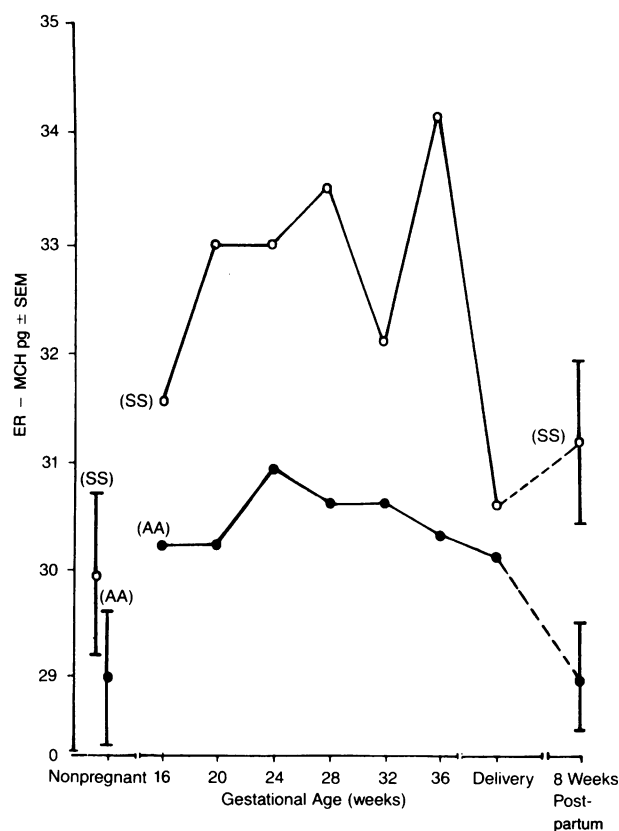**IN PREGNANT NIGERIANS WITH Hb AA AND Hb SS OR SC**

| | Nonpregnant (n=20) | Gestational Age in Weeks | | | | | | | 8 Weeks Postpartum |
		16	20	24	28	32	36	Delivery	
Hb SS or SC n=15	89.4 ±1.76	93.57 ±3.1	98.15 ±2.4	99.58 ±3.02	100.28 ±3.25	102.9 ±3.3	104.65 ±3.32	91.25 ±1.26	90.7 ±1.83
Hb AA n 20	88.36 ±1.89	90.44 ±1.35	92.52 ±1.48	92.93 ±1.21	93.24 ±1.53	93.70 ±0.62	94.01 ±0.97	92.51 ±1.62	89.28 ±1.87
Difference (t)	0.28	0.70	1.45	1.57	1.47	2.35	2.48	0.44	0.38
Significance (P)	>.5	>.1	>.1	>.1	>.1	<.05	<.02	>.5	>.5

*fl ± SEM

Figure 2. The effect of pregnancy on mean corpuscular volume (MCV) in patients with Hb AA and sickle cell disease (Hb SS or SC).

Figure 3. The effect of pregnancy on mean corpuscular hemoglobin (MCH) in patients with Hb AA and sickle cell disease (Hb SS or SC).

