The African Journal of MEDICINE and Medical Sciences

Volume 10
1981

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Melbourne

Afr. J. Med. med. Sci. (1981) **10**, 1–7.

IRON STUDIES IN PATIENTS WITH SICKLE CELL DISEASE

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Summary

The prevalence of iron deficiency anaemia during the first three decades of life was investigated in eighty-five patients with SS and SC haemoglobins. The parameters used were the haematocrit, serum iron, total iron binding capacity (TIBC), percentage saturation of transferrin and availability of iron in the bone marrow. The mean haematocrit values were similar throughout the three decades, but increased with age ($r = 0.41$). The mean serum iron was significantly lower ($P < 0.01$) in the first decade than in the second or third decade. Females had lower serum iron in the first and second decades and higher values in the third decade than their male counterparts. The transferrin saturation was significantly lower ($P < 0.01$) in the first decade than in the third decade. No haemosiderin was found in the marrow aspirates at a transferrin saturation of ≤15%. Of the eighty-five bone marrow aspirates studied for stainable iron, fifty-eight (68.2%) had nil iron. The data presented show that iron deficiency anaemia is a common finding in patients with haemoglobinopathies. The need to incorporate oral iron with folic acid and paludrine in the treatment of sickle cell disease is suggested.

Résumé

La prédominance de l'anémie attribuable à l'insuffisance du fer, au cours des premiers trente ans de vie, fut examinée chez quatre-vingt-cinq malades avec SS et SC hémoglobines. Les paramêtres appliqués furent l'hématocrite, le fer du sérum, la pâte d'attache à la capacité totale (TIBC), le pourcentage de saturation du transferrine, et la disponibilité de fer dans la moelle. Les valeurs moyennes d'hématocrite étaient semblables pendant toutes les trente années, mais elle s'augmentaient avec l'âge ($r = 0.41$). Le fer du sérum moyen était, d'une manière significative, plus bas ($P<0.01$) pendant les premières dix années que pendant la deuxième ou troisième période de dix ans. Chez les malades femmes les valeurs du fer du sérum furent plus basses pendant la première et deuxième période de dix ans, mais plus hautes pendant la troisième période de dix ans que chez les malades masculin. La saturation du transferrine fut, d'une manière significative, plus base ($P < 0.01$) au cours de la première période de dix ans qu'au cours de la troisième période. Aucun hémosidérire ne fut trouvé dans les aspirations de la moelle à la saturation du transferrine de ≤15%. Parmi les quatre-vingt-cinq aspirations de la moelle étudiées pour découvrir du fer colorable, cinquante-huite (68.2%) n'eurent nul fer. Les données expérimentales montrent que l'anémie due à l'insufissance du fer se trouve généralement chez les malades souffrants d'hémoglobinopathie.La nécessité d'incorporer du fer oral à l'acide folique et avec le cachet de paludrine est donc suggérée.

Introduction

There is a dearth of information in the literature on iron deficiency anaemia in patients with sickle cell disease, or the clinical and haematological response of these patients to therapeutic iron. Fullerton & Watson-Williams (1962) showed that megaloblastic anaemia due to folic acid deficiency is a common occurrence in patients with

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0309-3913/81/0600-0001 $02.00

haemoglobinopathies. Hence, prophylactic folic acid supplement is given routinely in the treatment of the anaemia. Iron supplement is often overlooked in the treatment because it is believed that these patients have chronic haemolysis of the red blood cells and the iron released is conserved in the body.

Fleming (1969) observed adequate iron in all his fifteen pregnant patients with haemoglobinopathies at a packed cell volume of 23% or below. But, Anderson (1972), and Roopnarineseigh (1976) have also reported high incidence of iron deficiency anaemia in pregnant patients with sickle cell disease.

Serjeant & Serjeant (1972) in their study of 438 asymptomatic sickle cell patients observed that the mean corpuscular haemoglobin concentration (MCHC) ranged from normochromic (>32) to mildly hypochromic values with a mean of 31. In a study of iron status in sickle cell patients (1975) Peterson *et al.* found eleven of the thirty-nine patients had no iron in the bone marrow. Administration of oral iron to two patients with iron deficiency anaemia produced a satisfactory haematological response, with haemoglobin concentration rising from 8.4 g/dl to 10.4 g/dl and from 9.5 g/dl to 13.4 g/dl respectively.

