

MALARIA AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AS RISK FACTORS FOR ANEMIA IN INFANTS IN KISUMU, WESTERN KENYA

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Abstract. The role of maternal and pediatric infection with human immunodeficiency virus type 1 (HIV-1) and malaria as risk factors for anemia was determined in a birth cohort of infants born to mothers participating in a study of the interaction between placental malaria and HIV infection, in Kisumu, Kenya. Between June 1996 and April 2000, 661 infants born to 467 HIV-seropositive and 194 HIV-seronegative mothers were monitored monthly from birth. At each visit a questionnaire was completed and a blood sample was collected for the determination of hemoglobin levels and detection of malaria and HIV. Anemia was common and increased from 13.6% at one month to 75% at six months and remained high throughout the second half of infancy. Placental malaria, infant malaria, and HIV infection of the infant were all associated with infant anemia in a multivariate model, adjusting for other co-variables found to be associated with infant anemia. The HIV-infected infants with malaria parasitemia had lower mean hemoglobin levels compared with HIV-uninfected infants, or HIV-infected infants without malaria, suggesting that HIV-infected infants are particularly vulnerable to the adverse consequences of malaria at this age. Early detection and prompt treatment of infant malaria and treatment of anemia as part of the study protocol failed to prevent most of the infants from becoming anemic. Although not proven effective in this study, micronutrient supplementation should be prospectively assessed in HIV-infected infants as a means of preventing anemia.

INTRODUCTION

Three-fourths of the preschool children in sub-Saharan Africa have anemia and the consequences are considerable.¹ Malaria-associated anemia is a major contributor to morbidity and mortality.^{2,3} Iron-deficiency anemia in infants has been associated with an impaired mental and physical development and alteration in the immune system.^{4–7} Children with severe anemia are at an increased risk of acquiring human immunodeficiency virus (HIV) because of the blood transfusions they may need.^{8,9} Nutritional deficiencies (iron, folic acid, and zinc), infectious diseases (including tuberculosis, hookworm infection, malaria, and schistosomiasis) and genetic red blood cell disorders (e.g., thalassemia, sickle cell disease) contribute to anemia, and interactions between causes have been described.¹⁰

Anemia has been reported as the most common hematologic consequence of HIV infection in children in the western hemisphere and has also been associated with HIV infection in developing countries.^{11–13} Significant differences were reported in mean hematocrit between HIV-infected and HIV-uninfected children in hospital-based, cross-sectional studies in Zaire.^{9,14} Results from longitudinal studies in Africa are lacking.

During the past two decades HIV/acquired immunodeficiency syndrome (HIV/AIDS) has emerged as a major problem in malaria-endemic areas. Malaria is a well known and important cause of anemia in infants in malaria-endemic areas. The prevalence of malaria increases with age in the first year of life, and is lowest in the first months when the transfer of maternal antibodies before delivery and the presence of fetal hemoglobin provide some protection against malaria infection, high-density parasitemia, and clinical malaria.¹⁵ However, in the second half of the first year of life, most maternal antibodies and fetal hemoglobin have disappeared, and infants are frequently reported to have the greatest burden of anemia and malaria.^{3,16,17} Little is known about the addi-

tional effect of malaria parasitemia in HIV-infected infants on anemia.

Adverse effects of malaria can start *in utero*, when placental malaria can lead to low birth weight, particularly in primigravidae.¹⁸ Iron stores are correlated with size at birth, and low birth weight has been associated with an earlier depletion of iron stores in the newborn and early development of iron-deficiency anemia.¹⁹ Studies in Malawi and Cameroon showed an association between placental malaria and anemia in infants at the age of two months and six months, respectively, and in Cameroon this was independent of low birth weight.^{17,20} This finding needs to be confirmed and gains importance with the increased risk for HIV-seropositive pregnant women to develop *Plasmodium falciparum* parasitemia and placental malaria, particularly in multigravidae.^{21–23}

We assessed the effect of HIV infection and placental malaria in the mother and HIV infection and malaria in the infant on anemia in the first year of life, using longitudinal data from a cohort study in an urban and periurban area in Kenya, which is endemic for both diseases.

MATERIALS AND METHODS

Study site, enrollment, and study population. This study was conducted at the Nyanza Provincial General Hospital in Kisumu town. Kisumu is located on the shores of Lake Victoria in western Kenya with a population of approximately 300,000. Malaria transmission is perennial and *P. falciparum* accounts for 98% of the malaria cases. Chloroquine resistance is widespread; 75% RII/RIII resistance of *P. falciparum* infections to chloroquine was reported in 1990.²⁴ Nyanza Provincial General Hospital is a 400-bed government referral hospital, providing health care mostly to the low-income population in this area.

Healthy pregnant women visiting the prenatal clinic of this hospital with an uncomplicated single pregnancy of 32 or more weeks and residing in the Kisumu area were invited to

participate in a study of the interaction between HIV and placental malaria. After informed consent was obtained, a questionnaire was completed to collect information on age, place of residence, education, socioeconomic status, and medical history. Participants were counseled about HIV infection, and blood was obtained by finger prick for HIV testing, hemoglobin, and a malaria smear. An appointment was made for post-test counseling and all women, irrespective of their HIV test result, were encouraged to deliver in the hospital. After delivery in the hospital, blood was again obtained from the mother by finger stick for a malaria smear and hemoglobin measurement. A placental blood smear was also collected. Gestational age of the newborn was assessed using a standardized modified Dubowitz scoring system.²⁵ The HIV-seropositive women with and without placental malaria were invited to visit the study clinic with their newborn every four weeks until the child was two years old. To avoid having study participants stigmatized as being HIV infected, and for comparison, 129 randomly selected HIV-negative women with placental malaria and 110 HIV-negative women without placental malaria were enrolled over the course of the study. In addition to recruitment in the prenatal clinic, eligible women could also be recruited directly from the labor ward. For these participants, blood smears and hemoglobin levels in the third trimester were not available. Hemoglobin of the newborn or cord blood was not measured at delivery. Blood for CD4 and CD8 counts was obtained from the enrolled mothers at one-month postpartum.

