

Predictors and consequences of anaemia among antiretroviral-naïve HIV-infected and HIV-uninfected children in Tanzania

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Abstract

Objective: Predictors and consequences of childhood anaemia in settings with high HIV prevalence are not well known. The aims of the present study were to identify maternal and child predictors of anaemia among children born to HIV-infected women and to study the association between childhood anaemia and mortality.

Design: Prospective cohort study. Maternal characteristics during pregnancy and Hb measurements at 3-month intervals from birth were available for children. Information was also collected on malaria and HIV infection in the children, who were followed up for survival status until 24 months after birth.

Setting: Dar es Salaam, Tanzania.

Subjects: The study sample consisted of 829 children born to HIV-positive women.

Results: Advanced maternal clinical HIV disease (relative risk (RR) for stage ≥ 2 v. stage 1: 1.31, 95% CI 1.14, 1.51) and low CD4 cell counts during pregnancy (RR for <350 cells/mm³ v. ≥ 350 cells/mm³: 1.58, 95% CI 1.05, 2.37) were associated with increased risk of anaemia among children. Birth weight <2500 g, preterm birth (<34 weeks), malaria parasitaemia and HIV infection in the children also increased the risk of anaemia. Fe-deficiency anaemia in children was an independent predictor of mortality in the first two years of life (hazard ratio 1.99, 95% CI 1.06, 3.72).

Conclusions: Comprehensive care including highly active antiretroviral therapy to eligible HIV-infected women during pregnancy could reduce the burden of anaemia in children. Programmes for the prevention of mother-to-child transmission of HIV and antimalarial treatment to children could improve child survival in settings with high HIV prevalence.

Keywords
HIV
Malaria
Anaemia
Child mortality
Sub-Saharan Africa

Anaemia is a major public health problem in most developing countries. Globally, anaemia is present in approximately 43% of children under the age of 4 years⁽¹⁾ with close to 75% of children under 5 years of age suffering from anaemia in East Africa⁽²⁾. Studies from sub-Saharan Africa have reported anaemia prevalence ranging from 42% to 75% among HIV-infected children⁽³⁾. Fe-deficiency anaemia is the commonest form of anaemia, and is estimated to constitute approximately 50% of the total burden of anaemia albeit with wide geographic variation^(4–6). It is a risk factor for delayed psychomotor development and cognitive function and also impairs

cell-mediated immunity^(7–10). Because of the long-term effects of Fe-deficiency anaemia during infancy it is important to prevent and treat it appropriately as early in infancy as possible. Little is known about the association between maternal factors during pregnancy among HIV-infected women and the development of anaemia in their children. Maternal factors that are treatable or preventable could be potential targets for public health interventions aimed at reducing the burden of anaemia in children.

Among HIV-infected adults, anaemia is known to be a risk factor for mortality in studies from both developed and developing countries^(11,12). Little is known about the association between anaemia and mortality in HIV-infected and uninfected children born to HIV-infected women in sub-Saharan Africa. We examined the associations between maternal and child factors and development

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of anaemia in children until 24 months of age. We also studied the relationship between anaemia in HIV-infected and uninfected children born to HIV-infected women and child mortality in the first two years of life.

Experimental methods

The current prospective study was conducted in Dar es Salaam, Tanzania within the setting of a randomized trial of vitamin supplementation of HIV-infected women during pregnancy and lactation. Details of the trial have been published elsewhere⁽¹³⁾. HIV-infected women were between 12 and 27 weeks of gestation (mean 20 (SD 3) weeks) and, after giving informed consent, were randomized in a double-blind factorial design to one of four arms: vitamin A, vitamin A and multivitamins, multivitamins excluding vitamin A or placebo. All women received standard antenatal care including daily supplementation with Fe and folic acid as well as weekly doses of chloroquine phosphate as per national guidelines in Tanzania. Women in the vitamin A arm received another high dose of vitamin A at delivery while others received a placebo. Antiretroviral therapy was not yet widely available in Tanzania during the period when the study was conducted and none of the mothers or children received antiretroviral therapy during the course of the study.