In a recent study in Ibadan, Nigeria, Oluboyede (1980) found evidence of iron deficiency anaemia by absence of iron in the bone marrow of a large majority of adult female patients (pregnant and non-pregnant) with sickle cell disease. Also, a common but constant observation in our environment on the peripheral films of patients especially children with sickle cell disease is hypochromia which is sometimes severe (E. Topley, personal communication).

Above observations have necessitated this study aimed at assessing the iron status of patients (children and adults) with sickle cell disease.

Materials and methods

Subjects

Male and female patients with sickle cell disease aged 3–30 years from both the children's anaemia clinic and the haematology clinic of the University Teaching Hospital, Ibadan, were the subjects of this study. All sickle cell patients are given appointments to attend the clinic at regular intervals even when asymptomatic and encouraged to attend the 'Day Care Unit' in the department of Haematology if crisis occurs between clinic appointments. All the subjects of this study were in steady state condition (asymptomatic); none was in crisis and none had been transfused recently.

Diagnosis

The grouping into SS or SC was established by electrophoretic method of Weatherall & Watson-Williams (1965).

Analysis

The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie & Lewis (1970). Serum iron concentration and total iron binding capacity were determined according to the method of Williams & Conrad (1966). Bone marrow aspiration was performed from anterior superior iliac crest in children and from sternum in adults. Smears made from the aspirates were immediately fixed in alcohol and subsequently stained with May-Grümmwald-Giemsa stain for cellular morphology and by Perl's prussian blue stain for iron. Marrow iron slides were numbered serially and read independently by two observers. The presence of iron was graded from 0 to 3 according to the method of Sorbie, Olatunbosun & Corbett (1971). A control iron stain (from a patient with over-transfused haemosiderosis) was run with each batch. Whenever there was disagreement in the grading, both observers met and reviewed the slide. Megaloblastic changes were recorded as normal, 0; moderate changes, 1+; and severe changes, 2++.

Results

A total of ninety-four patients were studied. Bone marrow aspirates were performed in eighty-five patients. Haematocrit values, serum iron, total iron binding capacity and percentage transferrin saturation were determined in sixty-seven patients. The age, sex and other relevant information about the patients are presented in Tables 1 and 2 and Fig. 1. Of the sixty-seven patients on whom complete data were available, seventeen patients (25.4%) were in the first

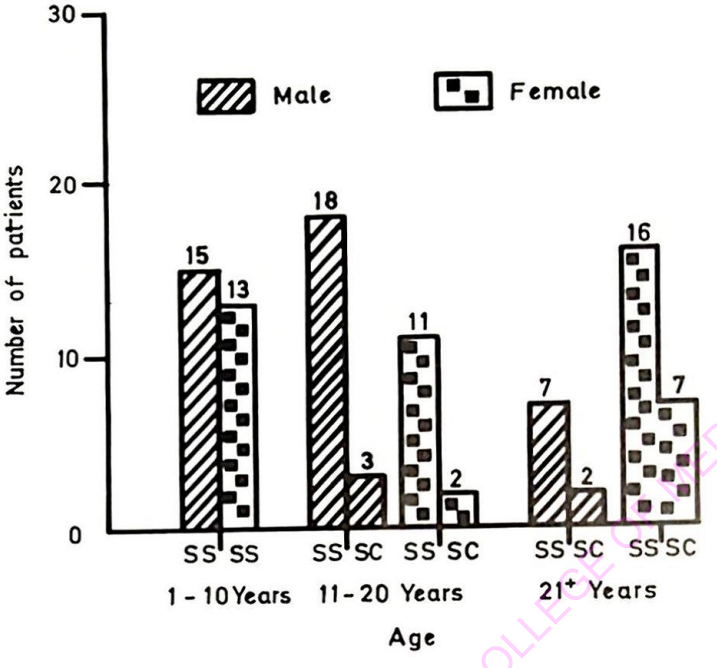

FIG. 1. Age and sex distribution of patients with SCD (SS and SC)

TABLE 1 Packed cell volume (PCV) in ninety-four cases under study

Age (years)	Male	Female
1 – 10	24.5±1.3 (n = 15)	20.6±1.0 (n = 12)
11 – 20	25.0±1.3 (n = 22)	23.6±1.3 (n = 12)
21+	29.0±1.9 (n = 9)	28.2±1.0 (n = 24)