During the monthly visits to the study clinic, information was collected on the health status of the infant during the past weeks using a standardized questionnaire. The infant was weighed (scale measuring to the nearest 0.1 kg) and the axillary body temperature was measured with a digital thermometer. Infant blood was obtained by finger prick for hemoglobin, a malaria smear, and detection of HIV DNA by the polymerase chain reaction (PCR). Infants with a positive malaria blood smear, regardless of symptoms and parasite density, were treated with sulfadoxine-pyrimethamine. Hematinics (ferrous sulfate/vitamin B complex syrup, 6 mg of elemental iron/kg/day, assuming 6 mg of elemental iron in 30 mg of ferrous sulfate) were prescribed for infants with a hemoglobin level < 9 g/dL; for logistic reasons (e.g., the number of bottles that had to be prepared and carried) an initial supply enough for two weeks was provided, and women were informed to return when this was finished.²⁶ Infants who did not return for their routine follow-up appointments were visited at home.

This study was carried out in a changing environment of perinatal HIV and malaria prevention. The study protocol was designed in 1994–1995, approved by the institutional review boards of the Kenya Medical Research Institute (Nairobi, Kenya), the Centers for Disease Control and Prevention (CDC) (Atlanta, GA), and the Academic Medical Center of the University of Amsterdam (Amsterdam, The Netherlands) in 1995, and started in 1996. The study protocol was reviewed yearly in Kenya and at the CDC. Studies in 1998 and 1999 have shown the benefit of short courses of anti-retroviral drugs in preventing perinatal transmission of HIV. During this review process, the Kenya Ministry of Health repeatedly determined that in the absence of national consensus and policy, it was inappropriate to introduce provision of services outside of the research questions being addressed (e.g., azi-

dothymidine for pregnant women) that were not standard of care and were not available to women outside of this study; Internal Review Board officials at participating institutions accepted this approach.

Laboratory procedures. Blood smears were stained with Giemsa and examined under oil immersion for malaria parasites. A thick smear was considered negative if 100 microscopic fields revealed no parasites. For positive smears, malaria parasites were counted against 300 leukocytes and parasite densities were estimated using an assumed count of 8,000 white blood cells per microliter of blood. Placental smears were counted the same way. Hemoglobin was measured using a HemoCue[®] machine (HemoCue AB, Angelholm, Sweden). The HIV testing of pregnant women in the prenatal clinic involved the use of two rapid test methods: SeroStrip HIV-1/2 (Saliva Diagnostic Systems, Pte. Ltd., Singapore) and Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Wicklow, Ireland). The HIV-1 testing of the infants was done by a nested PCR.²⁷ All PCRs were done in duplicate, and both negative and positive controls were included in each experiment. The PCR tests of PCR-negative infants were repeated at 12-week intervals. For infants who seroconverted the PCR was done retrospectively at monthly intervals to determine the month of the first positive PCR test result. The CD4 and CD8 counts were determined in peripheral blood by means of a standard flow cytometry protocol, with reagents obtained from Becton-Dickinson (San Jose, CA).²⁸

Definitions. For infants 24 weeks of age and older, anemia was defined as a hemoglobin concentration less than 11 g/dL.^{29,30} Because of the physiologic decrease in hemoglobin immediately after birth, for infants less than 24 weeks old an age-dependent definition was used: anemia was defined as a hemoglobin concentration more than two standard deviations below the mean of similarly aged infants from a reference population not exposed to malaria in the United States.³¹ A reference value for the age of five months was extrapolated by taking the midpoint from the reference values available for the ages of four and six months. Using these reference criteria, anemia at 4, 8, 12, 16, and 20 weeks of age was defined as, respectively, a hemoglobin concentration less than 10.7 g/dL, 9.4 g/dL, 9.5 g/dL, 10.3 g/dL, and 10.7 g/dL. Moderate anemia for infants was defined as a hemoglobin concentration between 9 and 7 g/dL and severe anemia as a hemoglobin concentration less than 7 g/dL.

A woman with a negative test result on screening with the SeroStrip HIV-1/2 was considered HIV seronegative. When both rapid test results for antibodies to HIV were positive a woman was considered HIV seropositive. In case of inconclusive rapid tests results, a positive Western blot test result indicated that the woman was HIV seropositive. The sequential rapid test algorithm was assessed previously by confirmation with Western blot; the sensitivity, specificity, positive predictive value, and negative predictive value for the combined two rapid tests were 99.8%, 98.9%, 99.8% and 98.9%, respectively (Steketee RW and others, unpublished data). An infant was defined as HIV-1 infected if the PCR test result was positive in two consecutive blood samples and HIV-1 uninfected if two consecutive samples were HIV negative and if the last three monthly blood sample that was obtained was also HIV negative. Malaria was defined as the presence of asexual stage parasites in thick blood smears, independent of

the presence or absence of clinical signs or symptoms. An axillary temperature of 37.5°C or higher at the time of the visit was considered documented fever.

An uncomplicated pregnancy was defined as a pregnancy without evidence of multiple gestation, hypertension, pre-eclampsia, polyhydramnios, an abnormal presentation of the fetus, a history of caesarian section, hemorrhage, or repeated miscarriages (> 2). Anemia in pregnant women was defined as a hemoglobin concentration < 11 g/dL and severe anemia as a hemoglobin concentration < 7 g/dL. The absence of electricity in the house was used as an indicator of low socioeconomic status.³² A premature baby was defined as a baby with a gestational age less than 37 weeks as assessed by the modified Dubowitz score.²⁵ A low birth weight baby was defined as a baby with a birth weight less than 2,500 grams. Small for gestational age (SGA) was defined as a sex-specific birth weight at or below the 10th percentile weight-for-gestational age of a reference population in the United States.³³ Rainy season included the months of April, May, and June (long rains) and October and November (short rains).

