Post-natal anaemia and iron deficiency in HIV-infected women and the health and survival of their children

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Postnatal anemia and iron deficiency in HIV-infected women and the health and survival of their children

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Abstract

Prenatal iron supplementation may improve pregnancy outcomes and decrease the risk of child mortality. However, little is known about the importance of postnatal maternal iron status for child health and survival, particularly in the context of HIV infection. We examined the association of maternal anemia and hypochromic microcytosis, an erythrocyte morphology consistent with iron deficiency, with child health and survival in the first two to five years of life. Repeated measures of maternal anemia and hypochromic microcytosis from 840 HIV-positive women enrolled in a clinical trial of vitamin supplementation were prospectively related to child mortality, HIV infection, and CD4 T-cell count. Median duration of follow-up for the endpoints of child mortality, HIV infection and CD4 cell count was 58, 17 and 23 months, respectively. Maternal anemia and hypochromic microcytosis were associated with greater risk of child mortality (HR for severe anemia=2.58, 95% CI: 1.66-4.01, *P* trend<0.0001; HR for severe hypochromic

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microcytosis=2.36, 95% CI: 1.27-4.38, *P* trend=0.001). Maternal anemia was not significantly associated with greater risk of child HIV infection (HR for severe anemia=1.46, 95% CI: 0.91, 2.33, *P* trend=0.08) but predicted lower CD4 T-cell counts among HIV-uninfected children (difference in CD4 T-cell count/μL for severe anemia:-93, 95% CI: -204-17, *P* trend=0.02). The potential child health risks associated with maternal anemia and iron deficiency may not be limited to the prenatal period. Efforts to reduce maternal anemia and iron deficiency during pregnancy may need to be expanded to include the postpartum period.

Keywords

anemia; iron deficiency; postnatal; HIV; child

Introduction

The HIV/AIDS epidemic is one of the most important challenges in global health today, with an estimated 33 million people worldwide living with HIV (Joint United Nations Programme on HIV/AIDS and World Health Organization, 2009). In sub-Saharan Africa, home to two-thirds of HIV-infected individuals, 60% of those infected are women (Joint United Nations Programme on HIV/AIDS, 2008). Despite progress in the prevention of mother-to-child-transmission of HIV and care for HIV-exposed children, children born to HIV-infected women continue to experience increased risks of morbidity and mortality, irrespective of HIV infection status (Newell et al., 2004, Shapiro et al., 2007, Thea et al., 1993). Low-cost interventions to improve the health and survival of children born to HIV-infected mothers are still needed.

Anemia is one of the most common hematological disorders in the world, affecting two billion people worldwide (World Health Organization et al., 2001). While there are many causes of anemia, iron deficiency is a major contributing factor, and women of reproductive age are among the most seriously affected due to blood loss during menstruation, increased demands during pregnancy to support fetal development, and insufficient dietary intake of iron and other nutrients. During pregnancy, anemia and iron deficiency are associated with increased maternal mortality, low birth weight, and preterm birth (Allen, 2000). In the context of HIV infection, anemia and iron deficiency are highly prevalent (Friis et al., 2001, Levine et al., 2001) and independent predictors of mortality and HIV disease progression (Mocroft et al., 1999, Moore et al., 1998, O'Brien et al., 2005).

The provision of iron supplements to pregnant women for the prevention of anemia is a widely practiced public health measure. Evidence suggests that maternal anemia during pregnancy is associated with increased risk of mortality among children (Chatterjee et al., 2007, Marchant et al., 2004). In addition, in developing countries where the prevalence of anemia is high, prenatal iron supplementation has been shown to increase birth weight (Christian et al., 2003, Menendez et al., 1994, Mishra et al., 2005). Little, however, is known about the importance of postnatal maternal iron status for children (Allen, 2000), particularly in the context of HIV infection. Very low maternal iron status may contribute to impaired child health and survival by reducing child iron stores and impairing cellular immunity during breastfeeding. During and after breastfeeding, maternal anemia and iron deficiency may reflect advanced maternal disease and adversely affect child health by impairing maternal care practices or increasing exposure to HIV and other pathogens co-infecting the mother.

We conducted a prospective study to examine the relationships of maternal anemia and iron deficiency during postnatal life with child mortality, HIV infection and immune status. The

study population consisted of HIV-infected women enrolled in a clinical trial to assess the impact of vitamin supplementation on maternal and child health outcomes in Tanzania.