At the time of enrolment detailed background information including educational levels were collected from all women using standardized questionnaires administered by trained research nurses, who also measured the women's weight, height and left mid upper-arm circumference using calibrated instruments and standardized techniques⁽¹⁴⁾. Detailed medical information was collected and a complete physical examination including vaginal examination was conducted by trained physicians. The women provided blood samples for determination of Hb, CD4 T-lymphocyte count and infection with *Plasmodium falciparum* malaria, as well stool samples for intestinal parasitic infestation. Birth weight was measured to the nearest 10 g using a standard beam balance (model 725; Seca, Hamburg, Germany) immediately after birth and gestational age was evaluated on the basis of maternal recall of last menstrual period. Children born to women enrolled in the trial were followed from birth through monthly visits to the study clinics; home visits were made in cases where a scheduled clinic visit was missed. The children provided blood samples at birth and every 3 months thereafter for determination of Hb and erythrocyte morphology as well as infection with *P. falciparum* malaria. CD4 T-lymphocyte counts were determined at birth and every 6 months thereafter, while HIV infection status was determined at birth, at 6 weeks and then at 3-month intervals.

Hb was measured using a CBC5 Coulter counter (Coulter Corporation, Miami, FL, USA). However, due to machine breakdown, the method was changed to the

cyanmethaemoglobin method using a colorimeter (Corning, Corning, NY, USA) in the later part of the study. Erythrocyte morphology was examined in thin blood films stained with Leishman's stain. For the analysis reported in the present paper, we considered the erythrocyte morphology to be microcytic and hypochromic if >75% of the cells examined showed microcytosis and hypochromasia. Infection with *P. falciparum* malaria was identified using Giemsa-stained thick and thin blood films. Parasite density was estimated by counting the number of parasites in 300 leucocytes and assuming a total leucocyte count of 8000/mm³ blood. Measurements of absolute counts of CD4 T cells were done using the FACScan and FACScout system (Beckton-Dickinson, San Jose, CA, USA). HIV-1 infection was diagnosed in the children on the basis of a positive peripheral mononuclear cell specimen by PCR before 18 months of age (using the Amplicor HIV-1 detection kit; Roche Diagnostics, Branchburg, NJ, USA) or a positive ELISA confirmed by a Western blot at or after 18 months of age. The time of HIV infection was designated as the midpoint between the last negative and the first positive HIV test. To the 1078 HIV-infected women enrolled in the trial, 984 live-born children were delivered. The study population consisted of 829 singleton children who had at least one Hb measurement available. There was no difference in the background characteristics of the 829 live-born children who were included in the analysis and those who were not included due to non-availability of at least one Hb measurement. For the present analysis child anaemia was defined in two different ways: anaemia (Hb < 8.5 g/dl) and anaemia suggestive of Fe deficiency (Hb < 8.5 g/dl and hypochromic microcytic erythrocyte morphology with >75% cells showing hypochromasia and microcytosis).

Generalized estimating equations (GEE; PROC GENMOD in the SAS statistical software package version 9.1 (SAS Institute, Cary, NC, USA)) were used to examine the association between maternal and infant factors and child anaemia and child anaemia suggestive of Fe deficiency separately⁽¹⁵⁾. A GEE model with exchangeable working covariance structure was used and a binomial distribution was specified. All covariates with $P \leq 0.20$ in the univariate models were entered into a multivariate model. Cox proportional hazards models (PROC PHREG in SAS) were fit to examine the association between child anaemia and mortality until 24 months of age. Child anaemia was defined in the same manner as for the analysis above and entered into univariate and multivariate models as a time-dependent covariate. The missing indicator method was used for covariates with missing data⁽¹⁶⁾.

The study protocol was approved by the Research and Publications Committee of Muhimbili University College of Health 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.

Results

A total of 3351 Hb measurements were available from 829 children over the 24 months of follow-up for an average of four measurements per child. The mean Hb level over all measurement occasions was 9.99 g/dl and the median was

9.70 g/d (25th, 75th percentile: 8.6, 11.2 g/dl). Twenty-three per cent of the Hb measurements were below 8.5 g/dl.

Maternal WHO HIV clinical disease stage ≥ 2 during pregnancy as compared with stage 1 was significantly associated with increased risk of anaemia in children up to 24 months of age (relative risk (RR) 1.31; 95% CI 1.14, 1.51; Table 1).

