

Research article

A prospective, cross-sectional study of anaemia and peripheral iron status in antiretroviral naïve, HIV-1 infected children in Cape Town, South Africa

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Abstract

Background: Anaemia is a common manifestation of paediatric HIV infection. Although there are many causes, anaemia of chronic diseases is the most frequent type. In poor countries iron deficiency is widespread. It is probable that many HIV-infected children in these countries are also iron deficient. This study describes the relationship between paediatric HIV infection and anaemia, and documents the peripheral iron status of antiretroviral naïve, HIV-infected children.

Methods: Sixty children were evaluated prospectively. Investigations included CD4+ count, haemoglobin concentration (Hb), red blood cell (RBC) morphology, and iron studies.

Results: Anaemia was present in 73% of children. Compared to mild HIV infection, median Hb was lower in children with moderate clinical infection (104 g/L v 112 g/L, $p = 0.04$) and severe clinical infection (96 g/L v 112 g/L, $p = 0.006$), and more children with severe infection were anaemic (92% v 58%, 0.04). There was a significant relationship between immunological status and Hb. 68% had abnormal RBC morphology. Significantly more children with moderate and severe disease, and severe immunosuppression had abnormal RBC morphology. 52% were iron-depleted, 20% had iron-deficient erythropoiesis and 18% iron deficiency anaemia (IDA). 16% (7/44) of anaemic children had microcytosis and hypochromia. Median soluble transferrin receptor concentration was significantly higher in those with microcytic hypochromic anaemia (42.0 nmol/L v 30.0 nmol/L, $p = 0.008$).

Conclusions: Both the proportion of anaemic children and the median Hb were associated with disease status. Iron depletion and IDA are major problems in HIV-infected children in South Africa.

Background

Anaemia is a common manifestation of paediatric HIV infection and is a significant negative predictor of survival [1,2]. Although there are many causes of HIV-associated

anaemia, anaemia of chronic diseases (ACD) is the most frequent type [3], inflammatory cytokines released during chronic diseases inhibit erythropoiesis, blunt the erythropoietin response, reduce red blood cell survival and pre-

vent the release of iron from the reticuloendothelial system, leading to the development of ACD [3,4].

Iron deficiency is widespread in children in developing countries. A recent nutritional survey showed that approximately 20% of South African children were anaemic and 10% iron deficient. In this survey HIV status was not recorded [5]. Another study conducted on Italian children documented iron deficiency, caused by intestinal malabsorption, in a large proportion of HIV-infected children [6]. Because of the high prevalence of iron deficiency among South African children, it is probable that many HIV-infected children are also iron deficient. This report documents the relationship between paediatric HIV infection and anaemia and describes the peripheral iron status of HIV-infected children in an economically deprived setting.

Methods

A prospective, cross-sectional study was performed on 60 clinically stable, antiretroviral naïve, HIV-infected children attending the HIV clinic at Red Cross War Memorial Children's Hospital in Cape Town. The ambulatory management of children who attend the clinic includes cotrimoxazole prophylaxis, treatment of minor infections and nutritional and micronutrient supplementation. Antiretroviral therapy is not routinely administered to HIV-infected children in South Africa. Children were considered eligible for inclusion if they were relatively well i.e. they had had no acute illness or febrile episode in the preceding 7 days, nor hospitalised in the preceding 14 days. Children were excluded if they were acutely ill. The first 2 or 3 children seen at each weekly clinic over an 8 month period were enrolled, after their parents were counselled and written consent obtained. The Research Ethics Committee of the University of Cape Town approved the study.

Children were diagnosed and classified into clinical and immunological categories according to CDC criteria [7]. CD4+ lymphocyte counts were measured on EDTA-anti-coagulated whole blood samples using an EPICS Profile II flow cytometer (Coulter Corporation, Hialeah, Florida) [8]. Haemoglobin concentration (Hb), red blood cell (RBC) indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution width (RDW)] and RBC morphology were determined on EDTA-anti-coagulated blood samples using a Coulter MAXM (Coulter Corporation), according to the manufacturer's instructions. Red blood cell morphology was validated by light microscopy, on May Grunewald Giemsa stained samples [9].