Analysis and statistical methods. For the analysis of anemia in infants, only mother-infant pairs with known HIV status were included. Sibling-infants (the second infant of mothers who participated with two infants [no twins] in the study) were excluded from the analysis. Differences in means were compared using the Student's *t*-test and differences in proportions were analyzed using chi-square tests.

For the analysis of the repeated measurements of hemoglobin, generalized estimating equations with Poisson regression and an autoregressive correlation structure were used. This allowed for the correlation that exists between repeated observations over time in the same person. Time-stationary and time-varying covariates of interest were included to estimate the strength of the association between risk factors and anemia over time. Each characteristic was first introduced in the model to calculate a univariate risk ratio (RR). For the final multivariate model, maternal and infant characteristics were included if an association had been reported in the literature or if they were significant in the univariate analysis, unless the use of the factor would give a substantial reduction in sample size that could distort the results (e.g., maternal hemoglobin level in the third trimester, infant blood smear result at the previous monthly visit). All HIV-infected infants were born to HIV-seropositive mothers, and the effect of HIV infection of mother or infant was assessed by the creation of a variable indicating either an HIV-infected infant of an HIV-seropositive mother, an HIV-uninfected infant of an HIV-seropositive mother, or an HIV-uninfected infant of an HIV-seronegative mother. Factors were removed according to their multivariate *P* value (*P* > 0.05). Gravity, although a biologic risk factor for placental malaria, was not significantly associated with infant anemia in univariate and multivariate analyses, either in a model with or without age, and no confounding was seen with placental malaria. For this reason, it was not included in the final model. Because of overlap between low birth weight and prematurity or SGA, only the factors prematurity and SGA were included in the model. To adjust for the difference in risk of anemia in the first half and the second half of infancy, an age factor was introduced, indicating either a visit prior to 24 weeks or a visit from age 24 to 52 weeks of life. Because of the interactions that were detected between age group and the factors examined (*P* <

0.05 for the interaction term), the results were presented stratified by age group. Factors only significant in one model were kept in both models to allow comparison of the RR between the two age groups.

The statistical programs SPSS (SPSS for Windows 9.0; SPSS, Inc., Chicago, IL) and SAS (SAS system for Windows 6.12; SAS, Inc., Cary, NC) were used for analyses. For all statistical tests, a two-sided *P* value < 0.05 was considered significant.

RESULTS

Characteristics of the study population. Between June 1996 and April 2000, 1,041 infant-mother pairs were enrolled in the study, and 661 infants (63.5%) participated in the analysis (Figure 1). For 351 excluded infants, the HIV status was not known; of these, 197 (56.1%) failed to make a clinic visit and 72 (20.5%) made only one visit; the HIV status could not be determined for these infants. Home visits to those who did not return for follow-up visits (269) identified 24 infant deaths in the first three months of life (8.9%). For an additional 82 infants, the HIV status was not yet known at the time of analysis. Nine sibling-infants were excluded. Compared with the 661 infants included in the analysis, the mothers of the 360 excluded infants were more likely to be HIV seropositive and married, but they were similar in other characteristics (gravity, age, Luo tribe, socioeconomic status, years of education, peri-urban or urban residence, low birth weight and prematurity). The proportions of boys was significantly higher among the excluded infants than among the 661 infants with complete data.

Fifty-four percent of the infants made visits up to 12 months of age and 57 infants (8.6%) died in the first year of life. An additional 59 infants had not finished one year of follow-up at the time of analysis. Together with the 545 infants who were alive when they left the study, they were expected to make 7,310 visits; 5,560 visits (76.1%) were actually made. The cumulative prevalence of HIV in the first year of life in infants born to HIV-seropositive mothers was 22.5% (105 HIV-infected infants of 467 HIV-seropositive mothers); 80 HIV-infected infants (76.2%) had their first positive PCR test result at ≤ three months and 98 (93.3%) had their first positive PCR result at ≤ six months.

Characteristics of the study population are shown in Table 1. The mean ± SD age of the mothers was 22.0 ± 4.6 years and the mean ± SD birth weight was 3,161 ± 431 grams. The malaria blood smear result was positive in 13.6% of the visits where a smear was available (753 of 5,543) and 370 infants (56.0% of the study population) became positive at least once during follow-up. The prevalence of infant malaria in this peri-urban population was low: 10.0% at 12 weeks and 15.3% at 24 weeks. The highest prevalence was at 44 weeks (22.3%). A parasite density ≥ 5,000/μL was reported in 123 visits (2.2%). In 98.8% of the infections, *P. falciparum* was reported as the only species present. A history of clinical malaria in the weeks between the routine visits, which was treated either in the study clinic (67.5%) or elsewhere, was obtained in 419 visits (7.5%) by 286 infants (43.3% of the study population). Hematinics were prescribed for 216 (32.7%) infants at 6.1% of the visits; antimalarials were prescribed for 428 (64.8%) infants at 16.1% of the clinic visits.

