Original Article

Iron Store of Pregnant Women with Hemoglobin SS and SC in Benin City

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ABSTRACT

Background: Anemia in pregnancy is common and morbidity is increased in the presence of sickle cell disease. There has been conflicting argument concerning routine iron replacement. However few, studies in Nigeria have comprehensively evaluated the iron status of these women. **Objective:** The study was carried out to determine the iron stores status of pregnant women with hemoglobin (Hb) Sickle cell S or Sickle cell C using the serum assay. C-reactive protein (CRP) was also assayed to rule out the effect of inflammation / infection on the serum level of ferritin. **Materials and Methods:** This was a prospective longitudinal study conducted at the University of Benin Teaching Hospital (UBTH), Benin City, over a period of eight months (from April 2011 to December 2011). The patients for the study were identified using the booking investigation results in the antenatal clinic records. Only those clinically stable pregnant women, in a healthy state, participated in this study.The hematological indices, serum ferritin assay, and the CRP were determined in both test (Hb SS / SC) and control (Hb AA) pregnant women. The data obtained were fed into a personal computer (PC) and analyzed using the Statistical Package for Social Sciences (SPSS) software version 16 for Windows. Categorical data were expressed as percentages and compared using the Chi-squared test, whereas, numerical data were expressed as mean (SD) and compared using the Student's *t*-test. The level of significance was set at $P \leq 0.05$. **Results:** A total of 23 Hb SS / SC pregnant women formed the test group. They were recruited for the study from the Sickle-Cell Center (attached to the Central Hospital, Benin City, the UBTH). The mean age, educational status, and the social class of both the test group and the control group were comparable. There was a significant difference in the mean hemoglobin concentration between the test group and control group, both at 16–20 weeks and 28 – 32 weeks of gestation ($P<0.001$), and the mean corpuscular volume (MCV) values at both gestational ages (GAs) ($P=0.097$ and 0.231, respectively). The values of the serum ferritin in the test group were also statistically and significantly higher than those of the control group ($P<0.001$ and $P=0.009$, respectively), at both GAs. **Conclusion:** This study demonstrated higher serum iron levels in Hb SS / SC pregnant women than in the controls (even after excluding those levels raised by the increased levels of CRP). In situations that may require iron supplementation, it may be reasonable to determine the iron status to ascertain the quantity that should be given.

KEY WORDS: Benin City, iron status, pregnancy, sickle-cell hemoglobin

INTRODUCTION

Anemia is the most common medical disorder in pregnancy and has a varied prevalence, etiology, and degree of severity in different populations, being more common in non-industrialized countries.[1] Inherited conditions that affect red blood cells (RBCs), such as, sickle-cell disease, constitute a significant etiological factor in the Negroid race.[2]

Pregnancy in a woman with sickling disorders, exposes both the mother and fetus to increased pregnancy complications associated with vaso-occlusive phenomenon, such as intrauterine growth restriction (IUGR), preterm labor, pre-eclampsia, and perinatal mortality.[3-5] In general, pregnancy outcomes are thought to be determined by the severity of the patient's anemia.[6] Although it is now accepted that with intensive prenatal care, many women can look forward to a successful pregnancy outcome; pregnancies associated with major sickle hemoglobinopathies are considered as 'high risk'. Thus with more women with sickle cell disease now surviving to childbearing age, there is need for a better understanding of this disease in pregnancy, its management, and management outcomes.

The World Health Organization (WHO) recommended universal oral iron supplementation for pregnant women

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(60 mg of elemental iron and 250 g of folic acid, once or twice daily).[7] However, the traditional teaching has been that iron deficiency is uncommon in sickle-cell disease patients, and routine iron supplementation is usually not given because of the fear of iron overload. This teaching has, however, been questioned by several authors,[8-10] especially in patients who have never been transfused.Thus, some authorities believe that routine antenatal administration of supplementary iron entails a negligible theoretical risk of iron overload for a substantial benefit,[8] and thus advise that iron supplementations must be given in the same manner as is given to other pregnant women at risk of iron deficiency anemia.

Several studies have been carried out in the past to assess the iron stores in pregnant women with sickle-cell disease,[11-15] with conflicting results and varied advice on the use of supplementary iron in these pregnancies. The differences in the outcomes could be attributed to the differences in their methodologies and study designs.