Materials and Methods

Study population

Between April 1995 and July 1997, 1078 HIV-infected pregnant women between 12–27 weeks gestation were enrolled in the Trial of Vitamins Supplementation Study in Dar es Salaam, Tanzania. Details of the design and methods have been published elsewhere (Fawzi et al., 1999). In brief, the study was a randomized, placebo-controlled trial to examine the efficacy of multivitamin and vitamin A supplements given pre- and postpartum to improve maternal and child health outcomes. In accordance with local guidelines for antenatal care, all women received a daily dose of 400 mg of ferrous sulfate (equivalent to 120 mg of ferrous iron) and 5 mg folate for anemia prophylaxis during pregnancy. All maternal hemoglobin levels after pregnancy were reported to study physicians for the appropriate management of severe anemia, if indicated. At the time of the study, antiretroviral therapy was not available to most women in Tanzania, including those who participated in this study.

Enrollment and follow-up

Detailed background information was collected from all women by research nurses using standardized questionnaires at enrollment. Maternal disease stage was assessed on the basis of a woman's morbidity history and physical examination according to the World Health Organization staging system for HIV disease (World Health Organization, 1990). Viral load assessment was done at baseline on a randomly selected subset of 387 women. Postnatal follow-up of mothers and children occurred through a 6-week postnatal visit and monthly visits thereafter and included physical examinations and anthropometric assessment. Mothers provided information on any illness since the last visit. Mortality surveillance of mothers and children was conducted through the monthly visits to study clinics. Home visits were made in the event that a scheduled clinic visit was missed.

Maternal blood specimens were provided at baseline of the parent study, six weeks postpartum, and every six months thereafter for the measurement of hemoglobin and peripheral blood picture assessment. Children provided a blood sample at birth, six weeks of age, and every three months thereafter for assessment of HIV infection. CD4 T-cell counts in children were determined at birth, six weeks of age and every six months thereafter.

Laboratory methods

Hemoglobin was measured using either a CBC5 Coulter Counter (Coulter Corp, Miami, FL) or the cyanmethaemoglobin method using a Colorimeter (Corning, Inc, Corning, NY). Thin blood films with Leishman stain were prepared and examined microscopically by trained laboratory technicians for the presence of hypochromasia and microcytosis. The prevalence of each characteristic among the sample of cells was coded into five levels of severity (absent, <25%, 25%–49%, 50%–75%, or >75%).

HIV infection was diagnosed in children before 18 months of age on the basis of a positive polymerase chain reaction test using the Amplicor HIV-1 DNA assay, version 1.5 (Roche Diagnostic Systems, Branchburg, NJ) or positive enzyme-linked immunosorbent assays and/or a Western Blot test at or after 18 months of age. The time of infection was designated as the midpoint between the last negative and first positive HIV test result. Absolute T-lymphocyte subset counting was done using the FACSCount and FACSCan systems (Becton Dickinson, San Jose, CA).

Statistical analysis

Maternal anemia was defined by hemoglobin levels according to criteria used for referral to district hospitals in Tanzania: severe anemia: < 8.5 g/dL, moderate anemia: 8.5 to < 11.0 g/dL, and absent: ≥11.0 g/dL (Johnson-Spear and Yip, 1994). Hypochromic microcytosis, an erythrocyte morphology consistent with iron deficiency, was used as a proxy to define four degrees of maternal iron deficiency: severe (hypochromasia ≥ 25% and microcytic cells observed), moderate (hypochromasia < 25% and microcytic cells observed), mild (any hypochromasia without microcytosis) or absent (hypochromasia absent). In the analysis of child HIV infection, we combined the two upper levels of hypochromic microcytosis due to the sparse occurrence of events.

We used the Cochrane-Armitage test for trend for proportions and the Kruskal-Wallis test for continuous measures to compare maternal characteristics by levels of anemia and hypochromic microcytosis at baseline of the parent study. We fit proportional hazards regression models to examine the relationship of time-varying maternal anemia and hypochromic microcytosis with time to child death, HIV infection, and the combined endpoint of death or HIV infection. We considered child mortality overall and within the first and second years of life. We considered two child HIV infection endpoints: total HIV infection (i.e. infection through all three routes: *in utero*, intrapartum and breastfeeding) and breastfeeding infection (i.e. infection after six weeks among those not known to be infected at six weeks). Follow-up time for each analysis was calculated as the time until the child outcome, maternal death, loss-to-follow-up or study closure (August 2003), whichever occurred first. Follow-up time for the HIV infection and HIV-free survival endpoints was equal to the time of the last HIV test if a child was still breastfeeding at the end of follow-up. We used generalized estimating equations (GEE) with an empirical variance estimator to model mean child CD4 T-cell counts by level of maternal anemia and hypochromic microcytosis (Fitzmaurice et al., 2004). To maintain the prospective nature of the analyses, the serial measurements of maternal anemia and hypochromic microcytosis were lagged, such that each outcome was related to the preceding maternal measurement.