Table 1 Maternal and child factors in relation to anaemia (Hb < 8.5 g/dl) among children born to HIV-infected women, Dar es Salaam, Tanzania

	Children at risk		Anaemia rate (/child-year)	Univariate relative risk*	95% CI	P value*	Adjusted relative risk†	95% CI	P value†
	n	%							
Maternal predictors									
HIV disease stage									
≥ 2	158	19	1.15	1.33	1.15, 1.54	0.0001	1.31	1.14, 1.51	0.0001
1	666	81	0.87	1.00			1.00		
CD4 cell count (/mm³)									
<350	293	38	0.96	1.06	0.92, 1.23	0.43	–	–	–
≥ 350	488	62	0.90	1.00					
Malaria parasite density (/mm³)									
≥ 1000	111	14	1.07	1.19	0.98, 1.43	0.08	1.22	1.04, 1.43	0.02
1–999	46	6	1.06	1.16	0.88, 1.52	0.30	1.07	0.84, 1.36	0.59
None	663	80	0.90	1.00			1.00		
Hb (g/dl)									
<8.5	222	27	0.93	1.00	0.85, 1.16	0.95	–	–	–
≥ 8.5	595	73	0.93	1.00					
Mid upper-arm circumference (cm)									
<22	22	6	1.14	1.36	1.05, 1.76	0.02	1.23	0.96, 1.57	0.10
≥ 22	779	94	0.90	1.00			1.00		
Weight (kg)									
<50	166	20	0.87	0.94	0.77, 1.14	0.51	–	–	–
≥ 50	645	80	0.94	1.00					
Ascaris infestation									
Yes	41	6	1.06	1.20	0.95, 1.52	0.12	1.15	0.94, 1.42	0.18
No	654	94	0.88	1.00			1.00		
Abnormal vaginal discharge									
Yes	106	13	1.03	1.15	0.95, 1.40	0.16	1.12	0.93, 1.35	0.25
No	712	87	0.91	1.00			1.00		
Age (years)									
>30	141	17	0.94	0.85	0.66, 1.10	0.22	0.94	0.73, 1.21	0.61
25–29	263	32	1.01	0.92	0.73, 1.15	0.44	0.97	0.78, 1.21	0.79
20–24	328	40	0.80	0.73	0.58, 0.92	0.007	0.74	0.60, 0.92	0.01
<20	97	11	1.10	1.00			1.00		
Education									
No education	62	7	1.12	1.20	0.91, 1.58	0.19	1.16	0.90, 1.49	0.26
Some education	767	93	0.92	1.00			1.00		
Child predictors									
Gestational age at birth (weeks)‡									
<34	60	7	1.32	1.46	1.18, 1.80	0.0004	1.24	1.02, 1.50	0.03
≥ 34	769	93	0.90	1.00			1.00		
Birth weight (g)									
<2500	78	10	1.25	1.39	1.12, 1.74	0.003	1.26	1.04, 1.54	0.02
≥ 2500	693	90	0.88	1.00			1.00		
Child-months at risk									
HIV-infected§									
Yes	1737		1.37	1.70	1.47, 1.97	<0.0001	1.61	1.40, 1.85	<0.0001
No	8316		0.84	1.00			1.00		
Malaria§									
Yes	681		1.34	1.52	1.25, 1.84	<0.0001	1.52	1.25, 1.85	<0.0001
No	9372		0.90	1.00			1.00		

*From separate univariate models using generalized estimating equations with a binomial distribution and a log link.

†From separate multivariate models using generalized estimating equations with a binomial distribution and a log link. The multivariate model included all the variables in the column except gestational age at birth and the following additional variables: maternal trial regimen, daily per capita amount of money spent on food and age child started semi-solid foods.

‡In an additional model gestational age at birth replaced birth weight.

§HIV infection and malaria were included as time-dependent covariates.

The risk of developing anaemia in the children was increased if their mothers had a malaria parasite density $\geq 1000/\text{mm}^3$ during pregnancy compared with no parasites (RR 1.22; 95% CI 1.04, 1.43). HIV infection in the child and malaria in the child were also independently associated with increased risk of anaemia (HIV infection: RR 1.61; 95% CI 1.40, 1.85 and malaria: RR 1.52; 95% CI 1.25, 1.85).