progressively throughout pregnancy and had returned by 8 weeks postpartum to the values taken before pregnancy. Figure 2 shows the effect of pregnancy on MCV in the two groups of pregnant women. The rise in MCV in the women with Hb AA was not significantly higher at various stages of pregnancy compared with the values measured before pregnancy or at 8 weeks postpartum. However, the rise in MCV in the women with Hb SS or SC was significantly higher than the values taken before pregnancy (P<.05 at 24 weeks; P<.05 at 28 weeks; P<.02 at 32 weeks; P<.01 at 36 weeks). Comparing both groups, the mean MCV values in subjects with Hb SS or SC was significantly higher (Table 3).
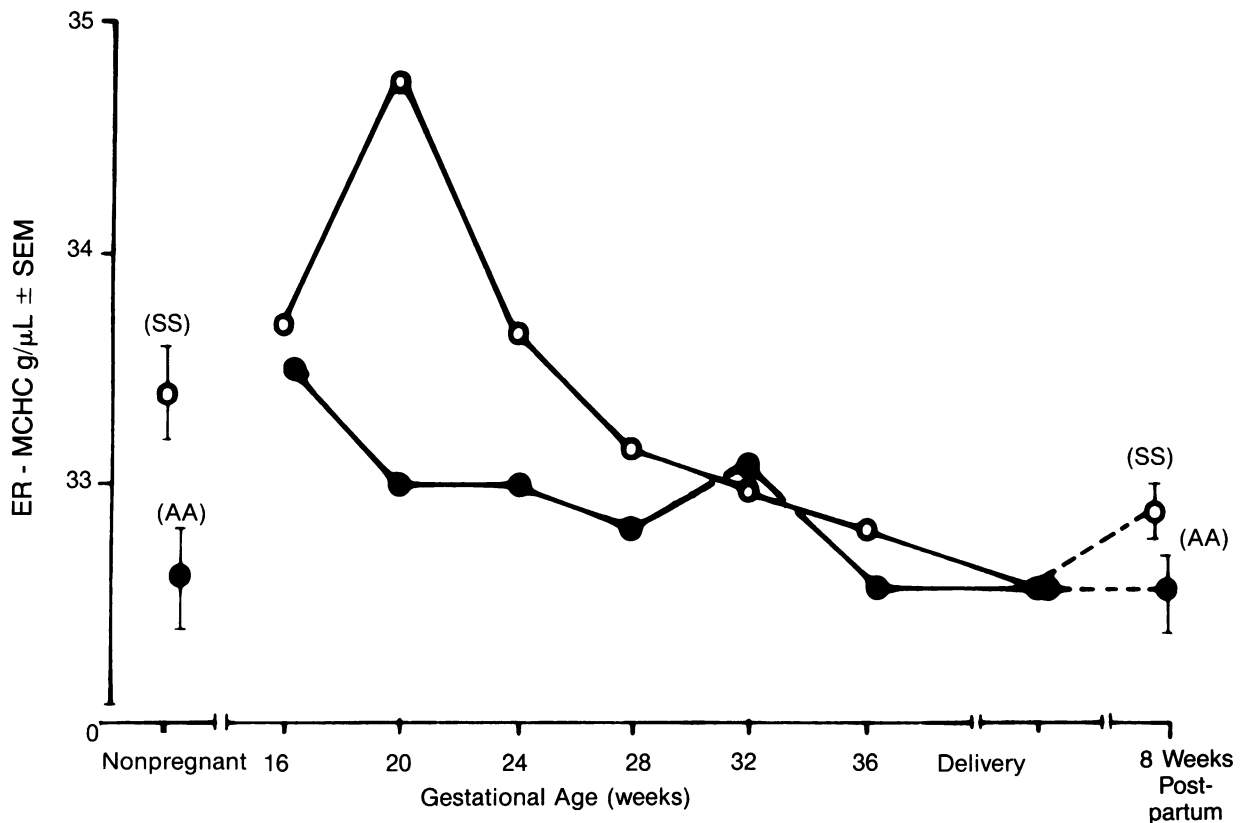
Mean Corpuscular Hemoglobin

The women with Hb AA had an insignificant increase in mean corpuscular hemoglobin (MCH) from the values measured before pregnancy to a maximum value of 30.9 ± 0.40 pg at 24 weeks. It fell gradually

TABLE 4. SERIAL MEAN CORPUSCULAR HEMOGLOBIN*
IN PREGNANT NIGERIANS WITH Hb AA AND Hb SS OR SC

	Nonpregnant (n=20)	Gestational Age in Weeks							8 Weeks Postpartum
		16	20	24	28	32	36	Delivery	
Hb SS or SC n=15	29.93 ±0.71	31.55 ±1.06	33.04 ±1.02	33.02 ±0.55	33.52 ±0.70	32.1 ±1.33	34.18 ±0.72	30.57 ±0.5	31.17 ±0.76
Hb AA n=20	28.86 ±0.70	30.2 ±0.59	30.2 ±0.53	30.9 ±0.4	30.6 ±0.47	30.6 ±0.36	30.3 ±0.39	30.1 ±0.51	28.78 ±0.55
Difference (t)	0.76	0.82	1.83	2.23	2.49	0.75	3.49	0.47	1.82
Significance (P)	<.5	<.5	<.1	<.05	<.02	<.5	<.001	>.5	<.1

*pg ± SEM

Figure 4. The effect of pregnancy on mean corpuscular hemoglobin concentration (MCHC) in patients with Hb AA and sickle cell disease (Hb SS or SC).

thereafter. The women with Hb SS or SC, however, had significant increases in MCH from the prepregnancy value throughout pregnancy beginning at 24 weeks, with the exception of a decrease at 32 weeks which appeared to be an artifact (Figure 3). The increases in MCH in the women with Hb SS or SC were also significantly higher ($P<.05$ at 24 weeks; $P<.02$ at 28 weeks; $P<.001$ at 36 weeks) than in subjects with Hb AA at similar stages of gestation. The MCH values were never below the level normally regarded as that indicative of iron deficiency in any of the subjects at any period of gestation (normal range, 26 to 30 pg).[10]