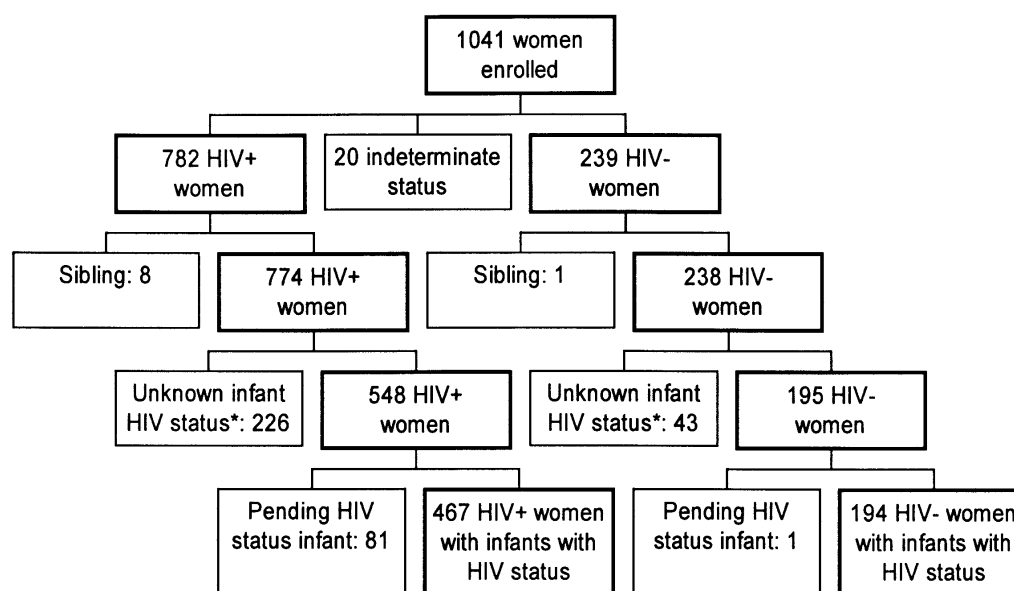


FIGURE 1. Flow chart of inclusion and exclusion of infants from data for analysis. *Unknown human immunodeficiency virus (HIV) status of the infant because the mother/infant pair never visited or made one visit only.

Hemoglobin level and prevalence of anemia. Mean hemoglobin levels were within the normal range for the first 12 weeks of life (Figure 2). However, mean hemoglobin levels continued to decrease until 32 weeks of age when they reached a nadir (9.9 g/dL). Anemia was common in the second half of infancy, observed in 78.6% of the visits (Figure 3). Severe anemia was not common and was recorded in only 59 infants (8.9%) during 79 visits (1.4%). The highest prevalence of severe anemia was 3.4% at nine months. A hemoglobin level less than 5 g/dL was observed in only four infants.

The HIV-infected infants had a lower mean hemoglobin level throughout the first year of life than HIV-uninfected infants (Figure 2) and significantly more anemia compared with HIV-uninfected infants (4–23 weeks: 33.6% versus 26.6%, respectively, RR = 1.27, 95% confidence interval [CI] = 1.09–1.47, and 24–52 weeks 88.5% versus 76.3%, RR = 1.16, 95% CI = 1.11–1.21). The highest prevalence of anemia in HIV-infected infants was seen at 48 weeks (95.7%), and HIV-infected infants were in particular more likely to have a hemoglobin level between 7 and 8.9 g/dL compared with HIV-uninfected infants (Figure 3).

Risk factors for anemia. Maternal risk factors for anemia in infancy in the univariate analysis included HIV infection, placental malaria, a hemoglobin level < 7 g/dL in the third trimester, and low socioeconomic status. Age of the mother, but not gravidity, was associated with anemia in infants (Table 2). Significant infant risk factors included male gender, low birth weight, HIV infection, and all characteristics that could indicate a present or past malaria infection (e.g., a history of treatment of malaria in the previous month, a history of fever, splenomegaly, documented fever, malaria parasitemia). The association between maternal HIV infection and anemia in HIV-uninfected infants was of borderline significance ($P = 0.07$). Additional characteristics not associated with infant anemia included marital status, years of education of the mother, Luo tribe, employment of the mother, a peri-urban (versus urban) residence, rainy season at the time of delivery, and a history of diarrhea in the infant.

TABLE 1

Characteristics of mothers and infants enrolled in the study by participation in the analysis in infants, June 1996–April 2000, Kismu, western Kenya (n = 661 mother-infant pairs)*

	Mothers	No.	%
Age (years)			
<20		212	32.1
20–29		399	60.5
≥30		50	7.6
Education (years of schooling)			
0–3		30	4.5
4–7		166	25.1
≥8		465	70.3
Low socioeconomic status		530	80.2
Semi-urban (versus urban place of residence)		98	14.8
Luo tribe (versus other tribes)		552	83.5
Gravidity			
Primigravidae		278	42.1
Secundigravidae		170	25.7
Gravidae ≥3		213	32.2
Mean ± SD Hb level, third trimester (g/dL)†			9.3 ± 1.7
Malaria parasitemia, third trimester†		126	23
Mean ± SD Hb level at delivery (g/dL)†			10.1 ± 2.3
Maternal peripheral parasitemia, delivery†		170	26.6
Placental malaria		225	34.0
CD4:CD8 ratio < 0.90†		324	56.9
HIV-seropositive mother		467	70.7
Infants			
Male		334	50.5
Low birth weight		41	6.2
Premature infant		48	7.3
Small for gestational age		72	10.9
HIV status mother and infant			
HIV-infected infant/mother HIV-seropositive		105	15.9
HIV-uninfected infant/mother HIV-seropositive		362	54.8
HIV-uninfected infant/mother HIV-seronegative		194	29.3

* Hb = hemoglobin; HIV = human immunodeficiency virus.

† Hemoglobin and malaria smear results in the third trimester were available for 550 women (83.2%), hemoglobin and malaria peripheral smears results at delivery were available for 639 women (96.7%), and CD4 and CD8 counts one month postpartum were available for 569 women (88.1%).