This study is, therefore, aimed at assessing the iron stores of healthy pregnant women with HbSS or SC, in Benin City, using the serum ferritin level, as recommended by the WHO/Centers for Disease Control and Prevention (CDC) expert consultation.[16] In addition, assay for C-reactive protein (CRP) must be performed to rule out the influence of infection/inflammation on the serum level of ferritin,which is an acute phase protein. Findings from this study will improve the knowledge of how best to care for Hb SS/SC pregnant patients with regard to iron replacement/prophylaxis.

MATERIALS AND METHODS

This was a prospective longitudinal study, conducted at the University of Benin Teaching Hospital (UBTH) and the Benin City and Specialist Hospital, also in Benin City, over a period of 8 months, from April 2011 to December 2011. The patients for the study were identified using the booking investigation results in the antenatal clinic records. Their haemoglobin genotypes were confirmed with a repeat Haemoglobin genotype screening at the Hematology Department of the UBTH, Benin City, using citrate agar gel electrophoresis. This allowed the HbS to be distinguished from HbD and HbG, and enabled the HbC to be distinguished from Hobe and HbA_2.

Only those in a clinically healthy state participated in the study. Those who were human immunodeficiency virus (HIV)-positive or had chronic inflammatory conditions like tuberculosis, recent history of febrile illness, history of blood transfusion in the last 12 months, and those on iron supplementation, were excluded from the study. Pregnant non-anemic Hb AA women, at comparable gestational age (GA), were recruited as the control and these were on routine hemantinics. The patients' bio-data, including the last menstrual period, GA, any history, and number(s) and date(s) of blood transfusion were obtained.

Blood samples were collected through venepuncture, with minimal stasis in the basal state, between 9 a.m. and 11 a.m., and placed in a specimen bottle containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant, for the determination of the hematological indices, and also put into a universal bottle for the assay for serum ferritin and CRP. Two separate samples were collected, the first at a GA of between 16 and 20 weeks, and the second at a GA of between 28 and 32 weeks. The hematological indices were determined on the day of collection, while the samples for serum ferritin and CRP were separated and the serum stored at a temperature below −20 C. The serum ferritin and CRP were later determined in batches.

The hematological indices were determined with the use of a Hematology Automated Analyzer — ERMA PCE 210, fully Auto Blood Cell Counter. The serum ferritin assay was determined using the automated Elecsys 2010 immunoanalyzer Hitachi/Roche Diagnostic System, whereas, the CRP was done with the high sensitivity the CRP enzyme immunoassay test kit. The reagents came along with 'reference standard sets,' which were for standardization and control. These investigations were carried out at the Hematology and Chemical Pathology Departments of the UBTH, Benin City.

The sample size was based on the reported incidence of sickle-cell disease in the general population, which was quoted as 2%.[17] The sample size was, therefore, determined using the statistical formula for a descriptive study[18,19] and was calculated to be 20.

The data obtained were fed into a personal computer (PC) and analyzed using the statistical package for social sciences (SPSS) software version 16 for Windows. The categorical data were expressed as percentages and compared using Chi-squared test and Fishers exact test, whereas, the numerical data were expressed as mean (SD) and compared using the Student's *t*-test and Mann Whitney where applicable. The level of significance was set at $P \leq 0.05$.

Ethical consideration

Ethical approval for the study was obtained from the Ethical Committee of the UBTH. The same was obtained from the Specialist Hospital, Benin City. Details of the study were carefully explained to each patient and informed consent was obtained from them before being recruited into the study. It was clearly explained to them that their

participation was entirely voluntary. Also, if they were unwilling at any point to participate, they were completely at liberty to refuse, and they were assured that it would not be held against them in any way, then or in future, in their management in UBTH or Central Hospital, Benin.

RESULTS

A total of 25 (17 Hb SS and 8 Hb SC) pregnant women met the criteria for the study and all gave a written consent to be recruited. However, two of these patients were withdrawn from the study due to spontaneous abortion before 16 weeks of gestation. Thus, a total of 23 patients formed the test group, whereas, 33 Hb AA pregnant women formed the control group. Majority of these patients were recruited from the Sickle-Cell Center (attached to the Central Hospital, Benin City). The others were recruited from the UBTH, Benin City.

Sociodemographic characteristics

Table 1 shows the sociodemographic characteristics of the study population. The age group in both the test group and control were comparable (P=0.425). The distribution of the parity between the test and control group were also comparable (P=0.141). The mean age of the test group was 28.65 (3.94) years, whereas, that of the control was 30.01 (4.17) years. There was no statistical difference (P=0.199). The educational status and the social class of both the test group and the control group were also comparable (P=0.929 and 0.783, respectively). The tribe and religious distribution between the test group and control group were also comparable [Table 1].