Evaluation of iron status was performed on heparinised plasma. Ferritin concentration was measured by commercial

immunoassay (Spectro Ferritin[®], Ramco laboratories, Houston, Texas), iron concentration (Fe) by direct spectrophotometry on a RA 1000 analyser, model TM34-E1120/115CV (Bayer – Technicon Instruments Corporation, New York) using a commercial kit (MPR Iron without deproteinisation, Roche Diagnostics Corp., Indianapolis), and transferrin concentration by nephelometry on a Behring nephelometer, model BN 100 with commercial antisera (Behringwerke AG, Marburg, Germany). Total iron binding capacity (TIBC) was calculated using the formula: TIBC ($\mu\text{g/dl}$) = (Transferrin \times 200) \div 8 [10]. Transferrin saturation (TS) was determined as follows: TS = (Plasma iron concentration \div TIBC) \times 100 [11]. Results were compared to locally derived normal age-related values. The following definitions were used in the analysis (i) iron depletion: ferritin $<$ 10 $\mu\text{g/L}$, (ii) iron-deficient erythropoiesis: ferritin $<$ 10 $\mu\text{g/L}$ and at least 3 of the following criteria: Fe $<$ 8.8 $\mu\text{mol/L}$, TIBC $>$ 71.6 $\mu\text{mol/L}$, TS $<$ 10%, MCV less than normal age-related values and MCH less than normal age-related values, and (iii) iron deficiency anaemia (IDA): criteria for iron-deficient erythropoiesis plus Hb less than the normal age-related range [12,13]. Soluble transferrin receptor concentration (sTfR) was measured on serum samples using a commercial immunoassay (Quantikine[™] IVD[™] sTfR assay, R&D Systems, Minneapolis, Minnesota). Soluble transferrin receptor concentration results were used in the comparative analyses.

Data was collated and analysed using Epi Info version 6.04, Division of Surveillance and Epidemiology, CDC, Atlanta, Georgia. Categorical and continuous data were compared using the Chi-squared and Kruskal Wallis tests respectively. The Spearman rank correlation test was used to determine the relationship between different continuous variables. A p value $<$ 0.05 was considered significant.

Results

The median age (quartiles) was 25.0 months (13.0, 37.8). The male to female ratio was 1.0:0.9. The diagnosis of HIV infection was established by polymerase chain reaction in 73% (44/60) i.e. children less than 18 months of age and by ELISA in 27% (16/60) i.e. children more than 18 months of age. Mild clinical features of HIV infection (Category A) were present in 32% (19/60), moderate features (Category B) in 48% (29/60) and severe features (Category C) in 20% (12/60). Twenty per cent (12/60) had no evidence of immunosuppression (Category I), 35% (21/60) were moderately immunosuppressed (Category II) and 45% (27/60) were severely immunosuppressed (Category III). The median age was significantly lower in category C when compared to category A (13.5 months ν 28.0 months, $p = 0.03$) and category B (13.5 months ν 30 months, $p = 0.02$). There was no significant age differences between the immunological categories.

Table I: Laboratory measurements of iron status (n = 60)

Variable	Median concentration (quartiles)	Percentage below normal age-range	Percentage above normal age-range
Hb (g/L)	104 (96, 111)	73	2
MCV (fL)	76.4 (74.1, 80.5)	20	0
MCH (pg)	26.3 (24.6, 27.7)	20	2
MCHC (g/dL)	34.3 (33.4, 35.0)	0	0
RDW (%)	16.2(14.9, 17.6)	0	62
Ferritin (µg/L)	9.0 (4.9, 36.5)	52*	2
Iron (µmol/L)	4.0(2.1, 7.7)	75	2
Transferrin (g/L)	2.6 (2.2, 2.9)	32	0
TIBC (µmol/L)	65.4 (55.4, 74.7)	0	33
TS (%)	6.0(4.1, 10.7)	70 ⁺	0

* Per cent < 10 µg/L, Per cent < 10%

Seventy three per cent (44/60) were anaemic by age-related reference values. The median Hb was 104 g/L (96, 110). Seventy two per cent had a Hb less than 110 g/L. Two children had a Hb less than 80 g/L. Compared to Category A, the median Hb was significantly lower in Category B (104 g/L *v* 112 g/L, *p* = 0.04) and Category C (96 g/L *v* 112 g/L, *p* = 0.006). Compared to Category A, more children in Category C were anaemic (92% *v* 58%, *p* = 0.04). The proportion of children with anaemia in Category B was greater than Category A (76% *v* 58%, *p* = 0.2), but this was not significant. Median Hb was significantly lower in Category III compared to Category I (99 g/L *v* 110 g/L, *p* = 0.003). The median Hb was lower in Category II than in Category I (104 g/L *v* 110 g/L, *p* = 0.07), but this was not significant. Compared to Category I, more children in Category II (76% *v* 42%, *p* = 0.04) and Category III (85% *v* 42%, *p* = 0.005) were anaemic. Median Hb did not correlate with age (*r* = 0.35, 95% confidence interval-0.14, 0.36).