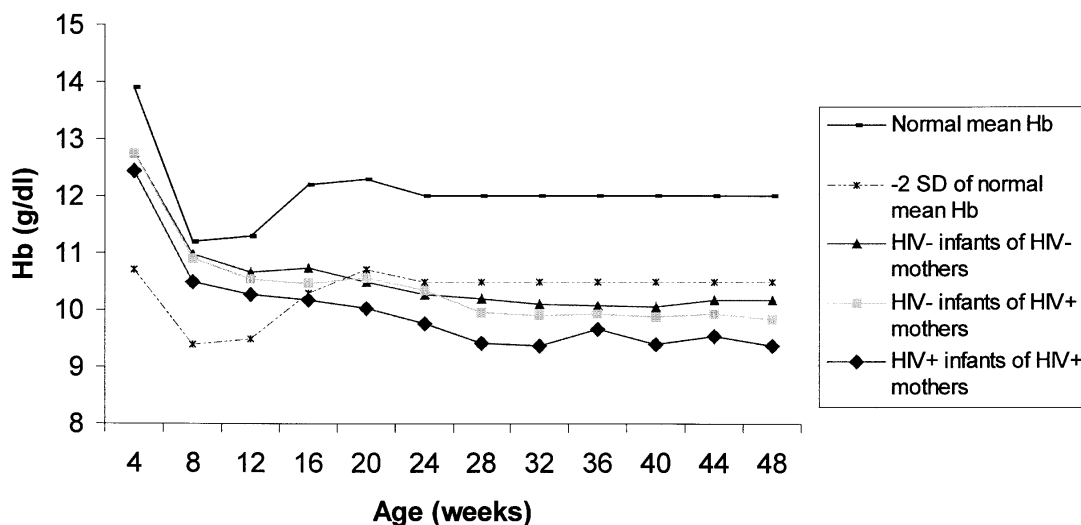


FIGURE 2. Mean hemoglobin (Hb) levels in the first year of life by human immunodeficiency virus (HIV) status of mother and the infant, Kisumu, 1996–2000. HIV+ = HIV-infected; HIV- = HIV-uninfected. Except for weeks 4, 10, and 12, mean Hb levels in HIV-infected infants were significantly less than those in HIV-uninfected infants of HIV-seronegative mothers, and at weeks 16–32 were also significantly less than those in HIV-uninfected infants of HIV-seropositive mothers. At 48 weeks, HIV-uninfected infants of HIV-seropositive mothers had significantly lower mean Hb levels compared with those in HIV-uninfected infants of HIV-seronegative mothers.

In the multivariate model (Table 3), the RRs were higher before 24 weeks, and decreased thereafter, when anemia changed from being a rare to a common event. An HIV infection of the infant was an important risk factor for anemia and this was true both early and late in infancy. For HIV-uninfected infants, the association between maternal HIV infection and anemia in infancy was of borderline significance. Placental malaria was a risk factor prior to 24 weeks of age, in the second part of infancy the association was of borderline significance ($P = 0.05$). To assess the time period of maximal effect of placental malaria, models were repeated monthly up

to 20 weeks. At four weeks of age, the adjusted RR was 1.35 (95% CI = 0.83–2.19), and this increased at eight and 12 weeks of age (RR = 1.68, 95% CI = 1.01–2.80 and RR = 1.77, 95% CI = 1.13–2.77, respectively) and then decreased at 16 and 20 weeks of age (RR = 1.02, 95% CI = 0.76–1.37 and RR = 1.10, 95% CI = 0.85–1.43, respectively).

Removal of placental malaria from the model for 4–23 weeks decreased the strength of the association between HIV-infected infants and anemia, indicating confounding or interaction; the P value for the interaction term between placental malaria and HIV-infected infants was 0.04. If one con-

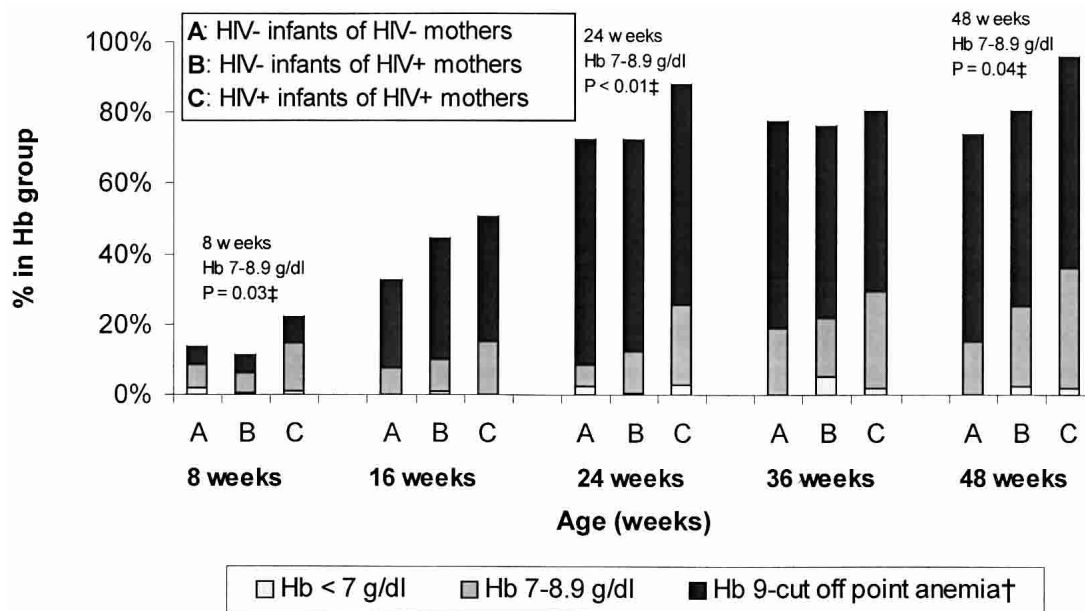


FIGURE 3. Anemia in the first year of life by human immunodeficiency virus (HIV) status of the mother and infant, Kisumu, 1996–2000. Hb = hemoglobin; HIV+ = HIV-infected; HIV- = HIV-uninfected. †Cut-off point anemia age 8 weeks: Hb < 9.4 g/dL, age 16 weeks: Hb < 10.3 g/dL, age 24 weeks and older: Hb < 11 g/dL. ‡Chi-square test for Hb = 7–8.9 g/dL vs Hb ≠ 7–8.9 g/dL, comparing the three groups.