Hematological indices

In both groups, the Hb concentration reduced as the GA advanced. This was, however, not statistically significant, (P=0.347 in the test group and 0.200 in the control group). There was, however, a significant difference in the mean hemoglobin concentration between the test group and control group, both at 16–20 weeks and 28–32 weeks of GA (both with a P<0.001).

The hematocrit also fell as pregnancies advanced in the both test and study groups. This fall was, however, not significant in the test group (P=0.238), whereas, in the control group, there was a significant difference (P=0.019).

The values of the mean corpuscular volume (MCV) rose with advancing gestation in both the test and control groups [Table 2]. This increase was, however, not significant (P=0.1683 and 0.3266, respectively). The MCV values at both GA in the test group (Hb SS/SC) were comparable with that of the control group at both GAs (P=0.969 and 0.231, respectively).

Serum ferritin and C-reactive protein

Table 3 shows the values of both the serum ferritin and C-reactive assays, in both the test and control groups. At 16 – 20 weeks of GA, the mean serum ferritin for the test group was 169.33 (112.64), which was significantly higher than the mean value for the control group, 44.02 (34.12) (P<0.001). At 28 – 32 weeks of GA, there were also significantly higher

Table 1: Sociodemographic characteristics of the study population

Demography	Test *n*=23 (%)	Control *n*=33 (%)	*P* value
Age			
20 – 30	15 (65.2)	18 (54.5)	0.425
30	8 (34.8)	15 (45.5)	
Parity			
Nullipara	15 (65.3)	13 (39.4)	0.141
Primipara	5 (21.7)	10 (30.3)	
Multipara	3 (13.0)	10 (30.3)	
Mean age	28.65 (3.94)	30.01 (4.17)	0.199
Educational status			
No formal education	0 (0.0)	0 (0.0)	0.929
Primary	2 (8.7)	4 (12.2)	
Secondary	9 (39.1)	11 (33.3)	
Tertiary	12 (52.2)	18 (54.5)	
Social class			
Class I	7 (30.4)	13 (39.4)	0.783
Class II	5 (21.7)	7 (21.2)	
Class III	8 (34.8)	9 (27.3)	
Class IV	2 (8.7)	4 (12.1)	
Class V	1 (4.3)	0 (0.0)	
Tribe			
Benin	11 (47.8)	12 (36.4)	0.894
Esan	7 (30.4)	9 (27.3)	
Ibo	4 (17.4)	9 (27.3)	
Hausa	1 (4.3)	1 (3.0)	
Urhobo	0 (0.0)	1 (3.0)	
Yoruba	0 (0.0)	1 (3.0)	
Religion			
Christian	22 (95.7)	32 (97.0)	1.000
Moslem	1 (4.3)	1 (3.0)	

Table 2: Hematological indices

Parameter	Test (*n*=23)	Control (*n*=33)	*P* value
16 – 20 weeks of gestation			
Hb concentration (g / dl)			<0.001
Mean	8.72 (1.09)	11.29 (1.16)	
Median	8.30	10.90	
Range	6.80 – 11	9.20 – 13.0	
HCT concentration			
Mean	28.67 (3.24)	35.81 (3.82)	<0.001
Median	28.80	35.50	
Range	22.5 – 34.2	29.6 – 45.3	
MCV (fL)			
Mean	87.30 (13.07)	87.41 (6.44)	0.969
Median	84.00	86.90	
Range	60.7 – 114.7	79.0 – 106.3	
28 – 32 weeks of gestation			
Hb concentration (g / dl)			<0.001
Mean	8.39 (1.26)	10.88 (0.94)	
Median	8.100	10.70	
Range	5.9 – 10.2	9.6 – 13.1	
HCT concentration			
Mean	27.59 (3.40)	33.93 (2.36)	<0.001
Median	28.80	33.30	
Range	19.6 – 32	30.2 – 40.7	
MCV (fL)			
Mean	92.39 (11.53)	89.08 (7.26)	0.231
Median	97.00	89.20	
Range	73.7 – 110.2	73.7 – 107.4	

Hb – Hemoglobin; HCT – Hematocrit; MCV – Mean corpuscular volume

levels of serum ferritin in the test group (152.25 (190.25) than the control group (36.53 (49.01), $P=0.009$.

