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Effects of Maternal HIV Infection on Early Kaposi Sarcoma-Associated Herpesvirus Seroconversion in a Kenyan Mother-Infant Cohort

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Background. We identified whether maternal human immunodeficiency virus (HIV) infection during pregnancy affects transplacental transfer of Kaposi sarcoma-associated herpesvirus (KSHV)-specific antibodies and subsequent infant infection.

Methods. We followed pregnant Kenyan women through delivery and their infants until age 2 years. Children were classified as HIV-exposed uninfected (HEU) or HIV-unexposed uninfected (HUU) based on maternal HIV status. Maternal venous and cord blood at delivery and child venous blood every 6 months were tested for antibodies to 20 KSHV antigens by multiplex bead-based immunoassay. Multiple comparisons were adjusted using false discovery rate (FDR).

Results. Maternal HIV infection was significantly associated with decreased transplacental transfer of antibodies against all KSHV antigens and lower cord blood levels for 8 antigens at FDR $P < .10$. Neither birth to 6-month antibody level changes nor 6-month levels differed in HEU and HUU, except for ORF50. By age 24 months, 74% of children KSHV seroconverted but HEU and HUU did not differ in time to seroconversion nor 2-year seropositivity after adjustment for child malaria infection.

Conclusions. Maternal HIV infection reduced a child's initial KSHV antibody levels but did not affect age of infection. Regardless of HIV exposure in utero, KSHV seroconversion in Kenyan children occurred early; associated factors must be identified.

Keywords. HIV; KSHV seroconversion; Kenya; antibodies; children; Kaposi sarcoma-associated herpesvirus (KSHV); mother-child pairs; oral shedding; pregnancy; transplacental antibody transfer.

In sub-Saharan Africa, seroprevalence of Kaposi sarcoma-associated herpesvirus (KSHV), the causative agent of Kaposi sarcoma (KS), is high and KSHV infection occurs early during childhood [1–3]. The factors leading to early KSHV seroconversion in KSHV endemic regions have yet to be adequately identified and are likely to be multifactorial. In a region that also has high human immunodeficiency virus (HIV) seroprevalence, childhood HIV infection has been associated with increased likelihood and earlier age of KSHV seroconversion [1, 4, 5], and some studies have found that children of women with HIV/AIDS (WHA) are more likely to be KSHV seropositive [4–6]. However, the role of exposure to HIV infection during pregnancy on the transplacental transfer of KSHV specific antibodies and subsequent susceptibility to KSHV infection in infants remains undefined.

Access to highly effective antiretroviral therapy (ART) combined with interventions to prevent mother-to-child HIV transmission has significantly reduced horizontal HIV transfer [7]. However, children born to WHA but not themselves infected have been reported to be more likely to have infection-related and noninfection-related illnesses [8–10], increased risk of hospitalization [11, 12], and increased rates of mortality [13–15], primarily at very early ages compared to HIV unexposed and uninfected children. Poorer health outcomes may be the result of a disrupted immune system due to increased infection exposures, fetal ART exposure, poor maternal health, or a variety of other environmental and biological factors, but the reasons remain unclear.

In the third trimester of pregnancy maternally derived immunoglobulin G (IgG) antibodies are transported across the placenta to provide passive humoral immunity in infants. For several

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infectious agents, the transplacental transfer of protective antibodies is reduced in pregnant WHA [16–25], thus increasing an infant's risk of early infection. Whether HIV infection during pregnancy reduces transplacental transfer of anti-KSHV–specific antibodies has not yet been reported. Studies of the impact of maternal HIV status on the risk of KSHV seroconversion in children in sub-Saharan Africa have reported mixed results. In Uganda, children of WHA were at a moderately increased probability of being KSHV seropositive [5]. However, studies based in Zambia and South Africa reported no associations between child KSHV serostatus and the mother's HIV status [1, 4, 26], nor by maternal exposure to ART [27]. To prevent early KSHV infection and reduce future KS risk, it is critical to identify factors that make children susceptible to KSHV.