TABLE 2

Maternal and infant characteristics associated in univariate analysis with anemia in infants, Kismumu, June 1996–April 2000, (n = 661 infants who made 5,459 monthly visits with a hemoglobin result)*

	No of visits	Number with anemia	Incidence density†	Risk ratio (95% CI)
Mothers				
Age (years)				
<20	1,678	953	56.8	1.16 (1.01–1.35)
20–29	3,324	1,730	52.0	1.08 (0.94–1.25)
≥30	457	219	47.9	Reference
Low socioeconomic status	4,401	2,400	54.5	1.15 (1.04–1.26)
Gravidity				
Primigravidae	2,228	1,217	54.6	1.05 (0.97–1.14)
Secundigravidae	1,338	722	54.0	1.06 (0.97–1.16)
Gravidae ≥3	1,893	963	50.9	Reference
Severe anemia, third trimester‡	430	258	60.0	1.16 (1.04–1.29)
Malaria parasitemia, third trimester	1,002	549	54.8	1.06 (0.97–1.17)
Hb level after delivery <7 g/dL‡	495	277	56.0	1.05 (0.94–1.18)
Malaria parasitemia delivery‡	1,394	762	54.7	1.03 (0.94–1.12)
Placental malaria	1,848	1062	57.5	1.12 (1.04–1.21)
CD4:CD8 ratio < 0.90‡	2,792	1543	55.3	1.13 (1.05–1.23)
HIV-seropositive mother	3,935	2140	54.4	1.11 (1.02–1.21)
Infants: time-independent variables				
Male	2,763	1,539	55.7	1.11 (1.03–1.19)
Low birth weight	310	190	61.3	1.17 (1.04–1.31)
Premature infant	324	192	59.3	1.12 (0.98–1.28)
Small for gestational age	595	362	60.8	1.16 (1.06–1.27)
HIV-infected infant	822	491	59.7	1.15 (1.05–1.25)
HIV status: infant and mother				
HIV+ infant/mother HIV+	822	491	59.7	1.21 (1.09–1.34)
HIV– infant/mother HIV+	3,113	1,649	53.0	1.08 (0.99–1.18)
HIV– infant/mother HIV–	1,524	762	50.0	Reference
Infants: time-dependent variables				
Malaria parasitemia	747	515	68.9	1.17 (1.10–1.25)
Parasite density (μL)				
≥5,000	121	103	85.1	1.32 (1.18–1.48)
1–1,4999	623	409	65.7	1.14 (1.07–1.22)
No. parasites detected	4,695	2,380	50.7	Reference
Malaria parasitemia previous visit	626	441	70.5	1.12 (1.06–1.19)
History of treated clinical malaria in the preceding month	416	313	75.2	1.29 (1.20–1.38)
History of fever	1,284	818	63.7	1.14 (1.08–1.20)
Documented fever (37.5°C)	348	248	71.3	1.21 (1.12–1.30)
Palpable spleen	252	199	79	1.26 (1.15–1.38)
Palpable liver	160	106	66.3	1.13 (1.01–1.27)

* Significant risk ratios are shown in **bold**. CI = confidence interval; Hb = hemoglobin; HIV = human immunodeficiency virus; HIV+ = HIV-infected; HIV– = HIV-uninfected.

† Incidence densities are expressed as the number of anemia diagnoses per 100 person-months of observation.

‡ Hemoglobin and malaria smear results in the third trimester were available for 550 women (83.2%), hemoglobin and malaria peripheral smear results were at delivery available for 639 women (96.7%), and CD4 and CD8 counts one month postpartum were available for 562 women (85.0%).

siders the small sample size for HIV-infected infants of mothers with placental malaria (n = 19), an interaction could not be excluded. When stratified by infant HIV status, the greatest effect of placental malaria prior to 24 weeks of life was on the prevalence of anemia in HIV-uninfected infants of HIV-seronegative mothers (adjusted RR = 1.42, 95% CI = 1.08–1.88). For HIV-uninfected infants of HIV-seropositive mothers and for HIV-infected infants, placental malaria was not a significant risk factor (adjusted RR = 1.19, 95% CI = 0.97–1.46 and RR = 0.91, 95% CI = 0.67–1.25, respectively).

To assess the effect of maternal immunodeficiency in HIV-seropositive mothers in terms of CD4:CD8 counts on anemia in HIV-uninfected infants, the model was repeated including only HIV-uninfected infants. A maternal CD4:CD8 ratio in HIV-seropositive mothers of less than 0.9 was significantly associated with anemia (RR = 1.14, 95% CI = 1.03–1.26) at 24–52 weeks of life, but not before 24 weeks (RR = 1.15, 95% CI = 0.90–1.47).

Of the factors that indicated past or present malaria in the infant, a history of treatment of clinical malaria, parasitemia

in the current visit, and a palpable spleen were consistent risk factors in the first year of life. To assess a confounding effect of the use of antimalarials and hematinics on the risk factors for anemia, the model was repeated with the exclusion of the first visit after an antimalarial or hematinic drug was prescribed (19.1% of the visits). Risk factors and RRs were very similar compared with the first model; however, before 24 weeks of age, higher RRs were seen for high-density parasitemia (2.08, 95% CI = 1.58–2.75) and splenomegaly (RR = 1.57, 95% CI = 1.26–1.96).