The mean concentration of CRP for the test group was not significantly different from that obtained for the control group, both at 16 – 20 weeks and 28 – 32 weeks of GA [Table 3], using serum levels of 0.068 – 8.2 mg/l as normal values for the CRP, based on the expected values of CRP among healthy individuals.[20] At 16 – 20 weeks of GA, three patients among the test values had raised values of CRP, whereas, among the control group, five patients had raised values of CRP. At 28–32 weeks of GA, two patients among the test group had raised values of CRP, whereas, three patients among the control group had raised values of CRP (two of these patients, one in each arm, had raised values at both GAs). Thus, a total of four patients in the test group were excluded from further analysis, based on their raised CRP. In the control group, a total of seven patients were excluded from further analysis based on their raised CRP.

Table 4 shows the serum level of ferritin compared between the two groups, after excluding those patients with raised CRP. The serum ferritin levels in women with Hb. SS/SC were significantly higher than those in the women with Hb AA, at both stages of pregnancy ($P<0.001$).

Table 5 shows the number of patients in both the test and control groups with low, normal, and raised levels of serum ferritin, based on the value showed in the table. At both GAs, no patient had a low level of serum ferritin (<15 µg / l). However, 15.4% (4/26) and 23.1% (6/26) patients in the control group had a low level of serum ferritin at both the GA groups, respectively.

Parity and a prior history of blood transfusion seemed to have no effect on the levels of serum ferritin among patients with Hb SS/SC [Table 6]. Similarly, GA had no significant effect on the levels of serum ferritin among Hb SS/SC patients. However, there was a slightly significant influence of gestation at age on the level of serum ferritin among hemoglobin AA patients [Table 7].

DISCUSSION

As expected, the hematocrit and Hb levels were significantly lower in a pregnant lady with Hb SS. This was due to chronic hemolysis (resulting in low red cell count, hematocrit concentration, and low hemoglobin concentration), which are characteristic of sickle-cell disease.[21] The low levels obtained in this study among Hb SS/SC patients reflected the above characteristics. The MCV was inversely proportional to the red cell count.[22] This relationship could account for the rise in the MCV observed in Hb SS/SC and Hb AA patients, as their hemoglobin concentration fell with increasing GA. This rise was, however, not statistically significant. This finding was similar to that of Oluboyede *et al*.[23] and Abudu *et al.*[14]

This study also showed that pregnant women with Hb SS/SC have higher levels of serum ferritin than women with normal hemoglobin. This finding is similar to the result of the study done by Abudu *et al.* However, the levels of serum ferritin obtained in this study were far lower than those obtained by Abudu *et al.* The mean level in this study, at 16 – 20 weeks of GA, was 137.92 µg/l and at 28 – 32 weeks of GA was 120.73 µg/l, those obtained by Abudu *et al*. were 900 – 916.1 and 680.0 µg/l, respectively. This difference

Table 3: Serum ferritin assay/C-reactive protein

Parameter	Test (*n*=23)	Control (*n*=33)	*P* value
16 – 20 weeks of gestation			
Serum ferritin (g/L)			<0.001
Mean	169.33 (112.64)	44.02 (34.12)	
Median	145.40	40.20	
Range	57.60 – 596.00	3.20 – 154.10	
CRP (mg/L)			
Mean	6.65 (3.91)	5.93 (3.40)	0.465
Median	5.70	5.89	
Range	2 – 22	1 – 13	
Normal (0.068–8.2) (%)	20 (87.0)	28 (84.8)	1.000
Raised (>8.2) (%)	3 (13.0)	5 (15.2)	
28 – 32 weeks of gestation			
Serum ferritin (g/L)			0.009
Mean	152.25 (190.25)	36.53 (49.01)	
Median	109.20	20.60	
Range	19.70 – 938.00	1.80 – 222.90	
CRP (mg/L)			
Mean	6.06 (3.17)	4.97 (3.44)	0.233
Median	6.50	3.81	
Range	1 – 15	1 – 17	
Normal (0.068–8.2) (%)	21 (91.37)	30 (90.9)	1.000
Raised (>8.2) (%)	2 (8.7)	3 (9.1)	

Table 4: Serum ferritin level compared (excluding patient with raised C-reactive protein)

Parameter	Test (*n*=19)	Control (*n*=26)	*P* value
16 – 20 weeks			
Mean	137.92(60.62)	46.36 (36.24)	<0.001
Median	124.4	40.20	
Range	57.60 – 238.00	6.20 – 154.1	
28 – 32 weeks			
Mean	120.73 (88.97)	27.81 (31.92)	<0.001
Median	108.00	19.85	
Range	19.70 – 315.8	1.80 – 151.60	