Multivariate regression was used to adjust for potential baseline confounders identified from the relevant literature, including maternal age, body mass index (BMI), viral load CD4 T-cell count, maternal WHO clinical stage of disease, malaria infection, number of previous births, compliance with prenatal iron and folic supplementation, defined as the percent of scheduled study visits attended during the prenatal period, treatment arm in the vitamin study, and child's time-varying HIV infection status. Continuous covariates were modeled using restricted cubic splines to allow for non-linearity. When non-linear associations were found, the corresponding spline terms chosen through stepwise selection were included in the model. To assess the statistical significance of an interaction between the maternal exposures and child HIV status, a partial likelihood ratio test was used in the time-to-event analyses and a score test in the GEE analyses. When the interaction was significant at $P \leq 0.05$, analyses were stratified by HIV-infected and uninfected person-time.

The study was approved by the Research and Publications Committee of Muhimbili University of Health and Allied Sciences, the Ethical Committee of the National AIDS Control Program of the Tanzanian Ministry of Health, and the Institutional Review Board of the Harvard School of Public Health and is in accordance with the Helsinki Declaration of 1975, as revised in 1983. Analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). *P* values were two-sided and considered statistically significant at $P \leq 0.05$.

Results

Of the 1078 women enrolled in the supplementation trial, there were 939 live born singleton infants. The study population for this analysis included the 840 women of these 939 for whom hematological information was available and their children. The characteristics of women, overall and by levels of anemia and hypochromic microcytosis at baseline of the parent study, are presented in Table 1. Mothers had a mean (SD) age of 25 (5) years and the majority were not HIV symptomatic at baseline (85% WHO HIV disease stage 1 and 62% had CD4 T-cell counts > 350 cells/μL). At baseline, 82% of women were anemic (hemoglobin < 11 g/dL), and 44% had evidence of hypochromasia. Maternal anemia and hypochromic microcytosis were not associated with socioeconomic indicators (education, parity, or money spent on food) but were related to clinical characteristics. Women with anemia or hypochromic microcytosis at baseline were more likely to have high viral load, malaria infection, and poor nutritional status.

The median (IQR) duration of follow-up for the mortality endpoint was 58 (16, 69) months. Nearly 30% of children died during follow-up (n = 225), with a median time until child death of 9 months. Overall, we found a nearly two-fold independent increase in risk of child mortality associated with moderate maternal anemia and a two and a half-fold increase associated with severe maternal anemia (Table 2). Maternal hypochromic microcytosis similarly predicted an increased risk of child mortality. The strong, overall elevated risks of child mortality were also noted within the first and second years of life. Children's HIV infection status did not modify the association between child mortality and maternal anemia (HR for severe anemia among HIV-infected children= 2.49, 95% CI: 1.43-4.32; HR for severe anemia among HIV-uninfected children = 3.31, 95% CI: 1.56-7.03; P for interaction = 0.16) or hypochromic microcytosis (HR for severe hypochromic microcytosis among HIV-infected children= 2.47, 95% CI: 1.19-5.13; HR for severe hypochromic microcytosis among HIV-uninfected children = 2.58, 95% CI: 0.77-8.68; P for interaction = 0.17).

Information on children's HIV status was available for 808 (96%) children. A total of 247 (31%) children were infected during follow-up, with 21% infected during breastfeeding (i.e. after six weeks of age). After multivariate adjustment, maternal anemia was not associated with increased risk of total child HIV infection or with breastfeeding transmission (Table 3). Maternal anemia, but not hypochromic microcytosis, was independently associated with the combined endpoint of child death or HIV infection (HR for moderate anemia=: 1.43, 95% CI: 1.05-1.94; HR for severe anemia = 1.48, 95% CI: 0.99-2.22; P trend = 0.03).

We next examined the association of maternal anemia and hypochromic microcytosis with child CD4 T-cell counts. We found the relationship to vary by child HIV infection status (P for interaction with anemia < 0.0001, P for interaction with hypochromic microcytosis = 0.009). Among children who were HIV-uninfected, maternal anemia was associated with a lower CD4 T-cell count (Table 4). There were also lower CD4 T-cell counts with maternal hypochromic microcytosis among HIV-uninfected children, but the trend was not significant. Neither maternal anemia nor hypochromic microcytosis was predictive of CD4 T-cell counts among HIV-infected children.