We also examined anaemia suggestive of Fe deficiency in the children based on Hb and erythrocyte morphology and found that maternal CD4 cell count <350 cells/ mm^3 during pregnancy was associated with a significantly increased risk (RR 1.58; 95% CI 1.05, 2.37; Table 2). The risk of anaemia suggestive of Fe deficiency was also increased if the child was HIV-infected or had malaria.

Table 2 Maternal and child factors in relation to anaemia suggestive of iron deficiency among children born to HIV-infected women, Dar es Salaam, Tanzania

	Children at risk		Anaemia rate (/child-year)	Univariate relative risk*	95% CI	P value*	Adjusted relative risk†	95% CI	P value†
	n	%							
Maternal predictors									
HIV disease stage									
≥ 2	158	19	0.17	1.36	0.83, 2.21	0.22	–	–	–
1	666	81	0.13	1.00					
CD4 cell count (/mm³)									
<350	293	38	0.18	1.59	1.05, 2.41	0.03	1.58	1.05, 2.37	0.03
≥ 350	488	62	0.11	1.00			1.00		
Malaria parasite density (/mm³)									
≥ 1000	111	14	0.21	1.68	1.03, 2.76	0.04	1.41	0.88, 2.26	0.15
1–999	46	6	0.15	1.27	0.48, 3.41	0.63	0.92	0.41, 2.11	0.85
None	663	80	0.12	1.00			1.00		
Hb (g/dl)									
<8.5	222	27	0.16	1.20	0.78, 1.83	0.41	–	–	–
≥ 8.5	595	73	0.14	1.00					
Mid upper-arm circumference (cm)									
<22	22	6	0.26	2.09	1.09, 4.00	0.03	1.51	0.85, 2.67	0.16
≥ 22	779	94	0.12	1.00			1.00		
Weight (kg)									
<50	166	20	0.14	1.20	0.75, 1.91	0.44	–	–	–
≥ 50	645	80	0.13	1.00					
Ascaris infestation									
Yes	41	6	0.11	0.91	0.38, 2.14	0.82	–	–	–
No	654	94	0.12	1.00					
Abnormal vaginal discharge									
Yes	106	13	0.11	0.86	0.46, 1.60	0.63	–	–	–
No	712	87	0.14	1.00					
Age (years)									
>30	141	17	0.14	0.82	0.39, 1.71	0.59	0.93	0.51, 1.53	0.84
25–29	263	32	0.11	0.62	0.32, 1.21	0.16	0.80	0.45, 1.42	0.44
20–24	328	40	0.15	0.86	0.46, 1.61	0.64	0.88	0.47, 1.84	0.65
<20	97	11	0.17	1.00			1.00		
Education									
No education	62	7	0.22	1.67	0.82, 3.43	0.16	1.36	0.73, 2.57	0.34
Some education	767	93	0.13	1.00			1.00		
Child predictors									
Gestational age at birth (weeks)‡									
<34	60	7	0.36	2.93	1.65, 5.20	0.0003	2.03	1.11, 3.69	0.02
≥ 34	769	93	0.12	1.00			1.00		
Birth weight (g)									
<2500	78	10	0.34	3.03	1.82, 5.07	<0.0001	2.62	1.60, 4.26	0.0001
≥ 2500	693	90	0.11	1.00			1.00		
Child-months at risk									
HIV-infected§									
Yes	2316		0.27	2.53	1.69, 3.79	<0.0001	2.37	1.59, 3.55	<0.0001
No	11 088		0.11	1.00			1.00		
Malaria§									
Yes	681		0.33	2.74	1.73, 4.33	<0.0001	2.85	1.74, 4.67	<0.0001
No	9372		0.12	1.00			1.00		

*From separate univariate models using generalized estimating equations with a binomial distribution and a log link.

†From separate multivariate models using generalized estimating equations with a binomial distribution and a log link. The multivariate model included all the variables in the column except gestational age at birth and the following additional variables: maternal trial regimen, daily per capita amount of money spent on food and age child started semi-solid foods.

‡In an additional model gestational age at birth replaced birth weight.

§HIV infection and malaria were included as time-dependent covariates.