Many children had abnormal RBC indices and altered peripheral iron results (Table 11). Red blood cell morphology showed anisocytosis in 68% (41/60), polychromasia in 17% (10/60), target cells in 15% (9/60) and microcytosis plus hypochromia in 12% (7/60). Thirty two per cent (19/60) had normal RBC morphology. In comparison to Category A more children in Category B (76% *v* 42%, *p* = 0.006) and Category C disease (92% *v* 42%, *p* = 0.02) had abnormal RBC morphology. Significantly more children in Category III than Category I had abnormal morphology (89% *v* 42%, *p* = 0.002). A larger proportion in Category II had abnormal morphology relative to Category I (67% *v* 42%, *p* = 0.4); this difference was not statistically significant.

Fifty two per cent (31/60) of the children were iron-depleted, 18% (11/60) had iron-deficient erythropoiesis

and 17% (10/60) IDA. Of the 11 patients with iron-deficient erythropoiesis 2 fulfilled all diagnostic criteria, 3 fulfilled 4 criteria and 6 fulfilled 3 criteria. Forty five per cent (20/44) of all anaemic children were iron-depleted. Table 2II compares anaemic children with low plasma ferritin concentrations and those with ferritin concentrations > 10 µg/L. With exception of transferrin and TIBC, measures of iron status are not significantly different between the two groups. Thirteen per cent (8/60) of the anaemic children had a ferritin concentration greater than 100 µg/L. Children with microcytic, hypochromic anaemia had significantly higher sTfR. The ferritin concentration of these children ranged from 4.9 µg/L to 49 µg/L. Four of these seven children had a ferritin concentration < 10 µg/L. Other measures of iron status were not significantly different between the groups of children with or without microcytic, hypochromic anaemia (Table 3III). Median sTfR was significantly higher in category C compared to category A (35.5 nmol/L *v* 26.0 nmol/L, *p* = 0.01). There was no association between sTfR and immunological category.

Discussion

A large number of stable, HIV-infected children were anaemic. Few had severe anaemia. Furthermore, both the proportion of children with anaemia and median haemoglobin concentration were associated with clinical and immunological status. There was no association between Hb and chronological age. Studies of infected children and adults indicate that anaemia independently predicts poor outcome [2,14,15]. The results of the present study are in agreement with these observations, as significantly more children with severe clinical features and severe immunosuppression were anaemic.

Many children had abnormal red blood cell morphology. Anisocytosis was the most frequent observation. It usually correlates with an increased red blood cell distribution

Table II: Comparison of anaemic children with plasma ferritin concentration < 10 µg/L (Group A) or ≥ 10 µg/L (Group B)

Variable	Group A (n = 20) Median (quartiles)	Group B (n = 24) Median (quartiles)	P value
Hb (g/L)	103 (97,108)	95 (87,104)	0.04
MCV (fL)	75.2(71.4,78.6)	76.3(71.9,81.6)	0.3
MCH (pg)	25.6(23.5,26.4)	26.3 (24.2, 28.3)	0.2
MCHC (g/dL)	33.6(32.9,34.3)	34.2 (33.2, 34.3)	0.2
RDW (%)	17.0(16.1,18.1)	16.6(15.4, 17.8)	0.7
Ferritin (µg/L)	4.9 (4.9, 7.0)	46.5(23.5, 117)	< 0.001
Iron (µmol/L)	3.7 (2.0, 5.7)	3.2 (2.0, 6.9)	0.8
Transferrin (g/L)	2.9(2.5,3.1)	2.2(1.9,2.6)	< 0.001
TIBC (µmol/L)	71.5(63.4,78.2)	54.9 (46.5, 65.9)	< 0.001
TS (%)	4.9 (3.5, 7.3)	5.1(4.1, 10.1)	0.5
sTfR (nmol/L)	32.0(29.3,38.5)	30.0 (22.8, 39.0)	0.3

Table III: Comparison of anaemic children with (Group I) or without (Group II) microcytosis plus hypochromia

Variable	Group I (n = 7) Median (quartiles)	Group II (n = 37) Median (quartiles)	P value
Hb (g/L)	96 (87, 99)	101(94,105)	0.2
MCV (fL)	67.2 (65.2, 68.2)	76.4(74.6,81.0)	< 0.001
MCH (pg)	22.0(21.1,23.2)	26.2 (25.0, 27.8)	< 0.001
MCHC (g/dL)	33.1 (32.6,34.1)	34.2 (33.4, 34.6)	0.08
RDW (%)	16.8(16.2, 18.0)	16.6(15.2,18.0)	0.4
Ferritin (µg/L)	8.0 (4.9, 38.0)	15.0(5.0,87)	0.3
Iron (µmol/L)	2.7 (2.0, 6.5)	3.5 (2.0, 5.7)	0.8
Transferrin (g/L)	2.8(2.6,3.1)	2.6 (2.0, 2.9)	0.1
TIBC (µmol/L)	69.3 (64.5, 77.5)	63.8(50.5,73.0)	0.1
TS (%)	3.7 (2.9, 9.0)	5.2(4.1,9.0)	0.1
sTfR (nmol/L)	42.0(35.0,45.0)	30.0 (25.0, 36.0)	0.008

width [16], present in many children. Anisocytosis is a non-specific feature that may be present in any red blood cell disorder, including IDA and ACD [17]. Interestingly, there was a statistically significant relationship between disease status and red blood cell morphology. More children with severe clinical disease and severe immunosuppression had abnormal morphology. These findings were similar to the relationship between disease status and anaemia, and reflect the connection between abnormal morphology and anaemia.

