Iron Deficiency Anaemia in Jamaican Children, Aged 1–5 Years, with Sickle Cell Disease

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ABSTRACT

Objective: *The aim of this study was to determine, using a combination of measures, the prevalence of iron deficiency anaemia (IDA) in children under five years-of-age who have sickle cell disease (SCD) and attend the Sickle Cell Clinic (SCU) of the Tropical Medicine Research Institute.*

Materials and Methods: *Children with homozygous sickle cell anaemia (Hb SS) or doubly heterozygous for Hb S and Hb C (Hb SC) disease who had not received a blood transfusion within three months prior to the iron measurements, were enrolled. The diagnosis of IDA was made if transferrin saturation was less than 16% with serum iron less than 10.7 µmol/l and a low mean corpuscular volume (MCV) for age.*

Results: *Twelve children (8.5%), seven with Hb SS and five with Hb SC had IDA. Adjusting for genotype, children with IDA had significantly higher red blood cell (RBC) counts (4.3 x10^9/l vs 3.0 x 10^9/l, $p < 0.001$) and total iron binding capacity (TIBC) (65.6 µmol/l vs 55.2 µmol/l, $p < 0.004$) but significantly lower reticulocyte (retic) counts (7.8 % vs 12.2%, $p = 0.001$) than children without IDA.*

Conclusion: *Iron deficiency anaemia is a clinical problem which affects children with SCD in Jamaica. The higher RBC counts in the IDA group may be due to decreased haemolysis and increased red cell survival whilst the lower reticulocyte counts may be due to impaired erythropoiesis. These observations need to be extended by clinical studies to establish improved diagnostic measures for IDA in SCD. Additionally, clinical trials are needed to determine whether treatment of IDA in children with SCD reduces morbidity and is associated with clinical benefits such as improvements in neurocognitive function.*

Anemia por Deficiencia de Hierro en los Niños Jamaicanos Entre 1 y 5 Años de Edad, que Padecen la Enfermedad de Células Falciformes

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RESUMEN

Objetivo. *El objetivo de este estudio fue determinar – mediante una combinación de medidas – la prevalencia de anemia por deficiencia de hierro (ADH) en niños menores de cinco años de edad que padecen la enfermedad de células falciformes (ECF), y asisten a la Clínica de Células Falciformes (CCF) del Instituto de Investigación de Medicina Tropical.*

Materiales y métodos. *Se inscribieron niños con anemia de células falciformes homocigóticas (Hb SS) o enfermedad doble heterocigoto por Hb S y Hb C (Hb SC), que no habían recibido transfusión de sangre por un período de tres meses antes de las mediciones de hierro. Se diagnosticaba ADH si la saturación de la transferrina era menos del 16%, con hierro en suero inferior a 10.7 mol/l, y un volumen corpuscular medio (VCM) bajo para la edad.*

Resultados. *Doce niños (8.5%), siete con Hb SS y cinco con Hb SC presentaban ADH. después del ajuste de las diferencias en el genotipo, los niños con ADH tuvieron conteos de glóbulos rojos (RBC) (4.3 x10^9/l vs 3.0 x 10^9/l, $p < 0.001$), y capacidad total de fijación del hierro (TIBC) (65.6 µmol/l vs 55.2 µmol/l, $p < 0.004$) significativamente más altos, pero conteos de reticulocitos (7.8% vs 12.2%, $p = 0.001$) significativamente más bajos que los niños sin ADH.*

Conclusión. *La anemia por deficiencia de hierro es un problema clínico que afecta a los niños con ECF en Jamaica. El hecho de que los conteos de RBC sean más altos en los grupos con ADH, puede*

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deberse a una disminución de la hemólisis y un aumento de la supervivencia de glóbulos rojos, en tanto que los conteos más bajos de reticulocitos pueden deberse a problemas de eritropoyesis. Estas observaciones necesitan ser ampliadas mediante estudios clínicos para establecer las medidas de diagnóstico mejoradas para ADH en ECF. Además de ello, se requieren ensayos clínicos a fin de determinar si el tratamiento de ADH en niños con ECF reduce la morbosidad y se halla asociado con beneficios clínicos tales como el mejoramiento de la función neurocognitiva.