Kisumu County in Kenya has a high prevalence of HIV (20% [7]) resulting in a large number of children born to WHA. This area also has one of the highest KSHV seroprevalences, with up to 80% in adults [28] and with 63% of children KSHV seroconverting by age 2 years [2]. We utilized data from a mother-child cohort based in a rural region of Kisumu County to identify whether maternal HIV infection reduces anti-KSHV antibody transplacental transfer, and leads to faster decay of maternally acquired anti-KSHV antibodies and earlier age of child KSHV seroconversion.

METHODS

Study Population

From 2011 to 2015, 370 pregnant women residing within 10 km of the Chulaimbo County Hospital, which serves predominately rural populations of Kisumu County, were enrolled at their first antenatal clinic and followed through pregnancy to delivery. WHA were enrolled through the Academic Model Providing Access to Healthcare site. Infants were prospectively followed through 24 months of age (Figure 1). We asked that children be brought to the study clinic whenever ill, where they received a medical examination and treatment. The methods have been described elsewhere [29, 30]. Written informed consent was obtained from all enrolled women. Protocol and consent forms were approved by the Scientific and Ethical Review Unit (SERU) at the Kenya Medical Research Institute (KEMRI), Colorado Multiple Institutional Review Board, University Hospitals Cleveland Medical Center Institutional Review Board, and SUNY Upstate Medical University.

Maternal and Child HIV Status

All women were tested for HIV at enrollment. Newly diagnosed HIV positive women were considered ART naive, while women with known HIV infection were considered ART experienced. Children were considered HIV negative and identified as HIV exposed uninfected (HEU) or HIV unexposed uninfected (HUU) based on maternal HIV status. All women who tested HIV positive and their infants received care and treatment. All WHA were given ART, and HEU infants were given cotrimoxazole based on Kenya Ministry of Health guidelines.

Sample Collection

Women provided 2–4 mL of venous blood at enrollment and within 12 hours of delivery (Figure 1). Cord blood was collected from the umbilical vein from the placental unit after delivery as previously described [31]. EDTA venous blood samples were collected from children at 6, 12, 18, and 24-month follow-up visits. Plasma was collected from whole blood and peripheral blood mononuclear cells (PBMCs) were separated over Ficol-Hypaque. Oral fluid samples were collected from women at 6 and 10 weeks postdelivery as described elsewhere [32]. All samples were stored at −80°C until analysis.

KSHV Antibody Testing

Maternal and child venous and infant cord blood samples were tested for IgG antibodies to 20 KSHV antigens (K8.1, ORF73, K3, K5, K10.5, K11, ORF6, ORF11, ORF25, ORF33, ORF37, ORF38, ORF50, ORF52, ORF55, ORF59, ORF61, ORF63, ORF65, ORF72) using a multiplex bead-based immunoassay as previously described [33]. These antigens were chosen based on the frequency of responses in diverse populations including those in sub-Saharan Africa, healthy donors, people with HIV, and patients with KSHV-associated disease. The median fluorescence intensity (MFI) across all counted beads was computed and recorded after subtracting background fluorescence. Cutoff values for each antigen were identified by receiver operating curve (ROC) analysis using the negative control sera on each plate as previously described [33]. Negative control sera were selected from healthy US-based blood donors considered at very low risk who also tested negative for KSHV by enzyme-linked immunosorbent assay (ELISA). Positive assay controls were sampled from US-based adult patients with active or history of KSHV-associated disease or detectable KSHV DNA in PBMCs. KSHV seropositivity was defined as detection of antibody to any of the 20 defined KSHV proteins above the specified cutoff in child venous blood samples taken at 12, 18, or 24 months. Samples collected at 6 months were excluded to prevent detection of maternal antibodies to KSHV. By 12 months, children were assumed to have no residual maternal anti-KSHV antibodies and anti-KSHV antibody detection was considered a seroconversion event.