Discussion

In this prospective study of children born to HIV-infected women, we found that maternal anemia and hypochromic microcytosis, an erythrocyte morphology consistent with iron deficiency, were associated with an increased risk of child mortality. Maternal anemia was also associated with lower CD4 T-cell counts among HIV-uninfected children.

Anemia has been shown to be an independent predictor of survival among HIV-infected adults (Mocroft et al., 1999, Moore et al., 1998, O'Brien et al., 2005), and previous studies (Kuhn et al., 2005, Marchant et al., 2004, Obimbo et al., 2004), including data from this cohort (Chatterjee et al., 2007), have suggested that maternal anemia during pregnancy may contribute to increased child mortality for up to two years of age. The findings of this study demonstrate that the potential risks associated with maternal anemia may extend through the postnatal period.

The literature on the role of maternal hypochromic microcytosis or iron deficiency in child survival is limited. A small number of studies from sub-Saharan Africa and recent evidence from the United States and Nepal suggest that prenatal iron supplementation may improve pregnancy outcomes and child survival (Christian et al., 2003, Christian et al., 2009, Mishra et al., 2005, Preziosi et al., 1997, Cogswell et al., 2003, Siega-Riz et al., 2006), but the potential effect of postnatal maternal iron status on child health and survival warrants further examination.

In addition to the increased risks of mortality associated with maternal anemia and hypochromic microcytosis, we found HIV-uninfected children born to women with postnatal anemia to have lower CD4 T-cell counts, compared to HIV-uninfected children born to women without postnatal anemia. The mechanisms for these associations are not clear. Severe maternal anemia has been associated with lower breast milk concentrations of iron (Kumar et al., 2008), and reductions in iron intake among children may result in reduced iron stores and impaired immunity. While the iron content of breast milk does not alone satisfy the high demand of infants, breast milk may be an important contributor to iron status in settings where typical complementary foods are low in iron. The relationship between iron deficiency and impaired cell-mediated immunity, including lower T-lymphocyte numbers, has been reviewed elsewhere (Oppenheimer, 2001, Beard, 2001). It is also possible that maternal anemia and iron deficiency are consequences of HIV infection and reflect the severity of maternal HIV disease. We adjusted for several indicators of HIV disease severity, including BMI, viral load, CD4 cell count, and WHO clinical stage, but we cannot exclude the possibility of residual confounding contributing to the observed associations. Advanced HIV disease during the postnatal period may reduce a mother's ability to care for her child, which could negatively impact a child's health through poor feeding practices or exposure to unhygienic environments. Additionally, high viral loads, co-infecting pathogens, and other nutrient deficiencies in breast milk are all common in advanced maternal HIV disease. If exposure to these factors contributes to impaired immune development in the first years of life, advanced maternal HIV disease in the postnatal period could similarly contribute to increased mortality and lower child CD4 T-cell counts.

We found the negative association between maternal anemia and child CD4 T-cell counts to be limited to HIV-uninfected children. In this cohort, Kupka et al. have shown that HIV-infected children experienced a gradual decline in CD4 T-cell counts during the first year of life, whereas HIV-exposed but uninfected children experienced a linear increase in CD4 T-cell counts over time (Kupka et al., 2009). It is possible that the risks of maternal anemia are important for the immunological development of HIV-uninfected children but introduce little additional risk to the already threatened immune systems of HIV-infected children. Lower CD4 T-cell counts may have contributed to an increased risk of childhood illness among HIV-uninfected children and likely mediate some of the increased risk of mortality observed in this study.

In this study, the association between maternal anemia and total child HIV infection (i.e. infection through all three routes: *in utero*, intrapartum and breastfeeding) was of borderline statistical significance. The magnitude of the multivariate results does, however, suggest that

maternal anemia could be associated with an increased risk of mother-to-child HIV transmission. In previous studies, anemia during pregnancy was associated with increased risk of mother-to-child transmission *in utero* and in the intrapartum period (Mehta et al., 2008, Naniche et al., 2008). It is possible that anemia is associated with increased viral shedding or viral load, both of which have been associated with increased risk of mother-to-child transmission of HIV (Fawzi et al., 2001, Garcia et al., 1999, Tuomala et al., 2003).