Number of patients studied (n) in parenthesis

TABLE 2 Other haematological data in patients with sickle cell disease

Age (years)	Mean serum iron (μg/100 ml)	Mean total iron binding capacity (μg/100 ml)	Mean percentage saturation of transferrin
1 – 10	69.0±14.3 (17)	450.3±30.1	15.2±2.8
11 – 20	87.9±17.3 (23)	484.3±29	20.3±3.4
21+	102.2±13.5 (27)	469.4±33.8	23.9±2.9
P values	0.01	0.05	0.01

( ) Number of patients in parenthesis

decade, twenty-three (34.3%) were in the second decade and twenty-seven (40.3%) were adults (over 21 years).

Haematocrit

The mean haematocrit value of 25.4% for the entire group is similar to the mean haematocrit values of 22.7%, 24.5% and 28.5%, in the first, second and third decades, respectively. However, the haematocrit values increase with age (r = 0.41). In all age groups, (Table 1), the males (SS or SC) have higher haematocrit values than females. Also, the haematocrit values for SC patients in all the three decades are significantly higher than values for SS patients (Fig. 2).

Serum iron

The mean serum iron level of the entire group is 88.9±16.8 μg/100 ml. This value is significantly higher (P <0.01) than the mean for the patients in the first decade but similar to values in patients in the second and third decades; which are also significantly higher than values in the first decade (P <0.01) (Table 2). Values for the female patients in the first and second decades were lower than their male counterparts. In the third decade, there was a higher value for female patients.

Total iron binding capacity

The TIBC values were similar in the three decades. (Table 2).

Transferrin saturation

The relationship between transferrin saturation and age is presented in Fig. 3. There was a very poor correlation between age and transferrin saturation (r = 0.14). In the age groups 1 – 10 years, 11 – 20 years and 21+ years, 59, 48 and 26%, respectively had a transferrin saturation below 15%. A significantly lower number (P<0.05) of adults had a transferrin saturation below 15%; also the number of individuals with transferrin saturation above 30% increased progressively from the first decade to adulthood. Furthermore, the mean transferrin saturation (Table 2) in first decade (15.2%) is similar to that in the second decade (20.3%) but is significantly lower (P<0.01) than the value for the adults (23.9%).

Bone marrow studies

Morphology. There was erythroid hyperplasia with decrease of the myeloid erythroid ratio to 1:1. The myeloid series were essentially normal with occassional large metamyelocyte. The megakaryocytes were adequate in the marrows and producing platelets.

Megaloblastic changes. Typical megaloblastic changes in the erythroid, myeloid and megakaryocytic series were looked for in all the eighty-five bone marrow aspirates studied. In eighty of the eighty-five marrow aspirates, no evidence of megaloblastic changes were seen. They were essentially normoblastic. Three marrows showed occasional megaloblast and giant metamyelocyte. They were classified as showing moderate megaloblastic changes. Two of the three marrows were from female patients in the

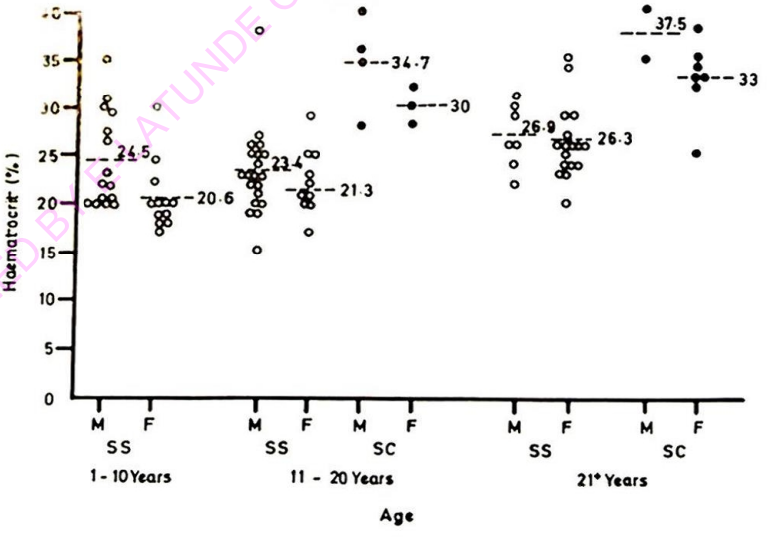

FIG. 2. Distribution of packed cell volume (PCV) in patients with SCD (SS and SC). ○, SS; ●, SC.