Measurement of KSHV DNA and Viral Load Testing in Oral Fluid

NucleoSpin DNA RapidLyse (Takara) was used to extract DNA from oral fluid according to manufacturer instructions. NanoDrop 2000 spectrophotometer (Thermo Scientific) was

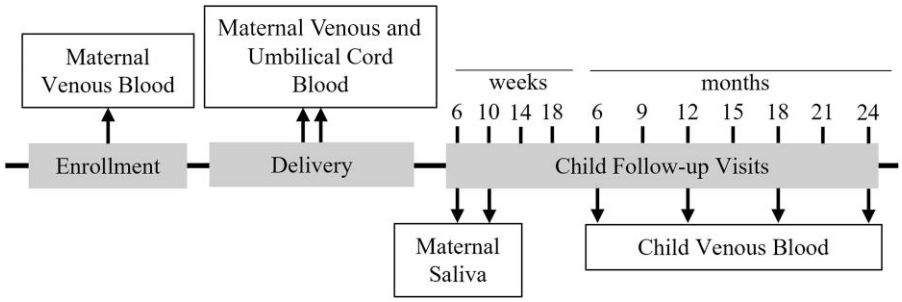

Figure 1. Scheduled visit and sample collection schedule for mother-child pairs enrolled in the study.

used to assess DNA purity and quantity. KSHV DNA was detected and quantified by quantitative polymerase chain reaction (qPCR) [34]. KSHV load was expressed as copies/mL of DNA and was log-transformed. KSHV shedding was defined as detection of KSHV DNA in oral fluids.

Statistical Analysis

We used *t* tests to compare log-transformed levels of (1) maternal antibody at delivery, (2) cord blood antibody, and (3) 6-month child antibody by maternal HIV status. χ^2 was used to compare proportions of women with KSHV oral shedding at 6 weeks postdelivery by HIV status and the Mann-Whitney *U* test was used to compare the median log-copies of KSHV found in oral fluid of KSHV shedders. We modeled (1) anti-KSHV antibody transplacental transfer (defined as the log ratio of cord-to-maternal antibody levels), and (2) loss of maternally acquired antibodies (defined as the log ratio of 6-month venous-to-cord blood antibody levels) in HEU versus HUU by multiple linear regression. KSHV seropositivity by 24 months was compared in HEU versus HUU by multiple logistic regression. *P* values were adjusted for multiple comparisons using false discovery rate (FDR). Time to KSHV seroconversion was defined as the age in months to first detection of any anti-KSHV antibody. Children were right censored at their last clinic visit with completed serology or at the end of the study, which was at 24 months of age, whichever came first. Outcomes were defined as KSHV seroconversion, no KSHV seroconversion, or loss to follow-up. Cox proportional hazards regression was used to model time at risk for KSHV seroconversion for HEU versus HUU children.

Potential confounders included maternal age at enrollment, education, parity, bed net use, *Plasmodium falciparum* infection (confirmed by qPCR or blood smear), or helminth infection during pregnancy, gestational age; and child sex, birthweight, *P. falciparum* infection (confirmed by qPCR), or sickness based on clinic visits. Child malnutrition measures of wasting, underweight, and stunting were defined by World Health Organization (WHO) Z-scores calculated using the WHO Anthro Survey Analyser [35]. All analyses were completed using SAS 9.4 (SAS Institute, Inc).

RESULTS

Participant Characteristics

Of the 369 enrolled women with complete serology, 356 (96%) were KSHV seropositive. These included 99 of 104 (95%) women with and 257 of 265 (97%) without HIV. Included in analyses were 253 infants delivered at the study hospital with matching cord and maternal venous blood samples or with a venous blood sample from at least 1 follow-up visit. Not all children had samples taken at every time point and so numbers varied for each analysis based on sample availability. WHA were older, more likely to be married, multiparous, to use a bed net during pregnancy, and were less likely to have a confirmed worm or *P. falciparum* infection during pregnancy compared to women without HIV (Table 1).

Among the 170 children with a follow-up visit between age 12 and 24 months there was no difference in the number of regularly scheduled visits attended (Table 1). However, HEU children were less likely to present to the study clinic while sick, present with a current or past fever, and had fewer sick visits on average. All children had at least 1 blood sample tested by qPCR for *P. falciparum* infection. However, HEU children had fewer samples tested and the likelihood of ever having *P. falciparum* detected by qPCR was lower than for HUU children. There was no difference in the 2 groups with regards to child WHO Z-scores, except that HEU were less likely to have had any stunting.