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INTRODUCTION

Iron deficiency is one of the most common nutritional deficiencies worldwide and is the leading cause of anaemia, especially in children and adult women (1, 2). Children in the developing world are especially vulnerable because of the increased requirements of growth (3), high helminth burden (2, 4) and diets with low iron bioavailabilty (2). In Jamaica, the prevalence of iron deficiency anaemia (IDA) in children is estimated at about 30% (5).

In sickle cell disease (SCD), the chronic haemolysis characteristic of the disorder results in an increased availability of iron from red cell destruction. Additionally, the reported increase in absorption of iron from the gastrointestinal tract (6) as well as the iron provided by blood transfusions (7, 8) would suggest that iron deficiency is unlikely in SCD. Indeed, Serjeant *et al* (9) have reported that serum iron levels were significantly higher in young children with Hb SS than in controls, standardized for age and gender. However, in contrast to these findings Rao *et al* (10) and Vichinsky *et al* (11) using different criteria have reported cases of IDA in SCD with prevalence of 12% and 8% respectively. The identification of IDA in children with SCD is important, as IDA contributes to worsening of anaemia (11) and may have negative long-term consequences on neurocognitive development (12, 13) and growth (3).

The diagnosis of iron deficiency is based primarily on laboratory measurements. However, conventional tests used, mean corpuscular volume (MCV), transferrin saturation and serum ferritin are limited because of varying ranges of sensitivities and specificities, as they may be modified by conditions other than iron deficiency such as age (14), chronic inflammation (15), genetic polymorphism (16) and by SCD (11, 17, 18). Current literature suggests that a low MCV for age, transferrin saturation less than 16% and serum ferritin less than 25 ng/ml are each 100% sensitive for IDA in SCD (10, 11). On the other hand, whilst serum ferritin less than 25 ng/ml is 100% specific for IDA in SCD, transferrin less than 16% and low MCV for age have specificities of 77–87% and 97% respectively (10, 11). Thus, it has been proposed that the use of a battery of tests to define iron status in a population improves precision in diagnosis of IDA. At the Sickle Cell Unit (SCU) in Jamaica, iron status is determined by the use of iron study tests: MCV, serum iron, total iron-binding capacity (TIBC) and transferrin saturation. Using this battery of tests, we sought to determine the prevalence of IDA in children under five years-of-age attending the SCU, and to describe differences between the IDA and non-IDA groups in terms of anthropometric and haematological variables.

MATERIALS AND METHODS

The sample consisted of children under five years-of-age with homozygous sickle cell anaemia (Hb SS) or doubly heterozygous for Hb S and Hb C (Hb SC) disease who attended the SCU during a two-year period (November 2001 – November 2003) and had iron measurements performed. Iron measurements are performed at the SCU on the initial visits of new patients and on clinical suspicion of IDA. Children who received a blood transfusion within three months prior to the iron measurements were excluded from the study. One hundred and forty-one children: 121 with Hb SS and 20 with Hb SC disease satisfied the study criteria.

The diagnosis of SCD was determined by Hb electrophoresis on cellulose acetate, pH 8.4, and citrate agar, pH 6.2. Quantitative HbA_2 levels by cellulose acetate membrane and HbF by Betke method confirmed the diagnosis. The haematological variables – haemoglobin (Hb), nucleated blood cell (NBC) count, platelet count (plts), red blood cell (RBC) count, and MCV, were determined using a Coulter MAX-M. Reticulocyte counts (retics) were performed by tube test method (staining technique). Serum iron and iron-binding capacity (TIBC) were determined using an Abbott Alycon autoanalyser. Transferrin saturation was calculated from serum iron and TIBC. The child was classified as having IDA if all three criteria: transferrin saturation less than 16%, serum iron less than 10.7 µmol/l, low MCV for age: 0.5–2 yr < 70 fl, 2–5 yr < 73 fl (19) were present. Cut-off points were based on laboratory standards as well as other studies (10, 11). Height and weight measurements performed at the time of iron measurements were recorded and body mass index (BMI) calculated.