Next we examined anaemia in the children as a time-dependent predictor of child mortality to 24 months of age. There were a total of 177 deaths over 15 472 child-months of follow-up. Anaemia suggestive of Fe deficiency was associated with a statistically significant twofold increased risk of death in the total cohort (hazard ratio (HR) 1.99; 95% CI 1.06, 3.72; Table 3). Child anaemia defined as Hb < 8.5 g/dl did not show a statistically significant association with mortality overall (HR 1.38; 95% CI 0.95, 2.01). However, Hb < 8.5 g/dl was associated with a borderline statistically significant increased risk of death in the HIV-uninfected subgroup (HR 1.86; 95% CI 0.96, 3.61; *P* = 0.07; Table 4).

Discussion

We found a positive association between advanced maternal HIV disease stage during pregnancy defined

clinically as per WHO criteria and development of childhood anaemia (Hb < 8.5 g/dl). Maternal CD4 cell count < 350 cells/mm³ during pregnancy was associated with development of anaemia suggestive of Fe deficiency in children in the first two years of life. There were fewer children classified as having Fe-deficiency anaemia and the smaller number of ‘events’ in the present analysis could be a potential reason why maternal HIV disease stage did not show a statistically significant association even though the point estimate from the univariate analysis showed an increased risk. Advanced clinical and immunological maternal HIV disease during pregnancy has been shown to be associated with increased risk of mortality in children^(17–22). Advanced maternal HIV disease could interfere with the transfer of Fe that occurs from the mother to the fetus and also fetal haematopoiesis is affected by maternal HIV infection^(23–25). Placental malaria has been shown to be associated with child anaemia at 2 months⁽²⁶⁾ and 6 months of age⁽²⁷⁾ among

Table 3 Association of child anaemia with overall mortality among children born to HIV-infected women, Dar es Salaam, Tanzania

Child anaemia	Child-months at risk	No. of deaths	Hazard ratio* (univariate)	95% CI	<i>P</i> value*	Hazard ratio† (adjusted)	95% CI	<i>P</i> value†
Hb (g/dl)								
<8.5	3590	43	1.30	0.91, 1.86	0.16	1.38	0.95, 2.01	0.10
≥8.5	11 882	134	1.00			1.00		
Hb < 8.5 g/dl and hypochromic microcytosis								
Yes	548	12	2.34	1.29, 4.23	0.005	1.99	1.06, 3.72	0.03
No	14 924	165	1.00			1.00		

*From separate univariate Cox proportional hazards models with anaemia as a time-dependent covariate.
 †From separate multivariate Cox proportional hazards models with anaemia as a time-dependent covariate. The multivariate models also included the following adjusting variables, with indicator variables for individuals missing data on a particular variable: maternal trial regimen, CD4 cell count during pregnancy, education, daily per capita amount of money spent on food and the child variables of birth weight, age started semi-solid foods, time-varying CD4 cell counts, malaria parasitaemia and HIV infection status.

Table 4 Association of child anaemia with mortality stratified by HIV status among children born to HIV-infected women, Dar es Salaam, Tanzania

Child anaemia	Child-months at risk	No. of deaths	Hazard ratio* (univariate)	95% CI	<i>P</i> value*	Hazard ratio† (adjusted)	95% CI	<i>P</i> value†
HIV-infected								
Hb (g/dl)								
<8.5	939	29	0.93	0.60, 1.44	0.75	0.97	0.61, 1.54	0.91
≥8.5	2466	85	1.00			1.00		
Hb < 8.5 g/dl and hypochromic microcytosis								
Yes	150	9	2.00	1.00, 4.00	0.05	2.06	0.94, 4.54	0.07
No	3254	105	1.00			1.00		
HIV-uninfected								
Hb (g/dl)								
<8.5	2652	14	1.64	0.86, 3.12	0.13	1.86	0.96, 3.61	0.07
≥8.5	9416	49	1.00			1.00		
Hb < 8.5 g/dl and hypochromic microcytosis								
Yes	398	3	2.01	0.62, 6.48	0.24	1.42	0.43, 4.69	0.56
No	11 669	60	1.00			1.00		

*From separate univariate Cox proportional hazards models with anaemia as a time-dependent covariate.
 †From separate multivariate Cox proportional hazards models with anaemia as a time-dependent covariate. The multivariate models also included the following adjusting variables, with indicator variables for individuals missing data on a particular variable: maternal trial regimen, CD4 cell count during pregnancy, education, daily per capita amount of money spent on food and the child variables of birth weight, age started semi-solid foods, time-varying CD4 cell counts and malaria parasitaemia.

children born to HIV-uninfected mothers. Our study extends these findings by demonstrating that the increased risk of child anaemia due to maternal malaria during pregnancy may extend into the second year of life among children born to HIV-infected women.