There are several limitations to this study. First, the interpretation of anemia and hypochromic microcytosis in defining nutritional iron status remains uncertain. Anemia can result from a variety of causes in addition to iron deficiency, including other micronutrient deficiencies (folate, vitamin B12, vitamin A), infection, blood loss, or hemoglobinopathies. At baseline, nearly one-half of anemic women showed no evidence of hypochromasia (data not shown), suggesting that causes of anemia other than iron deficiency may also be important in this population. Hypochromic microcytosis is consistent with and can be a useful indicator of iron deficiency anemia (Massawe et al., 1999), but this morphology can result from causes other than iron deficiency, including thalassemia. Second, the low sensitivity of hemoglobin and erythrocyte morphology to detect early changes in individual iron stores limited our ability to assess the full range of maternal iron deficiency as it relates to child health and survival. Studies that include other biochemical measures of iron status, such as ferritin or soluble transferrin receptor, may be warranted to further elucidate the relationship between the full range of maternal iron deficiency and child health. Third, at the time of this study, anti-retroviral treatment for HIV was not available in Tanzania, which may limit the generalizability of these findings since treatment has become more available in Dar es Salaam since 2004. Finally, pre-natal iron supplementation was provided to all women in the parent trial according to local standard of care. We have no direct data on compliance with these supplements but calculated a proxy indicator of compliance, defined as the percent of scheduled study visits attended during the prenatal period. This measure was included in multivariate analyses, but the possibility of residual confounding by prenatal supplementation cannot be excluded. Such supplementation may have reduced variation in maternal iron status and power to detect significant associations with the maternal hypochromic microcytosis exposure.

In this study of HIV-infected women and their children, we found maternal anemia measured through postnatal life strongly predicted child mortality and immune status. Further research to identify the factors contributing to the high burden of anemia in this population is necessary. The possibility of adverse effects of high iron status among HIV-infected individuals (Gordeuk et al., 2006, McDermid et al., 2007, Salmon-Ceron et al., 1995) currently cautions against the routine provision of iron supplements. If iron deficiency is found to be an important contributor to anemia in HIV-infected pregnant women, further studies including clinical trials to examine the safety and efficacy of targeted postnatal maternal iron supplementation in the context of HIV infection may be warranted. Such studies could be used to inform the expansion of programs to reduce maternal anemia during pregnancy to the postnatal period. The increasing availability of programs for the prevention of mother-to-child-transmission of HIV may provide the setting in which interventions to support maternal iron status can be implemented to improve the health and survival of children born to HIV-infected mothers.

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Key Messages

Prenatal iron supplementation may improve pregnancy outcomes, however, little is known about the importance of postnatal maternal iron status for child health and survival. We found maternal anemia measured through postnatal life strongly predicted child mortality and immune status among children born to HIV-infected women. The potential child health risks associated with maternal anemia and iron deficiency may not be limited to the prenatal period. Efforts to reduce prenatal maternal anemia and iron deficiency may be expanded to include the postpartum period.

Table 1

Maternal characteristics by levels of anemia and hypochromic microcytosis at baseline of the parent study