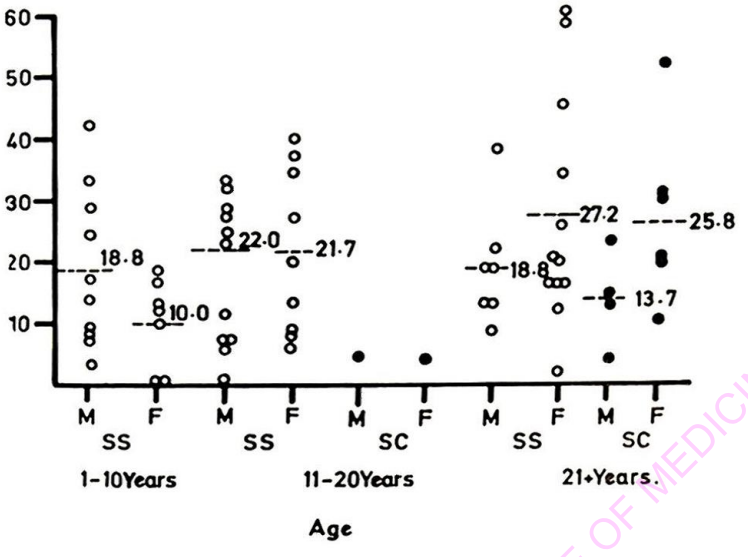

FIG. 3. Distribution of transferrin saturation in patients with SCD (SS and SC) ○, SS; ●, SC.

age group 1 – 10 years. The third marrow was from a male patient also in the age group 1 – 10 years. Two marrows showed severe megaloblastic changes. Both marrows were from female patients in the age group 11 – 20 years.

Iron stain

Eighty-five marrow aspirates were stained for iron. Fifty-eight (68.2%) had nil to scanty stainable iron, while twenty-seven (31.8%) had normal to excess iron; (Table 3). Twenty-two of these eighty-five marrow aspirates were from the first decade and 16/22 (72.7%) had nil to scanty iron, three had normal iron and in three others, iron was found in excess. Thirty marrow aspirates were studied from patients in the second decade and of these twenty-two (73.3%) had nil to scanty iron, six had normal iron and two had excess iron. In the adults, thirty-three marrow aspirates were studied and twenty (61%) had nil to scanty iron, six had normal iron and seven had excess iron. At a transferrin saturation below 20%, the number of individuals (78%) with scanty to nil iron in the bone marrow is significantly greater ($P<0.005$) than those with normal to excess iron stores (22%). However, with increasing transferrin saturation (>30%), there was increase in the amount of haemosiderin in the bone marrow ($P<0.01$). Of all patients with nil iron in the bone marrow, the mean transferrin saturation for those in the first decade (10.2%) is significantly lower ($P<0.05$) than the value for those in the second decade (16.3%) or the value for the adults (18.0%).

Bone marrow iron and transferrin saturation

Figure 4 shows the comparison of bone marrow iron availability to the transferrin saturation. In the majority of cases iron was absent from the bone marrow at a transferrin saturation of <15%.

Discussion

The findings of this study show that iron deficiency anaemia is common in the first three decades of life in patients with sickle cell disease. Although it is generally believed that the anaemia occurring in sickle cell disease is that of megaloblastic anaemia of folate deficiency and that this situation exists because of the chronic haemolysis of the red blood cells followed by increased deposition of haemosiderin in the reticulo-endothelial system including the bone marrow, liver, and spleen, evidence of iron deficiency has also been reported by Peterson *et al.*, (1975). O'Brien

TABLE 3 Bone marrow iron studies in patients with sickle cell disease

Bone marrow score	Age (years) 1–10 Male	1–10 Female	11–20 Male	11–20 Female	21–30 Male	21–30 Female	Total number	Percentage of total
Nil 0	7	6	13	6	5	12	49	57.6
Scanty 1+	2	1	2	1	—	3	9	10.6
Normal 2+	3	—	5	1	2	4	15	17.6
Excess 3+	1	2	—	2	2	5	12	14.2
	13	9	20	10	9	24		100
Total	22		30		33		85	