TABLE 5. SERIAL MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION* IN PREGNANT NIGERIANS WITH Hb AA AND Hb SS OR SC

| | Nonpregnant (n=20) | Gestational Age in Weeks | | | | | | | 8 Weeks Postpartum |
		16	20	24	28	32	36	Delivery	
Hb SS or SC n=15	33.43 ±0.21	33.7 ±0.28	34.78 ±0.49	32.68 ±0.32	33.16 ±0.31	33 ±0.07	32.8 ±0.53	32.55 ±0.2	32.9 ±0.12
Hb AA n=20	32.62 ±.23	33.49 ±0.29	32.96 ±0.22	33 ±0.12	32.75 ±0.14	33.1 ±0.15	32.55 ±0.21	32.56 ±0.18	32.57 ±0.17
Difference (t)	1.84	0.37	2.56	0.73	0.91	0.45	0.34	0.03	1.14
Significance (P)	>.05	>.5	<.02	<.5	<.5	>.5	>.5	>.5	>.1

*g/dL ± SEM

Mean Corpuscular Hemoglobin Concentration

The mean corpuscular hemoglobin concentration (MCHC) rose initially to a maximum value at 16 weeks of gestation in the women with Hb AA (Figure 4) and fell thereafter. The maximal value of MCHC was reached at 20 weeks in the women with Hb SS or SC. Although the maximal rise in the women with Hb AA was significant ($P<0.05$), the rise in the women with Hb SS or SC was insignificant. The only significant difference observed was at 20 weeks in the two groups (Table 5). In both groups, at no period of gestation was the mean value of MCHC below that normally regarded as indicative of iron deficiency (normal range 30% to 36%).[10]

Serum Ferritin

Table 6 shows the median serum ferritin values in both groups and also the mean values after logarithmic transformation of the actual individual values obtained in each gestational period. Serum ferritin values in women with Hb SS or SC were significantly higher than in women with Hb AA at all stages of pregnancy and both before and after pregnancy. Among the women with Hb SS or SC, there was an initial significant increase in serum ferritin compared with the prepregnancy values. Figure 5 shows the effect of pregnancy on serum ferritin levels in both groups of women.

There was a highly significant difference ($P<.001$) between cord and maternal serum ferritin values, both

TABLE 6. SERIAL SERUM FERRITIN* IN PREGNANT NIGERIANS WITH Hb AA AND Hb SS OR SC

| | Nonpregnant (n=20) | Gestational Age in Weeks | | | |
		16	20	24	28
HB SS or SC n = 15					
Median	393.3	916.1	900	710.4	680
Range	(175-900)	(285-2400)	(320-2320)	(160-1800)	(176-1560)
Mean Log Transformation ± SEM	5.87 ±0.15	6.64 ±0.19	6.55 ±0.18	6.08 ±0.29	6.30 ±0.21
Hb AA n=20					
Median	89.8	107.7	104.4	70.43	79.94
Range	(13-250)	(37-230)	(15-190)	(17-155)	(15-180)
Mean Log Transformation ± SEM	4.24±0.20	4.5±0.14	4.4±0.17	4.1±0.15	4.2±0.18
Difference (t)	4.9	6.48	5.97	4.5	5.38
Significance (P)	<.001	<.001	<.001	<.001	<.001

*μg/L

in the Hb AA group and Hb SS or SC group. However, there was no significant difference in cord serum ferritin values between the women with Hb AA and Hb SS or SC. Also, there was no significant correlation between maternal serum ferritin and cord serum ferritin in either group.

Because 11 of the 13 patients with Hb SS had blood transfusions in the immediate postpartum period for severe crises or postcesarean operation, we did not estimate the postpartum serum ferritin in these patients.

DISCUSSION

This article presented the results of a serial study of iron status in pregnant Nigerian women with sickle cell disease using routine hematological indices (ie, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin) and a more sensitive measurement of iron stores by radioimmunoassay of serum ferritin.[9]