		Anemia[a]				Hypochromic microcytosis[a]				
	Total[b] n (%)	Absent n (%)	Moderate n (%)	Severe n (%)	*p*[c]	Absent n (%)	Mild n (%)	Moderate n (%)	Severe n (%)	*p*[c]
All	840 (100)	147 (18)	475 (57)	218 (26)		467 (56)	256 (31)	64 (8)	53 (6)	
Age (y)					0.07					0.31
< 20	103 (12)	15 (10)	57 (12)	31 (14)		58 (12)	28 (11)	10 (16)	7 (13)	
20-24	340 (41)	55 (37)	199 (42)	86 (40)		193 (41)	96 (38)	29 (45)	22 (42)	
25-29	259 (31)	53 (36)	127 (27)	79 (36)		131 (28)	91 (36)	19 (27)	18 (34)	
≥ 30	138 (16)	24 (16)	92 (19)	22 (10)		85 (18)	41 (16)	6 (9)	6 (11)	
Education					0.42					0.39
None	59 (7)	7 (5)	34 (7)	18 (8)		32 (7)	15 (6)	7 (11)	5 (9)	
Primary	694 (83)	121 (82)	399 (84)	174 (80)		386 (83)	215 (84)	48 (75)	45 (85)	
Secondary or higher	91 (10)	19 (13)	42 (9)	26 (12)		49 (11)	26 (10)	9 (14)	3 (6)	
Parity					0.20					0.66
0	265 (32)	45 (31)	144 (31)	76 (36)		149 (32)	75 (30)	24 (39)	17 (34)	
1-2	404 (49)	71 (49)	231 (50)	102 (48)		225 (49)	128 (51)	27 (44)	24 (48)	
≥ 3	153 (19)	28 (19)	92 (20)	33 (16)		87 (19)	46 (19)	11 (18)	9 (18)	
Shillings for food /day[d]					0.71					0.26
< 500	450 (60)	76 (59)	259 (61)	115 (58)		256 (61)	138 (60)	28 (54)	28 (55)	
≥ 500	305 (40)	52 (41)	169 (40)	84 (42)		164 (39)	94 (41)	24 (46)	23 (45)	
Mean (SD) gestational age at study entry (wk)	20.3 (3.4)	20.0 (3.4)	20.3 (3.3)	20.5 (3.5)	0.18	20.2 (3.4)	20.5 (3.4)	20.4 (3.5)	20.4 (3.0)	0.38
HIV clinical stage					0.34					0.16
Stage 1	711 (85)	126 (86)	404 (85)	181 (83)		403 (86)	211 (82)	54 (84)	43 (81)	
Stage 2	120 (14)	20 (14)	66 (14)	34 (16)		60 (13)	42 (16)	9 (14)	9 (17)	
Stage 3	8 (1)	1 (1)	5 (1)	2 (1)		4 (1)	2 (1)	1 (2)	1 (1)	
Stage 4	1 (0)	0 (0)	0 (0)	1 (1)		0 (0)	1 (0)	0 (0)	0 (0)	
CD4 count (cells/μL)					0.06					0.01
≤ 350	300 (38)	42 (30)	175 (39)	83 (40)		147 (33)	101 (42)	30 (49)	22 (43)	
> 350	494 (62)	96 (70)	279 (62)	119 (59)		295 (67)	139 (58)	31 (51)	29 (57)	

		Anemia[a]				Hypochromic microcytosis[a]				
	Total[b] n (%)	Absent n (%)	Moderate n (%)	Severe n (%)	P[c]	Absent n (%)	Mild n (%)	Moderate n (%)	Severe n (%)	P[c]
Viral load (copies/mL)					0.01					0.007
< 50,000	189 (51)	43 (67)	102 (48)	44 (45)		120 (57)	51 (45)	10 (37)	8 (38)	
≥ 50,000	184 (49)	21 (33)	109 (52)	54 (55)		91 (43)	63 (55)	17 (63)	13 (62)	
Malaria infection					0.03					0.01
No	674 (81)	122 (83)	389 (83)	163 (75)		390 (84)	198 (79)	45 (70)	41 (77)	
Yes	158 (19)	25 (17)	79 (17)	54 (25)		74 (16)	53 (21)	19 (30)	12 (23)	
Body mass index (kg/m^2)					0.06					0.009
< 18.5	20 (2)	4 (3)	12 (3)	4 (2)		11 (2)	6 (2)	2 (3)	1 (2)	
18.5-24.9	588 (72)	103 (71)	324 (69)	161 (77)		310 (67)	187 (75)	53 (86)	38 (78)	
25-29.9	178 (23)	28 (19)	109 (23)	41 (20)		113 (25)	49 (20)	6 (10)	10 (20)	
≥ 30	36 (4)	11 (8)	22 (5)	3 (1)		27 (6)	8 (3)	1 (2)	0 (0)	

[a] Anemia was categorized as absent: Hb≥ 11.0 g/dL; moderate: Hb 8.5 to < 11.0 g/dL; and severe: Hb < 8.5 g/dL. Hypochromic microcytosis anemia was categorized as absent: hypochromasia absent; mild: any hypochromasia without microcytosis; moderate: hypochromasia < 25% and microcytic cells observed; or severe: hypochromasia ≥ 25% and microcytic cells observed.

[b] Totals may be less than 840 due to missing values.

[c] *P* value is from the Cochran-Armitage test for trend for proportions and the Kruskal-Wallis test for continuous measures.

[d] At the time of the start of the study in 1995, the mean exchange rate was 1 USD = 575 Tanzanian shillings.