The effect of concurrent malaria on anemia by age can be seen in Figure 4, stratified by HIV status of the infant. The HIV-infected infants without parasitemia had significantly lower hemoglobin levels than HIV-uninfected infants without parasitemia but higher hemoglobin levels than HIV-uninfected infants with parasitemia (linear regression > 12 weeks, comparing HIV-uninfected infants with malaria with HIV-infected infants without parasitemia; *P* = 0.07). Anemia was particularly common in HIV-infected infants with parasitemia at or after 16 weeks of age (RR of malaria for anemia in

TABLE 3

Maternal and infant characteristics associated with anemia in infants in a multivariate model, Kisumu, June 1996–April 2000*

Variable	Age 4–23 weeks Risk ratio (95% CI)	Age 24–52 weeks Risk ratio (95% CI)
Time stationary variables for mothers		
Age <20 years	1.21 (1.04–1.40)	1.05 (0.99–1.12)
Low socioeconomic status	1.23 (1.02–1.47)	1.09 (1.01–1.18)
Placental malaria	1.21 (1.04–1.40)	1.06 (0.99–1.13)†
Time stationary variables for infants		
Male	1.20 (1.04–1.38)	1.07 (1.01–1.13)
SGA	1.32 (1.10–1.58)	1.07 (0.99–1.16)
Premature	1.29 (1.00–1.65)	1.04 (0.94–1.15)
HIV-status mother and infant		
HIV+ infant	1.34 (1.09–1.64)	1.20 (1.10–1.31)
HIV– infant of HIV+ mother	1.18 (0.99–1.40)†	1.07 (0.99–1.16)†
HIV– infant of HIV– mother	Reference	Reference
Time varying variables for infants		
Parasite density (μL)		
≥5,000	1.73 (1.25–2.38)	1.09 (0.99–1.18)†
1–4,999	1.15 (0.97–1.37)	1.08 (1.02–1.13)
No parasites detected	Reference	Reference
History of treatment for clinical malaria in the preceding month		
History of fever	1.75 (1.44–2.12)	1.11 (1.06–1.17)
Documented fever (37.5°C)	1.26 (1.02–1.55)	1.06 (0.99–1.13)
Palpable spleen	1.44 (1.17–1.77)	1.11 (1.06–1.17)

* Significant risk ratios are shown in bold. CI = confidence interval; SGA = small for gestational age; HIV = human immunodeficiency virus; HIV+ = HIV-infected; HIV– = HIV-uninfected.

† Borderline significance: $P = 0.0529$ for placental malaria, $P = 0.0589$ for HIV-uninfected infants of HIV-seropositive mothers before 24 weeks, $P = 0.0746$ for HIV-uninfected, 24–52-week-old infants for HIV-seropositive mothers, $P = 0.0538$ for a parasite density of 5,000 parasites/μL.

HIV-infected infants 16 weeks of age = 1.13, 95% CI = 1.00–1.27), and the hemoglobin level was significantly decreased compared with HIV-uninfected infants, and HIV-infected infants without parasitemia ($P < 0.01$).

DISCUSSION

Anemia was a major problem in this urban birth cohort. It was rare in the first weeks of life, but increased from 13.6% at four weeks of age to 75% at six months of age and remained high throughout the second half of infancy. Although the mean hemoglobin level was slightly higher than that reported in infants from our rural study site in Asembo, the shape of the mean hemoglobin curve in the first year of life was similar to that seen in Asembo.³⁴ This included a lack of nadir at two months of age, decreasing hemoglobin levels up to 8–10 months of age, and little variation in the mean hemoglobin level in the second half of the first year. A similar age-dependent pattern was also reported in Tanzania.³⁵

An HIV infection of the infant was a consistent risk factor for anemia throughout infancy, and we estimate that in the HIV-infected infants participating in this study 16–17% of the anemia could be attributed to the presence of HIV (attributable fraction). As expected, malaria was a risk factor for anemia. This was also true in early infancy when most infants are protected from severe malaria through passively transferred maternal antibodies and the presence of fetal hemoglobin.

Importantly, HIV-infected infants with malaria had lower mean hemoglobin levels from 16 weeks of age onwards compared with HIV-uninfected infants with and without malaria, and HIV-infected infants without malaria, suggesting that HIV-infected infants are particularly vulnerable to the adverse consequence of malaria at this age. This is consistent with our observation in pregnant women with HIV-infection and malaria (Ayisi JG, unpublished data).

This study also showed that maternal factors play an important role in infant anemia. Maternal infection with HIV was an additional risk factor, not only directly, through mother-to-child transmission of HIV, but also indirectly, as suggested by the finding that infant anemia was worse in HIV-uninfected infants when born to HIV-seropositive mothers compared with those born to HIV-seronegative mothers (borderline significance). The effect was particularly evident in the infants born to mothers with more advanced immunodeficiency (low CD4:CD8 ratios). This may be the result of different infant feeding habits by HIV-seropositive mothers or an effect of health status of the mother and potential mother-to-child transmission of other pathogens.³⁶

Placental malaria was a risk factor for early infant anemia that was independent of prematurity and SGA. This confirms earlier reports from Malawi and Cameroon,^{17,20} but is in contrast with recent findings from our rural study site near Kisumu; however, low birth weight and premature infants were excluded in that analysis.³⁴ Microscopically detectable congenital malaria (cord blood) was rare in this study (1.7%) and by itself cannot explain the increased risk of infant anemia. The increased risk with placental malaria might reflect behavioral differences (e.g., frequent travel with the infant to the rural area) or increased environmental exposure to malaria in households (e.g., house construction and location of residence), which might be less amendable to anti-malarial interventions during pregnancy. However, the observation that the association between placental malaria and infant anemia is present only in the first few months of life and absent in the last six months of infancy, both in the current study and in a previous study in Cameroon, may indicate a biologic association.¹⁷ Brabin suggested that exposure of the fetus to malaria antigens due to damage of the placental barrier may make the newborn more susceptible to immunologically mediated hemolysis or to dyserythropoiesis.³⁷

This study has several limitations that should be considered. First, estimates of the prevalence of anemia are not applicable to the general infant population in Kisumu because of the study design, which resulted in a relatively high proportion of HIV-seropositive women, and thus HIV-infected infants, as well as relatively more women with placental malaria. However, the associations between the maternal and infant factors described are likely to be valid in a wider context as well. Second, the HIV status could not be determined in 26% of the infants because they were lost to follow-up before their first sample, or they contributed only a single sample, whereas two consecutive samples were required. The prevalence of HIV among these infants is likely to be higher than among infants with known HIV status, since a higher proportion of their mothers were HIV seropositive, and the early infant mortality among these 26% was high. Third, infants who did not return for visits may have been different from infants who returned, e.g., they may have been more healthy so the mother may not have thought it necessary to visit, or they