Maternal HIV Infection and Transplacental Transfer of Anti-KSHV Antibodies

There were 169 mother-child pairs of KSHV-seropositive mothers with matched maternal venous and cord blood at delivery. Maternal anti-KSHV antibody levels at delivery did not differ by maternal HIV status except for antibodies against ORF50, which were significantly lower in women with versus without HIV (mean log-transformed MFI, 2.59 [SD 0.46] and 2.92 [SD 0.57], respectively; FDR $P = .010$) (Supplementary

Table 1. Maternal and Newborn Characteristics for Maternal-Child Pairs

Characteristics	Women With HIV	Women Without HIV
Maternal		
Total	n = 85	n = 168
Age in years, mean (SD)	27.8 (6.0)	21.8 (5.9)
Tribe, Luo vs other	83 (97.6)	163 (98.2)
Marital status, married vs not	66 (77.6)	106 (63.9)
Education, upper primary school or higher vs lower primary school or lower	73 (85.9)	143 (86.1)
Gravidity		
Nulliparous	9 (10.6)	72 (43.4)
Primiparous	23 (27.1)	54 (32.5)
Multiparous	53 (62.4)	41 (24.7)
Bed net used during pregnancy, yes vs no	77 (90.6)	120 (72.3)
Insecticide-treated bed nets, yes vs no	55 (64.7)	109 (65.7)
Any worm infection during pregnancy, yes vs no	28 (32.9)	91 (54.8)
Any *P. falciparum* infection during pregnancy by qPCR or blood smear, yes vs no	33 (38.8)	109 (65.7)
KSHV seropositive, yes vs no	82 (96.5)	165 (98.2)
Neonate		
Total	HEU (n = 85)	HUU (n = 168)
Sex, female vs male	41 (48.2)	82 (48.8)
Birthweight, mean (SD)	3209 (538)	3156 (460)
Low birthweight, <2500 g vs 2500+ g	6 (7.9)	9 (6.1)
Gestational age, mean (SD)	38 (3.8)	38.5 (3.6)
Preterm, <38 wk vs 38+ wk gestational age	31 (36.9)	60 (36.1)
Child		
Total	HEU (n = 61)	HUU (n = 109)
Number of regularly scheduled visits, mean (SD)	8.0 (1.8)	7.8 (2.0)
Ever came for a sick visit, yes vs no	56 (91.8)	108 (99.1)
Number of sick visits, mean (SD)	4.5 (3.5)	6.8 (4.0)
Child ever had a fever, yes vs no[a]	52 (85.2)	107 (98.2)
Number of visits with *P. falciparum* qPCR completed, mean (SD)	4.8 (2.2)	5.6 (2.0)
Any *P. falciparum* infection by qPCR, yes vs no	13 (21.3)	42 (38.5)
WHO Z-score[b]		
Wasting, weight-for-height < −2 SD	10 (16.4)	17 (15.6)
Underweight, weight-for-age < −2 SD	16 (26.2)	29 (26.6)
Stunting, height-for-age < −2 SD	48 (78.7)	103 (94.5)

Data are No. (%) unless otherwise specified. All variables missing <2% except birthweight (HUU n = 20, HEU n = 9).

Abbreviations: HEU, HIV-exposed uninfected; HIV, human immunodeficiency virus; HUU, HIV-unexposed uninfected; KSHV, Kaposi sarcoma-associated herpesvirus; qPCR, quantitative polymerase chain reaction; WHO, World Health Organization.

[a]Ever fever included clinical presentation with temperature >38°C or caregiver report of a fever within 2 days of clinical presentation.

[b]WHO Z-scores were calculated using the WHO Anthro Survey Analyser.

Table 1). In women with compared to without HIV infection, we found statistically significant lower cord-to-maternal antibody level ratios (CMR) for all but 2 anti-KSHV antibodies (K11 and ORF65), which had marginally lower CMRs (Figure 2).

Among WHA, maternal antibody levels at delivery were not statistically different by ART status (naive vs experienced) (Supplementary Table 2). No differences were seen in the CMR of anti-KSHV antibodies by ART status, except the CMR of anti-K5 antibodies was higher in ART naive compared to ART experienced women (Supplementary Table 3).