Table 2

Association of maternal anemia and hypochromic microcytosis with child mortality

	Hazard ratio (95% Confidence Interval) for child mortality				
	# Events / Y	**Unadjusted HR**	***P, trend***	**Adjusted HR[a]**	***P, trend***
Anemia[b]					
Complete follow-up[c]					
Absent	62 / 1786	1.00	<0.0001	1.00	<0.0001
Moderate	112 / 1185	2.16 (1.55, 3.01)		1.81 (1.27, 2.57)	
Severe	51 / 278	3.81 (2.54, 5.70)		2.58 (1.66, 4.01)	
0-11 months of age					
Absent	22 / 139	1.00	<0.0001	1.00	0.002
Moderate	76 / 182	3.26 (1.88, 5.65)		2.22 (1.20, 4.08)	
Severe	39 / 58	5.29 (2.88, 9.69)		2.97 (1.50, 5.87)	
12-23 months of age					
Absent	23 / 387	1.00	0.0004	1.00	0.005
Moderate	21 / 239	1.82 (1.00, 3.29)		1.57 (0.84, 2.93)	
Severe	9 / 49	4.18 (1.93, 9.06)		3.78 (1.60, 8.93)	
Hypochromic microcytosis[b]					
Complete follow-up[c]					
Absent	128 / 2397	1.00	<0.0001	1.00	0.001
Mild	62 / 607	1.78 (1.29, 2.47)		1.58 (1.12, 2.33)	
Moderate	19 / 159	2.62 (1.57, 4.37)		1.45 (0.80, 2.62)	
Severe	15 / 85	3.01 (1.69, 5.36)		2.36 (1.27, 4.38)	
0-11 months of age					
Absent	69 / 269	1.00	<0.0001	1.00	0.002
Mild	44 / 80	2.53 (1.65, 3.88)		2.02 (1.26, 3.24)	
Moderate	13 / 17	3.61 (1.88, 6.95)		3.45 (1.50, 7.93)	
Severe	11 / 13	2.64 (1.29, 5.42)		1.90 (0.83, 4.37)	
12-23 months of age					
Absent	39 / 538	1.00	0.09	1.00	0.45

	Hazard ratio (95% Confidence Interval) for child mortality				
	# Events / Y	**Unadjusted HR**	***P, trend***	**Adjusted HR[a]**	***P, trend***
Mild	9 / 101	1.27 (0.62, 2.62)		0.93 (0.41, 2.12)	
Moderate	3 / 24	1.70 (0.52, 5.50)		1.05 (0.30, 3.62)	
Severe	2 / 12	3.17(0.76, 13.18)		3.02 (0.67, 13.58)	

[a]Multivariate-adjusted hazard ratio from a proportional hazards model adjusting for maternal age (<20, 20-24, 25-29 ≥30 y), BMI at baseline (kg/m^2), viral load at baseline (copies/mm^3), CD4 at baseline (cells/mL), WHO clinical stage at baseline (1, 2-4), malaria infection at baseline, parity (0, 1-2, ≥ 3), prenatal compliance with iron and folic acid supplementation (percent of scheduled prenatal visits attended), regimen, and child HIV status (time-varying).

[b]Anemia was categorized as absent: Hb ≥ 11.0 g / dL; moderate: Hb 8.5 to < 11.0 g/dL g/ dL; and severe: Hb < 8.5 g/dL. Hypochromic microcytosis anemia was categorized as absent: hypochromasia absent; mild: any hypochromasia without microcytosis; moderate: hypochromasia < 25% and microcytic cells observed; or severe: hypochromasia ≥ 25% and microcytic cells observed.

[c]Median duration of complete follow-up for the child mortality endpoint was 63 months (inter-quartile range: 19, 71).

Table 3

Association of maternal anemia and hypochromic microcytosis with child HIV infection or death

		Hazard ratio (95% Confidence Interval)			
	# Events / Y	**Unadjusted HR**	***P, trend***	**Adjusted HR[a]**	***P, trend***
Anemia[b]					
Total HIV transmission					
Absent	58 / 627	1.00	0.0005	1.00	0.08
Moderate	134 / 424	1.76 (1.25, 2.49)		1.43 (1.00, 2.04)	
Severe	55 / 93	2.06 (1.33, 3.20)		1.46 (0.91, 2.33)	
Breastfeeding transmission					
Absent	42 / 612	1.00	0.006	1.00	0.29
Moderate	72 / 380	1.73 (1.16, 2.59)		1.29 (0.84, 1.98)	
Severe	17 / 74	1.92 (1.04, 3.55)		1.30 (0.66, 2.53)	
Total HIV transmission or death					
Absent	82 / 1284	1.00	<0.0001	1.00	0.03
Moderate	185 / 783	1.74 (1.30, 2.35)		1.43 (1.05, 1.94)	
Severe	74 / 156	2.12 (1.45, 3.11)		1.48 (0.99, 2.22)	
Hypochromic microcytosis[b]					
Total HIV transmission					
Absent	147 / 896	1.00	0.03	1.00	0.51
Mild	71 / 188	1.42 (1.03, 1.95)		1.17 (0.84, 1.62)	
Moderate or Severe	29/ 59	1.40 (0.87, 2.26)		1.07 (0.63, 1.82)	
Breastfeeding transmission					
Absent	90 / 851	1.00	0.20	1.00	0.83
Mild	33 / 165	1.49 (0.97, 2.29)		1.03 (0.64, 1.66)	
Moderate or Severe	8 / 50	1.11 (0.48, 2.54)		0.84 (0.36, 1.99)	
Total HIV transmission or death					
Absent	200 / 1686	1.00	0.006	1.00	0.26
Mild	98 / 392	1.38 (1.04, 1.83)		1.14 (0.85, 1.52)	
Moderate	24 / 101	1.51 (0.90, 2.53)		1.13 (0.63, 2.02)	
Severe	19 / 44	1.67 (0.94, 2.95)		1.32 (0.73, 2.42)	