Distinguishing IDA from ACD in chronic inflammatory diseases may be a problem, as many of the laboratory measurements used to evaluate iron status are affected in a similar manner [18]. Ferritin concentration remains the most practical measurement for evaluating iron stores. A low concentration (< 10 µg/L) unequivocally identifies iron-depleted stores. Ferritin is an acute phase reactant.

Therefore, levels may be falsely normal in chronic inflammatory diseases such as HIV infection, despite the presence of iron depletion [12,13]. Elevated ferritin levels, often exceeding 1000 µg/L have been recorded in adults with acquired immunodeficiency syndrome [3]. In the present study, 45% (20/44) of all anaemic children were iron-depleted. A comparison of the laboratory results of anaemic children with or without hypoferritinaemia (Table 2II) showed few statistically significant differences between the two groups. In particular, sTfR was not significantly different between the two groups. Failure to show a clear distinction between these groups confirms the complex relationship between iron depletion, iron deficiency anaemia and anaemia of chronic disorders in HIV infection.

The present study employed strict criteria to identify children with IDA. These criteria were adapted from previous-

ly published guidelines. Although locally derived normal age-related values were taken into consideration the criteria were similar to those used internationally [12,13]. The results probably under-represent the true extent of IDA. For example, only four children with microcytic, hypochromic anaemia had low ferritin concentrations. They were included in the IDA group. The other three with microcytic, hypochromic anaemia were excluded, although they had significantly elevated soluble transferrin receptor concentrations, in keeping with IDA (Table 3III). Despite these omissions, the results suggest that IDA is more prevalent in HIV-infected children than in the general paediatric population in South Africa [5].

Soluble transferrin receptor is not an acute phase reactant. It is considered more reliable than ferritin to distinguish IDA from ACD in acute and chronic inflammatory diseases. In IDA sTfR is elevated and in ACD sTfR remains normal [13,18]. In the present study, sTfR of anaemic children with or without hypoferritinaemia were not significantly different. However, those with microcytic, hypochromic anaemia had significantly elevated sTfR. The results suggest that many HIV-infected children with anaemia probably have a combination of ACD and iron depletion, a smaller proportion have IDA and some children with normal ferritin concentrations have IDA. Only those with classic findings of IDA had predictably high soluble transferrin receptor concentrations.

The biggest weakness of the present study was the failure to compare the iron status of HIV-infected children with non-HIV-infected controls. However, iron depletion was widespread, and IDA was far more prevalent than was documented in a recent national study of more than 6000 South African children [5]. That study was undertaken in 1994, when the prevalence of paediatric HIV infection was low [19]. The findings of the present study were comparable to results obtained in an Italian study evaluating iron status in HIV-infected children. In the Italian study iron deficiency, defined as low serum iron concentration, was present in 48% of HIV-infected children. The present study employed stricter criteria to define iron status. Therefore we can conclude that iron depletion and IDA are significant problems in HIV-infected children in South Africa.

Many aetiological factors probably contribute to the development of low iron status in HIV-infected children, including reduced dietary intake, the quality of dietary iron and altered iron absorption [20]. The Italian study showed that intestinal malabsorption is a major factor [5]. Whether iron therapy causes deleterious effects in paediatric HIV infection has not been established. In general, while the relationship between infection and iron status remains contentious, iron overload is associated with in-

creased susceptibility to certain infections [21]. Therefore, liberal iron therapy or prophylaxis in HIV-infected children may facilitate the development of opportunistic infections. For the present time, HIV-infected children with IDA should receive therapeutic iron replacement. However, more research is required to establish the benefits and or deleterious effects of iron therapy and prophylaxis in antiretroviral naive, HIV-infected children, particularly those with iron depletion.

Abbreviations

ACD Anaemia of chronic diseases

EDTA Ethylene diamine tetra-acetic acid

CDC Centers for Disease Control

Fe Iron concentration

Hb Haemoglobin concentration

HIV-1 Human immunodeficiency virus – type 1

IDA Iron deficiency anaemia

MCH Mean corpuscular haemoglobin

MCHC Mean corpuscular haemoglobin concentration

MCV Mean corpuscular volume

RBC Red blood cell

RDW Red blood cell distribution width

sTfR Soluble transferrin receptor concentration

TIBC Total iron binding concentration

TS Transferrin saturation

Competing interests

None declared.

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