Maternal HIV Infection and Loss of Maternally Acquired Antibodies From Birth to Age 6 Months

Paired cord and 6-month venous blood were available for 138 children. Overall, cord blood antibody levels were significantly lower in HEU versus HUU children for K5, ORF50, ORF61, and ORF72, and marginally lower for ORF63, ORF38, K3, and K11 (Figure 3). By age 6 months there were no statistically significant differences in anti-KSHV antibody levels for HEU versus HUU children (all FDR $P = .999$). The change in antibody levels from birth to age 6 months, measured as the log-ratio of 6-month venous blood-to-cord blood levels did not differ in children based on their mother's HIV status, except HEU children had a less drastic decrease in anti-ORF50 antibody levels from birth to age 6 months (Figure 4).

Among HEU, cord blood levels of anti-ORF50 were higher in children whose mothers were ART naive versus ART experienced (mean log-transformed MFI, 2.63 [SD 0.45] and 2.21 [SD 0.34], respectively; FDR $P = .020$) but not for other antibodies and no differences in antibody levels at 6 months of age by maternal ART status were seen (Supplementary Table 4). Maternal ART status was not associated with changes in child anti-KSHV antibodies from delivery to age 6 months (Supplementary Table 5).

Maternal HIV Infection and Age of KSHV Seroconversion

Of 170 children with at least 1 follow-up between 12 and 24 months of age, 125 (74%) seroconverted for KSHV by 24 months, 23 (14%) did not seroconvert, and 22 (13%) were lost to follow-up. Antibodies against only 1 antigen were detected in 50% of KSHV seroconverters, 2 antigens in 17%, 3 antigens in 10%, 4–9 antigens in 21%, and 13–15 antigens in the remaining 2% (Supplementary Figure 1). The most commonly detected antibodies were against ORF37, ORF33, ORF52, ORF61, and K11. Overall KSHV seropositivity by 24 months was significantly lower in HEU children (64%) than HUU children (79%) ($P = .034$) (Figure 5*A*). HEU children were significantly less likely to be KSHV seropositive than HUU children (odds ratio [OR], 0.47; 95% confidence interval [CI], 0.23–0.94; $P = .033$). However, this association was no longer statistically significant after adjustment for child *P. falciparum* infection detected by qPCR (adjusted OR, 0.60; 95% CI, 0.29–1.25; $P = .174$). We also found no difference in the time to KSHV seroconversion in HEU versus HUU children after adjustment for child *P. falciparum* infection (adjusted hazard ratio 0.98; 95% CI, 0.66–1.47; $P = .980$) (Figure 5*B*). Among HEU children