These results are in agreement with earlier smaller studies from sub-Saharan Africa, which reported a high prevalence of anaemia among children with HIV infection who were not on antiretroviral therapy^(28–31) and noted HIV infection to be associated with a decrease in Hb levels over time⁽³²⁾. HIV infection in children could cause anaemia by interfering with haematopoiesis or absorption of Fe and the presence of opportunistic infections⁽³³⁾.

We noted a positive association between anaemia in the children and mortality over the period of follow-up. However, only anaemia suggestive of Fe deficiency was statistically significantly related to mortality. In Uganda, antiretroviral-naïve HIV-infected children with anaemia (Hb < 9 g/dl) at 9 months had a 59% increased risk of death by 36 months of age⁽³⁴⁾ and anaemia was also a predictor of disease progression among HIV-infected children in another study⁽³⁵⁾. A recently published meta-analysis has shown an association between Hb levels and mortality among antiretroviral-naïve HIV-infected children⁽³⁶⁾. The children in our study were of a younger age group than those in the two studies mentioned. The study from Uganda reported the association between one measure of Hb and mortality whereas our study captured Hb levels over a longer period of time by using multiple measurements available for each child from birth to 2 years of age. Two separate Fe and folic acid supplementation trials were carried out among children aged 1–36 months born to women who were presumably HIV-negative^(37,38). In the trial from Pemba⁽³⁷⁾, adverse events due to malaria-related causes and infection-related causes were increased in the children who received Fe and folic acid supplementation compared with those who received placebo. In the trial from Nepal there was no effect of supplementation on mortality⁽³⁸⁾. The children enrolled in these studies are different from those studied in the present study, which only included children born to HIV-infected women. Moreover, our results are in agreement with those reported among the subgroup of children who had Fe deficiency and anaemia in the Pemba trial, which demonstrated a protective effect of Fe and folic acid supplementation against serious adverse events in that subgroup of children⁽³⁷⁾. Also, in the Nepal trial, there were statistically non-significant reductions in respiratory and diarrhoea morbidity in the group that received Fe and folic acid as compared with placebo⁽³⁸⁾.

There are some potential limitations in our study methodology. Gestational age was determined using maternal recall of last menstrual period, which might not be totally accurate. Due to logistical reasons Hb measurements were done using two different methods – using CBC5 Coulter counter and colorimeter. However,

both these methods give comparable results⁽³⁹⁾. Biochemical measurements such as serum ferritin would have given a more accurate diagnosis of Fe-deficiency anaemia; however, erythrocyte morphology is also used to make a clinical diagnosis of Fe-deficiency anaemia in the absence of more definitive biochemical measurements⁽⁴⁰⁾. We did not have detailed information on the diet of the children and the role it may have played in their development of anaemia. However, we controlled for quality of diet using as a proxy indicator, the daily per capita amount of money spent on food.

The results from the present study have important public health implications for countries with a high prevalence of HIV infection and a high burden of anaemia among children. Childhood anaemia is known to be associated with impaired psychomotor and cognitive development^(7,8). The results from our study show that anaemia among children born to HIV-infected women, especially when related to Fe deficiency, is also associated with increased risk of mortality. HIV-exposed children receiving follow-up care within programmes for prevention of mother-to-child transmission of HIV and paediatric AIDS should receive a comprehensive package of child survival interventions including screening and treatment for anaemia. Assessment of HIV-positive pregnant women for antiretroviral therapy for their own health, including clinical staging of HIV disease and CD4 cell counts, should be scaled up on a priority basis; only about 12% of HIV-positive pregnant women received this assessment in 2007⁽⁴¹⁾. These interventions, when implemented at scale, can have a significant impact on child survival and development in sub-Saharan Africa.

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