Statistics

Values are expressed as means ± sd. Differences in mean values between the IDA group and the non-IDA group adjusting for genotype effects were determined by ordinary linear regressions. The Stata statistical package version 8 for Windows™ (Stata Corporation, College Station, TX) was used for data-analysis.

RESULTS

Using our IDA criteria, 12 children, seven with Hb SS and five with Hb SC had IDA resulting in a prevalence of 8.5%. There was a significantly greater than expected prevalence in

patients with Hb SC (42%) *vs* Hb SS (5.8%). The distributions of serum iron concentration and transferrin saturation were skewed to the right with median, minimum and maximum values being 9.7 µmol/l, 0.2 µmol/l, 40.7 µmol/l and 18%, 1%, 71% respectively (Figure). In contrast, the distribution of MCV for the total sample was left skewed with median, minimum and maximum values being 83 fl, 57 fl and 102 fl respectively (Figure).

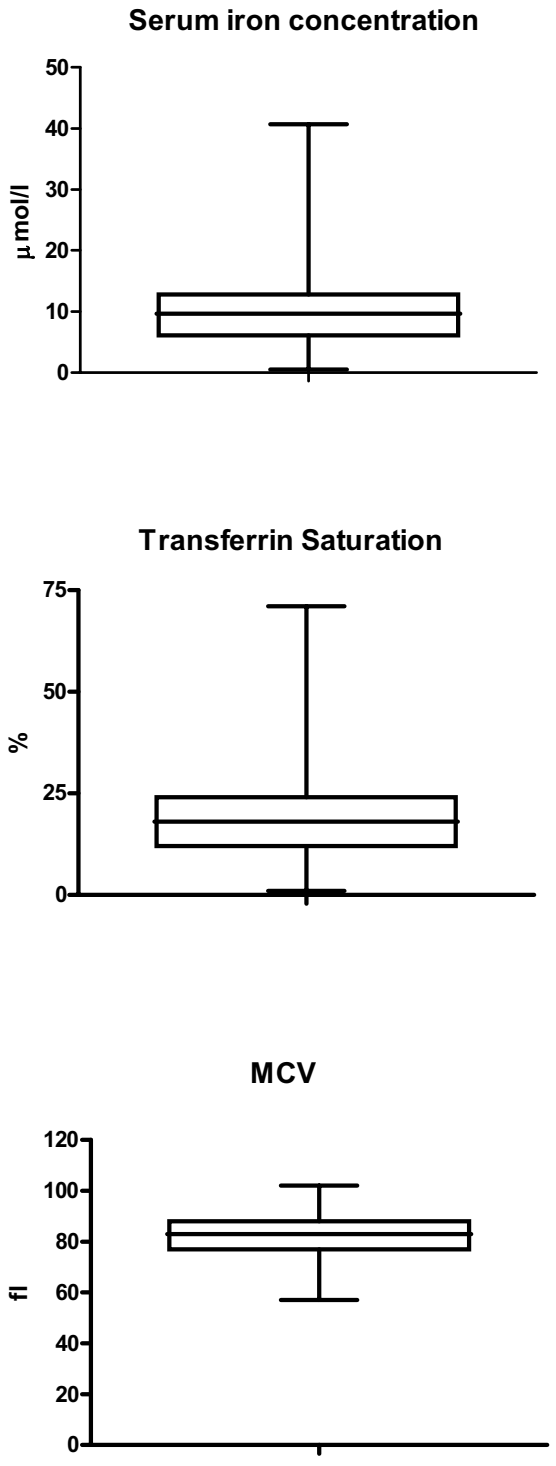

Figure: The distribution of iron concentration, transferrin saturation, and mean corpuscular volume in a group of children with sickle cell disease.

The anthropometric characteristics of the IDA and non-IDA groups are shown in Table 1. There was no significant difference in the means of the anthropometric variables between the two groups. The haemoglobin concentration ranged from 5.5 g/dl to 12.1 g/dl with a mean of 9.3 g/dl in the IDA group and from 5.6 g/dl to 11.4 g/dl with a mean of 8.3 g/dl in the non-IDA group (Table 2). This difference (9.3 g/dl *vs* 8.3 g/dl) in mean values was statistically significant ($p < 0.03$). As expected, patients with Hb SC had significantly higher Hb (~ 2gm/dl) than did patients with Hb SS, this being independent of IDA status. However, in a regression model with haemoglobin concentration as the outcome variable and IDA status and genotype entered simultaneously as independent categorical factors there was no significant difference in mean haemoglobin concentration of IDA groups, adjusting for genotype (Table 2).