Table 5: Serum ferritin level compared [excluding patient with raised C-reactive protein from both cases and control (>8.2 mg/L)]

Parameter	Test *n*=19 (%)	Control *n*=26 (%)	*P* value
16 – 20 weeks of gestation			
Low level (<15g/L)	0 (0.0)	4 (15.4)	0.207
Normal (15 – 300g/L)	19 (100.0)	22 (84.6)	
Raised (>300 g)	0 (0.0)	0 (0.0)	
28 – 32 weeks of gestation			
Low level (<15g/L)	0 (0.0)	6 (23.1)	0.030
Normal (15 – 300g/L)	18 (94.7)	20 (76.9)	
Raised (>300g)	1 (5.3)	0 (0.0)	

Table 6: Effect of parity and history of blood transfusion on the mean (SD) levels of ferritin (µg/L) among SS/SC patients (excluding those with raised C-reactive protein)

Parameter	Gestational age	
	16 – 20	28 – 32
Parity		
Nullipara (*n*=13)	158.28 (61.16)	146.62 (93.46)
Primipara (*n*=4)	78.58 (21.54)	87.10 (36.54)
Multipara (*n*=2)	124.40 (0.00)	19.70 (0.00)
P value	0.057	0.116
History of blood transfusion		
Yes (*n*=9)	142.64 (71.45)	121.19 (88.03)
No (*n*=10)	133.67 (52.63)	120.31 (94.56)
P value	0.757	0.984

Table 7: Effect of gestational age on the mean (SD) level of serum ferritin (µg / L) (excluding those with raised C-reactive protein)

Parameter	16 – 20 weeks	28 – 32 weeks	*P* value
SS / SC group			
Serum ferritin (*n*=19)	137.92 (60.62)	120.73 (88.97)	0.49
AA group			
Serum ferritin (*n*=26)	46.36 (36.24)	27.81 (31.92)	0.056

could be attributed to the differences in methodology and also to the effect of the CRP used as a discriminant analyzer in this study.

The finding that iron stores were not depleted in the bone marrow of pregnant SS women, in this study, are in contrast to those in the study by Anderson in the West Indies,[11] and Oluboyede in Ibadan.[12] Apart from the differences in methodology, the different natural histories of sickle-cell disease patients, from different parts of the world, could account for the differences in their outcomes. It was also shown in this study that parity, increasing GA, and previous history of blood transfusion, had no influence on the level of iron stores, even after excluding the influence of CRP. These findings raise questions about the belief that Hb SS/SC patients, who had never been transfused were the ones likely to have iron deficiency, and therefore, needed iron supplementation in pregnancy.[8-10]

We also found a fall in the level of serum ferritin among patients in both groups with advanced GA, in this study. This was similar to the findings of Aken'Ova *et al*., who demonstrated a decreased level of serum ferritin in pregnant women with sickle-cell disease.[15] However, the levels of serum ferritin obtained in this study, with advancing GA, were far above the levels suggestive of iron depletion. This tends to counter the argument that pregnant sicklers need supplementary iron or may come down with iron-deficiency anemia in pregnancy.

The finding from Fleming's study,[24] which demonstrated adequate iron store in pregnant women with sickle-cell disease, is supported by the results of this study. Thus, the traditional teaching that endogenous iron from hemolysis of RBCs in sickle-cell-diseased patients is stored and re-used in time of higher iron demand, is probably true.

CONCLUSION AND RECOMMENDATION

This study has demonstrated adequate levels of iron store, based on the serum ferritin levels, among patients with Hb SS/SC, even after excluding those levels raised by increased levels of CRP. None of the Hb SS/SC patients showed depleted levels of iron store (serum ferritin level <12– 15µg/L).

From the results of this study, it may be plausible to say that it is correct not to give pregnant women with Hb SS/SC routine iron supplementation. However, there could be medical or surgical disorders that may predispose pregnant sicklers to iron depletion and it is reasonable to obtain iron status of these cases to determine the exact quantity needed. Better still,due to the increasing availability of the assay (including auto-analyzers) for the determination of serum ferritin, it may be a sound clinical judgment to recommend monthly checks of serum ferritin for all pregnant Hb SS/SC patients. The results thus obtained would help in identifying those that will benefit from iron supplementation. It is further recommended that a serum assay of CRP, where available, be done at the same time, to avoid the spurious effect of inflammation on the level of serum ferritin. Further studies are required with larger sample size to determine the effect of some obstetric complications, and dietary supplementation (unsaturated fatty acids) on the iron stores of these women.

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