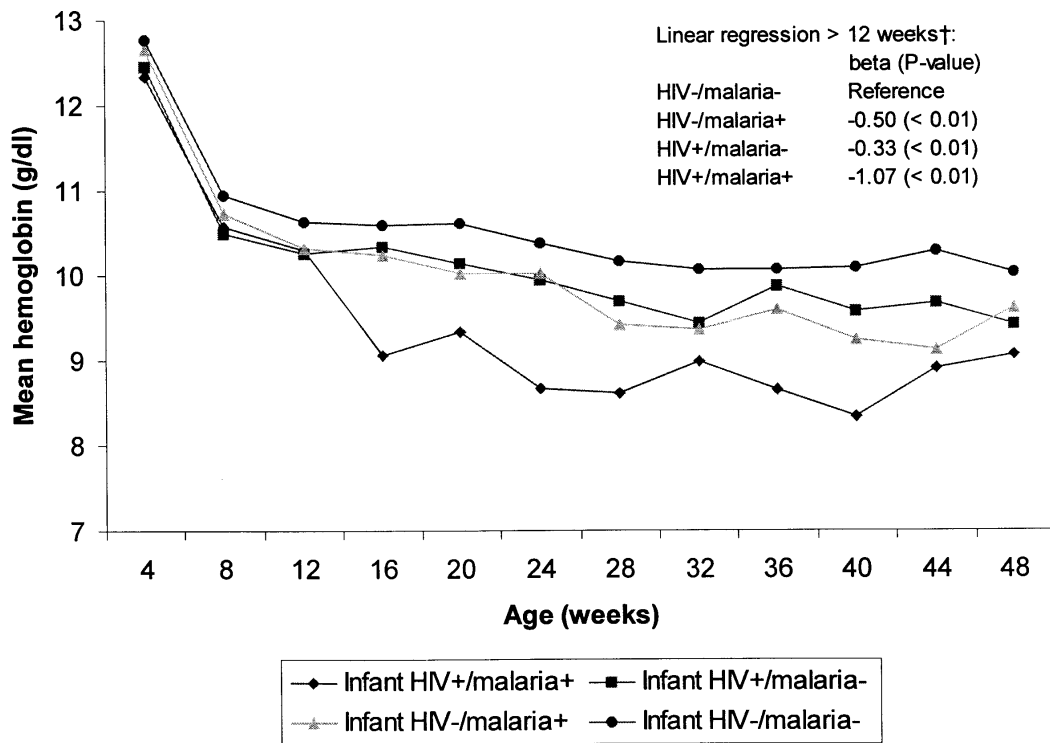


FIGURE 4. Malaria parasitemia and hemoglobin (Hb) levels in infants by human immunodeficiency virus (HIV) status, Kisumu, 1996–2000. HIV+ = HIV-infected; HIV- = HIV-uninfected; malaria+ = parasitemia detected in blood smear; malaria- = no parasites detected in blood smear. †Linear regression > 12 weeks, adjusted for maternal age, socioeconomic status, placental malaria, sex, small for gestational age, prematurity, history of fever, enlarged spleen, and documented fever.

may have been too sick so the mother may not have thought it useful to visit the study clinic. This may have skewed the results.

A hemoglobin level < 11 g/dL was used as a cut-off value for the definition of anemia in the second six months of infancy, according to the definitions of the World Health Organization.³⁰ No suitable standard reference values are currently available for young infants in whom physiologic hemoglobin concentrations show important variations with age. The use of a separate hemoglobin standard for African and Caucasian persons is still under discussion.³⁸ A single cut-off value of 11 g/dL would inevitably have resulted in an underestimate of anemia in infants less than two months old and an overestimate in infants 3–5 months old. We therefore chose to use an age-specific reference population to define anemia in infants less than six months old.³¹ The definition of anemia did not influence the relationship between maternal and infant factors on infant anemia, the primary objective of this study.

Lastly, the study aimed to determine the role of maternal and infant infection with HIV and malaria on infant anemia, but did not assess other known causes of anemia, including iron and other nutritional deficiencies, or hereditary red blood cell disorders. Blood cultures were not routinely prepared, and we cannot define the contribution of bacterial pathogens, which are likely to play an important role in HIV-infected infants. Helminthic infections were not routinely assessed, but were determined in a sub-sample of the study infants (n = 315) as part of a sub-study of the etiology of infant diarrhea (Eberhard M, unpublished data). Only 3.5% had evidence of helminthic infections in their stool; no association with anemia was seen in this subgroup.

Because of their increased vulnerability for developing anemia, in particular in the presence of malaria, infants born to HIV-seropositive mothers, especially if they are infected with HIV, may need to be targeted with specific interventions. However, it will be important to assess if interventions that have been proven to be beneficial in HIV-uninfected infants in developing countries, e.g., iron supplementation, are also beneficial in HIV-infected infants because causes of anemia and response to treatment may differ between HIV-infected and HIV-uninfected infants. The association between placental malaria and early infant anemia found in this study suggests that prevention of maternal malaria might be beneficial in preventing anemia in the first six months of life, particularly when combined with other interventions that impact beneficially on birth weight and the maternal micronutrient status. A concern was that close monitoring, combined with early detection and prompt treatment of infant malaria and anemia alone, was not sufficient as a strategy to prevent infant anemia in this study population. All infants were regularly examined by a physician and treated free of charge for anemia, malaria (any parasitemia, regardless of symptoms) and other infectious diseases, but this apparently failed to prevent most of the infants from becoming anemic. This suggests that more research is needed in this high-risk population, and in particular in HIV-infected infants, to assess how micronutrient supplementation combined with malaria interventions, either intermittent treatment with antimalarials, regular chemoprophylaxis, or the use of impregnated bed nets or other protective measures can improve hemoglobin levels and decrease anemia in a more sustainable manner.

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