Oluboyede et al[10] have documented hematological parameters in a normal Nigerian female population at Ibadan as MCV (77 to 96 fl), MCH (26 to 30 pg), MCHC (30% to 36%), and serum ferritin (8 to 90 μg/L). Our findings in nonpregnant normal women in Lagos are similar, except that the median serum ferritin of 89.8 μg/L (range 13 to 250 μg/L) we found is slightly higher. We found that nonpregnant women with sickle cell disease in this study had a high level of serum ferritin; this finding is in agreement with the
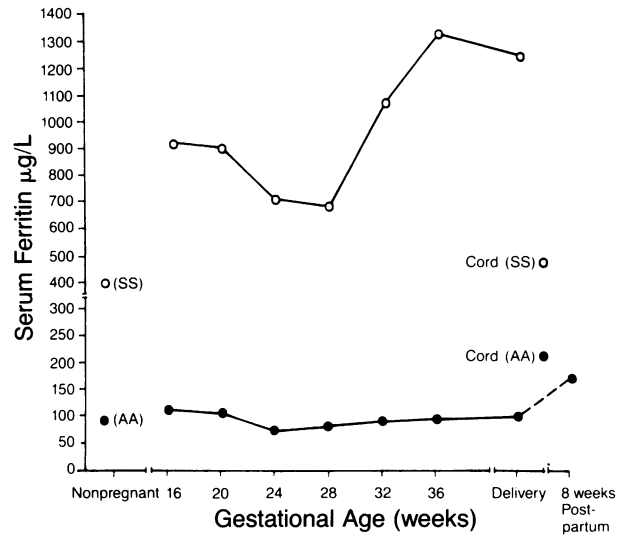
Figure 5. The effect of pregnancy on serum ferritin concentration in patients with Hb AA and sickle cell disease (Hb SS or SC).

findings of Peterson et al[11] and Oluboyede et al.[10]

Our results show that pregnant women with sickle cell disease have high serum ferritin levels. This is in sharp contrast to Oluboyede's[5] findings at Ibadan. The difference may be due to the fact that the mean

TABLE 6. SERIAL SERUM FERRITIN* IN PREGNANT NIGERIANS WITH Hb AA AND Hb SS OR SC (continued)

Gestational Age in Weeks				8 Weeks
32	36	Delivery	Cord	Postpartum
1071 (152-3200)	1328 (132-2560)	1240 (190-4200)	470.8 (108-880)	—
6.30 ±0.34	6.72 ±0.30	6.53 ±0.30	6.12 ±0.27	—
86.83 (16-230)	88.74 (27-179)	93.25 (38-240)	263.3 (54-580)	163.42 (40-360)
4.25 ±0.13	4.32 ±0.14	4.41 ±0.12	5.46 ±0.16	4.88 ±0.16
4.4 <.001	5.45 <.001	5.04 <.001	1.53 >.1	—

*μg/L

concentration of serum ferritin in Oluboyede's cross-sectional study at 23 weeks of gestation corresponds with the period of maximal hemodilution in pregnant women with sickle cell disease. Studies from Jamaica[3,4] using bone marrow suggest that the iron stores in pregnant women with hemoglobinopathy could be inadequate. Patients from various parts of the world with sickle cell disease are known to have a different natural history.[12] The methodology in our study was different from the Jamaican studies. We studied a group of homozygous patients with sickle cell disease, whereas Roopnarinesingh's[4] study included anemic patients with heterozygous (AS) disease.

Chronic hemolysis resulting in low PCV, red blood cell count, and hemoglobin concentration are characteristic of sickle cell hemoglobin disease. Mean corpuscular volume and mean corpuscular hemoglobin are inversely proportional to the red blood cell count, and this relationship may account for the significant rises observed in pregnant patients with Hb SS or SC.

In this study, we documented serial changes in hematological indices and serum ferritin in pregnant patients with hemoglobin SS or SC who were in steady clinical states throughout pregnancy. Our hope was to help evolve a more rational approach to the management of these groups of patients.

From the data presented, it would not seem justifiable to give pregnant patients with sickle cell disease iron supplementation. Fleming's[2] claim of adequate iron stores in pregnant Nigerians with sickle cell disease is supported by our findings. Hendriscke et al[13] has found no evidence of increased hemolysis during pregnancy in pregnant sickle cell patients. Although there is evidence of hyposthenuria[14] in West African patients with sickle cell disease, there is no evidence yet of increased iron loss in the urine[15] or excessive iron loss via the skin. It, therefore, seems reasonable to assume that the iron from hemolysis of red cells in sickle cell disease is stored and probably reused in time of high iron demand. However, the iron status of individual patients with Hb SS or SC should be assessed before giving iron, so that wasteful, unnecessary, and injurious prescription of iron is avoided.

The lack of correlation between maternal serum ferritin and cord serum ferritin is in agreement with a previous report.[16] The lack of a significant difference in the cord serum ferritin concentration between babies born to mothers with hemoglobin SS or SC and hemoglobin AA may indicate that the baby takes just the amount of iron it requires, even if there are excess maternal iron stores.

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