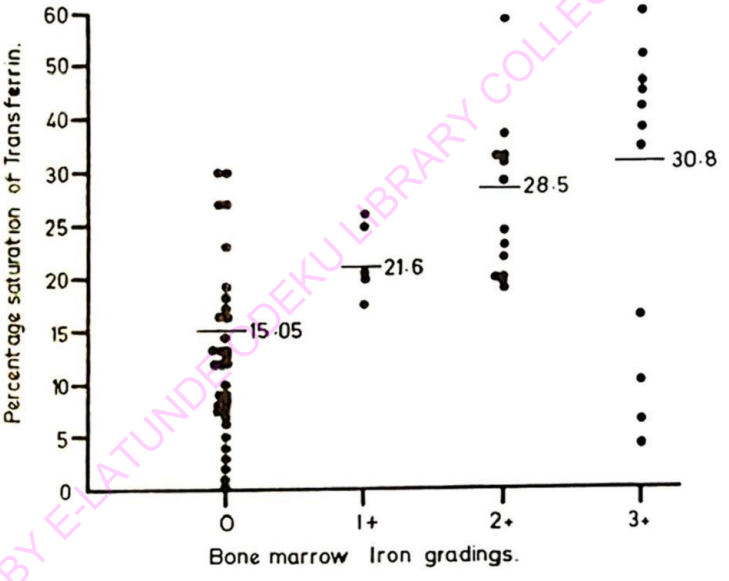

FIG. 4. Comparison of available bone marrow iron and transferrin saturation

(1978) reported lack of iron overload in patients with sickle cell anaemia in the first two decades of life. Washington & Boggs (1975) reported abnormally high urinary iron excretion in patients with sickle cell disease. The cause of the high incidence of iron deficiency in our patients is not known. The following suggestions can be made. Firstly, that the high incidence of iron deficiency in this group is part of the generally observed high incidence of iron deficiency in the population (O.A. Oluboyede & E. Topley, unpublished data); secondly, the possibility of an increased urinary iron loss in this group of patients can not be ruled out as previously documented by Washington & Boggs (1975). Daily iron intake in the Western State of Nigeria still remains high (36 – 42 mg) as previously reported by Gilles, Watson-Williams & Ball (1964). The observed higher levels of haemoglobin concentration, haematocrit and transferrin saturation among our SC patients agree with the reports of Serjeant & Serjeant (1972).

Haematological response to oral iron therapy by sickle cell patients with proven iron deficiency has been reported by Peterson *et al.*, (1975). The report did not record clinical observation while the patients were on oral iron. It has been suggested (Lincoln, Aroesty & Morrison, 1973) that 'in sickle cell anaemia, a reduction in haemoglobin concentration in the red blood cell should lead to a reduction in sickling which could in turn increase these patients' red cell life span to near normal'. This suggestion does not take into effect the increased activity of the bone marrow subsequent to anaemia with increased production of abnormal red blood cells. Also, Greenberg, Cass & Castle (1957) have observed 'that in AS disease persons with iron-deficiency anaemia did not show sickling phenomenon but as their mean corpuscular haemoglobin concentration were brought within normal range, an increasing proportion of their red blood cells would sickle when deprived of oxygen, although the relative proportions of A and S haemoglobins remained unchanged.' This is probably due to cellular re-arrangement of haemoglobin within the red cell with increased membrane haemoglobin, increased membrane rigidity which will subsequently lead to increased sickling.

Although the regime of intermittent blood transfussion improves the haemoglobin, dilutes the abnormal red blood cells and suppresses the bone marrow hyper-activity; in our environment, intermittent blood transfusion regimen can not be followed because of the limitations on blood supply.

Presently therefore, our sickle cell patients with high incidence of iron deficiency provide a unique group for a double blind trial of therapeutic oral iron to evaluate not only the haematological and clinical response but also to characterise precisely the relationship between corpuscular haemoglobin concentration and sickling phenomenon in SS and SC patients.

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(*Received* 3 *March* 1980; *accepted* 3 *November* 1980)