[a] Multivariate-adjusted hazard ratio from a proportional hazards model adjusting for maternal age (<20, 20-24, 25-29 ≥30 y), BMI at baseline (kg/m^2), viral load at baseline (copies/mL), CD4 at baseline (cells/μL), WHO HIV clinical stage at baseline (1, 2-4), malaria infection at baseline, parity (0, 1-2, ≥ 3), prenatal compliance with iron and folic acid supplementation (percent of scheduled prenatal visits attended), and regimen.

[b] Anemia was categorized as absent: Hb≥ 11.0 g / dL; moderate: Hb 8.5 to < 11.0 g/ dL; and severe: Hb < 8.5 g/dL. Hypochromic microcytosis anemia was categorized as absent: hypochromasia absent; mild: any hypochromasia without microcytosis; moderate: hypochromasia < 25% and microcytic cells observed; or severe: hypochromasia ≥ 25% and microcytic cells observed.

Table 4

Association of levels of maternal anemia and hypochromic microcytosis with child CD4 T-cell count (cells/μL)

	CD4 T-cell Count (cells/μL)			
Anemia[a]	**Age Adjusted**	***P,trend***	**Multivariate Adjusted**[b]	***P,trend***
HIV-infected				
Absent, mean ± SD[c] (ref)	1209 ± 432	0.84		0.99
Moderate, mean difference	39 (-38, 116)		40 (-38, 118)	
Severe, mean difference	-59 (-196, 77)		-40 (-179, 99)	
HIV-uninfected				
Absent, mean ± SD[c] (ref)	1712 ± 565	0.01		0.02
Moderate, mean difference	-66 (-118, -14)		-61 (-114, -8)	
Severe, mean difference	-89 (-195, -16)		-93 (-204, 17)	
Hypochromic microcytosis[a]				
HIV-infected				
Absent, mean ± SD[c] (ref)	1238 ± 453	0.59		0.37
Mild, mean difference	31 (-63, 125)		39 (-58, 135)	
Moderate, mean difference	-46 (-205, 112)		-48 (-211, 115)	
Severe, mean difference	111 (-107, 330)		181 (-42, 403)	
HIV-uninfected				
Absent, mean ± SD[c] (ref)	1663 ± 541	0.14		0.19
Mild, mean difference	-12 (-81, 57)		-6 (-77, 65)	
Moderate, mean difference	-2 (-117, 112)		-2 (-123, 119)	
Severe, mean difference	-234 (-433, -34)		-232 (-422, -22)	

[a] Anemia was categorized as absent: Hb≥ 11.0 g / dL; moderate: Hb 8.5 to < 11.0 g/ dL; and severe: Hb < 8.5 g/dL. Hypochromic microcytosis anemia was categorized as absent: hypochromasia absent; mild: any hypochromasia without microcytosis; moderate: hypochromasia < 25% and microcytic cells observed; or severe: hypochromasia ≥ 25% and microcytic cells observed.

[b] Multivariate-adjusted mean difference from generalized estimating equations adjusting for maternal age (<20, 20-24, 25-29 ≥30 y), BMI at baseline (kg/m^2), viral load at baseline (copies/mL), CD4 at baseline (cells/μL), WHO HIV clinical stage at baseline (1, 2-4), malaria infection at baseline, parity (0, 1-2, ≥ 3), prenatal compliance with iron and folic acid supplementation (percent of scheduled prenatal visits attended), and regimen.

[c] Data are mean ± SD of the average measurement during follow-up for each child.