Table 1: Age and anthropometric characteristics of IDA and non-IDA groups of young children with SCD

Characteristic	IDA	Non-IDA
Age (months)	33.9 (16.2)	37.7 (17.3)
Weight (kg)	13.5 (3.1)	13.7 (3.6)
Height (cm)	99.3 (6.3)	99.1 (10.5)
BMI (kg/m^2)	15.7 (3.6)	15.4 (1.1)

Values are mean (SD)

Children with IDA also had significantly higher RBC counts (4.3 x10^9/l vs 3.0 x 10^9/l, $p < 0.001$) and TIBC (65.6 µmol/l *vs* 55.2 µmol/l, $p < 0.004$) than children without IDA, independent of genotype (Table 2). On the other hand, reticulocyte counts were significantly lower in the children with IDA than in children in non-IDA group (7.8% *vs* 12.2%, $p = 0.001$), this difference also being independent of genotype (Table 2).

Compared to the tri-test measure for the diagnosis of IDA in this study, each individual criterion was as sensitive but specificity varied from 46% to 64% (Table 3). Low MCV for age had the best test performance characteristics and serum iron the worst.

DISCUSSION

In this cross-sectional study, a battery of tests was used in order to increase the specificity in diagnosing IDA. Using these tests, data demonstrated that of the 141 children with Hb SS and Hb SC who had had serum iron determination within the last two years at the SCU, 8.5% of children tested would be considered to have IDA. The data further demonstrate that there were significant differences in haematological and biochemical indices with RBC counts ($p < 0.001$) and TIBC ($p < 0.004$) being significantly higher in the IDA group versus the non-IDA group. On the other hand, reticu-

Table 2: Haematological variables by genotype and IDA group

Variables	IDA			Non-IDA		
	SC n = 5	SS n = 7	ALL n = 12	SC n = 15	SS n = 114	ALL n = 129
Hb (g/dl)†	10.5 (0.5)	8.4 (2.3)	9.3 (2.0)	10.2 (0.6)	8.1 (1.2)	8.3 (1.3)
NBC (x 10^9/l) †	10 (2.5)	12.6 (2.8)	11.5 (2.9)	9.3 (2)	16.3 (7.6)	15.4 (7.6)
RBC (x 10^{12}/l) †	4.6 (0.3)	4 (0.9)	4.3 (0.8)**	3.9 (0.4)	2.9 (0.6)	3 (0.7)
Plts (x 10^9/l)	285.4 (52.8)	386.9 (133.9)	344.6 (116.3)	390.4 (176.2)	430.3 (161)	421.6 (160.5)
Retics (%) †	6.4 (3.4)	8.7 (4.3)	7.8(4)*	7.1 (2.6)	12.9 (4.3)	12.2 (4.5)
TIBC (µmol/l)	63.1 (7.2)	67.4 (8.4)	65.6(7.9)*	57.4 (13.1)	55 (12)	55.2 (12)

Values are mean (sd). Abbreviations: TIBC – total iron binding capacity; Hb- haemoglobin; RBC – red blood *c*ells; Plts– platelets; NBC – nucleated blood cells; Retics – reticulocytes. † effect of genotype $p < 0.05$; * $p < 0.004$, ** $p < 0.001$ comparing means for IDA group *vs* non-IDA group, adjusting for genotype effects.

Table 3: Test Performance characteristic of individual criterion for diagnosing iron deficiency anaemia in young children with sickle cell disease compared with the tri-test criteria.

	Transferrin saturation criterion	Serum Iron criterion	Low MCV age criterion
Sensitivity %	100	100	100
Specificity %	64.3	46	95
Positive Predictive Value %	20.7	14.6	66.6
Likelihood ratio positive	2.8	1.84	21.5

Sensitivity is the proportion of diseased patients correctly identified.

Specificity is the proportion of healthy patients correctly identified.

The positive predictive value (PPV) is the probability of a patient having the disease following an abnormal test result assuming a prevalence of 8.5%.

The likelihood ratio of a positive test is the ratio of the probability (likelihood) of a positive test result in an abnormal patient and in a normal patient = Sensitivity/(1- specificity).

locyte counts were significantly lower in the IDA than in the non-IDA group.

The diagnosis of IDA in SCD is difficult and it is possible that subjects in the IDA and non-IDA group may have been misclassified. At present, the absence of stainable iron in marrow is considered to be the gold standard for diagnosing iron deficiency. However this test is invasive and is not offered routinely. Further, in SCD, the distribution of iron within the body may be highly compartmentalized, thereby reducing the specificity of this measure in diagnosing IDA (7, 20, 21). Alternatively, serum ferritin concentrations and/or ratios based on transferrin receptor concentration to ferritin concentration, have been proposed as the best non-invasive measure of iron status in individuals (14, 22, 23). However, serum ferritin levels are increased independent of changes in iron stores by acute inflammation (15). As the natural history of sickle cell disease is characterized by intermittent crises with associated acute inflammatory reactions, the utility of serum ferritin as a marker of iron status is limited to steady state periods (24). Further, the usefulness of serum ferritin concentration as a marker of iron stores in SCD is confounded by the observation that serum ferritin concentration is affected by blood transfusions (7, 8). Therefore, there is the urgent need to develop and validate a non-invasive but specific measure of iron stores in SCD. Serum hepicidin, a 25-amino acid peptide made by hepatocytes and whose physiological function is to regulate iron absorption, may emerge as this marker (25).

At the SCU, serum ferritin assays are unavailable. Therefore, in this study, the authors used three criteria, low serum iron concentration, low transferrin saturation and low MCV for age for diagnosing IDA. Individually, each measure has been shown to be as sensitive but with much lower specificity than serum ferritin and the tri-test measure in diagnosing IDA in SCD (11, 26). Using the tri-test measure, the proportion of individuals with IDA in this study (8.5%) is similar to the proportion of persons with IDA in case series reported in the literature (10, 11). Therefore, assuming that the true prevalence of IDA in young children with SCD is 8.5% then the minimum specificity of the tri-test measure would be ~91%. This compares favourably with the test performance characteristics of serum ferritin (11).

Iron deficiency anaemia in SCD is associated with decreased haemolysis and increased red cell survival (27, 28). The mechanism of this is thought to be due to the lowering of the intracellular concentration of deoxyhaemoglobin S (MCHC-S) because of insufficient iron for haem synthesis. The lower MCHC-S results in a decreased rate of polymerization of the sickle haemoglobin when deoxygenated (27). In the present study, the higher RBC levels in the IDA group than in non-IDA group may have been due to decreased haemolysis and increased red cell survival in the IDA group. Additionally, the lower reticulocyte count in the IDA group than in the non-IDA group suggests that erythropoiesis was impaired in the IDA group.

Whether the reduction in haemolysis and increased red cell survival associated with IDA in SCD is accompanied by clinical benefit is unclear (27). Clinically, there is evidence

that IDA contributes to worsening of anaemia (11) and has negative long-term consequences on neurocognitive development (12, 13), especially if it develops during early childhood. Children with SCD have impaired neurocognitive development from various postulated factors including cerebrovascular accidents (CVAs) – clinical CVAs and silent infarcts (29), an encephalopathic process (30), and chronic anaemia (31). It can therefore be argued that development of IDA in children with SCD would exacerbate neurocognitive impairment. Additionally, IDA is associated with impaired growth (3) and this may further worsen the growth deficit observed in SCD (32).

In summary, IDA is a clinical problem, which affects children with SCD in Jamaica. However, the highly selective nature of the sample requires one to exercise caution in generalizing the findings of this study. Nonetheless, these observations need to be extended by clinical studies to establish improved diagnostic measures of IDA in SCD and further to determine whether treatment of IDA reduces morbidity and improves neurocognitive development in children with SCD.

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