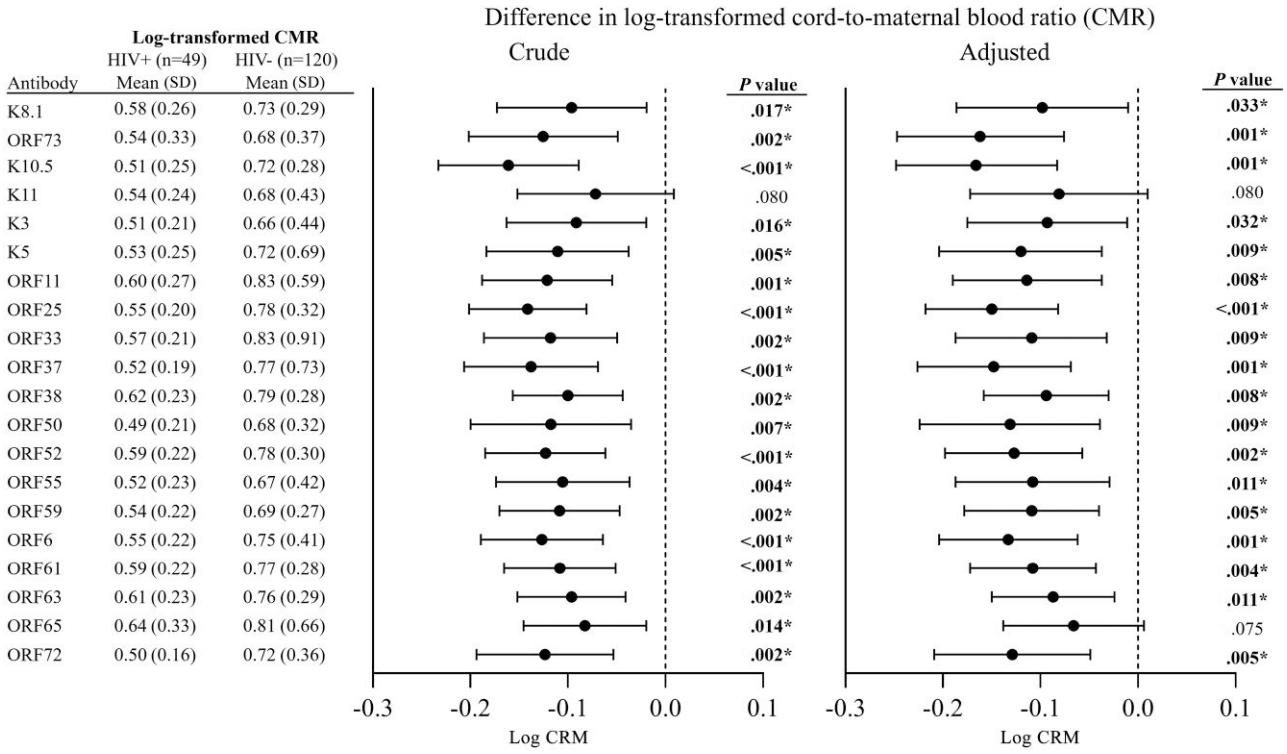

Figure 2. Comparison of transplacental transfer of anti-KSHV antibodies, measured by log-transformed CMR in mother-child pairs of women with HIV compared to women without HIV. All *P* values are multiple comparison adjusted using false discovery rate. Adjusted models include maternal age, any worm infection during pregnancy, and any *Plasmodium falciparum* infection (by qPCR or blood smear) during pregnancy. **P* < .05 considered statistically significant. Abbreviations: CMR, cord-to-maternal ratio; HIV, human immunodeficiency virus; KSHV, Kaposi sarcoma-associated herpesvirus; qPCR, quantitative polymerase chain reaction.

(n = 35), maternal ART status was not significantly associated with overall KSHV seropositivity nor time to KSHV seroconversion.

Maternal HIV Infection and Maternal KSHV Shedding in Oral Fluids

There was no difference in the prevalence of KSHV oral fluid shedding for women with and without HIV (19/65 [29%] vs 40/126 [32%]; *P* = .722). Among KSHV shedders, WHA had lower median oral viral load (median, 3.5 log-copies; interquartile range [IQR], 3.4–5.8 and median, 4.7 log-copies; IQR, 3.6–5.3; *P* = .038). Among WHA, ART status was not associated with KSHV shedding.

DISCUSSION

We found that the transplacental transfer of anti-KSHV antibodies was reduced in HEU infants, suggesting lowered protection for infants against KSHV seroconversion early in life. However, these differences in anti-KSHV antibody levels disappeared by the time the infants were 6 months of age. We found no difference in the age of KSHV seroconversion for infants born to women with and without HIV infection, but did find that a larger proportion of children born to women without HIV infection were KSHV seropositive by 24 months in crude analysis. However, the relationship between HIV exposure and KSHV seropositivity was attenuated after adjusting for childhood *P. falciparum* infection, suggesting a confounding effect of malaria on HIV and KSHV seroconversion in this cohort.

We found that WHA had reduced transfer of anti-KSHV antibodies to their infants, potentially reducing an infant's first line of defense against early infection. HIV infection during pregnancy has been associated with reduced transplacental transfer of antibodies for several other antigens including *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, group B *Streptococcus*, tetanus toxoid, pertussis, measles, polio, varicella zoster virus, malaria, and others [16–25]. The mechanism behind this is unknown but may result from an increased likelihood of hypergammaglobulinemia in WHA, leading to saturation of placental neonatal Fc receptors (FcRn). Receptor saturation reduces the ability of further binding of antibodies to FcRn, which could prevent transport of anti-KSHV–specific IgG across the placental barrier [19, 36]. The mechanisms leading to reduced transplacental KSHV antibody transfer in WHA require further study.

Although HEU neonates began life with lower anti-KSHV antibody levels than HUU neonates, we did not observe

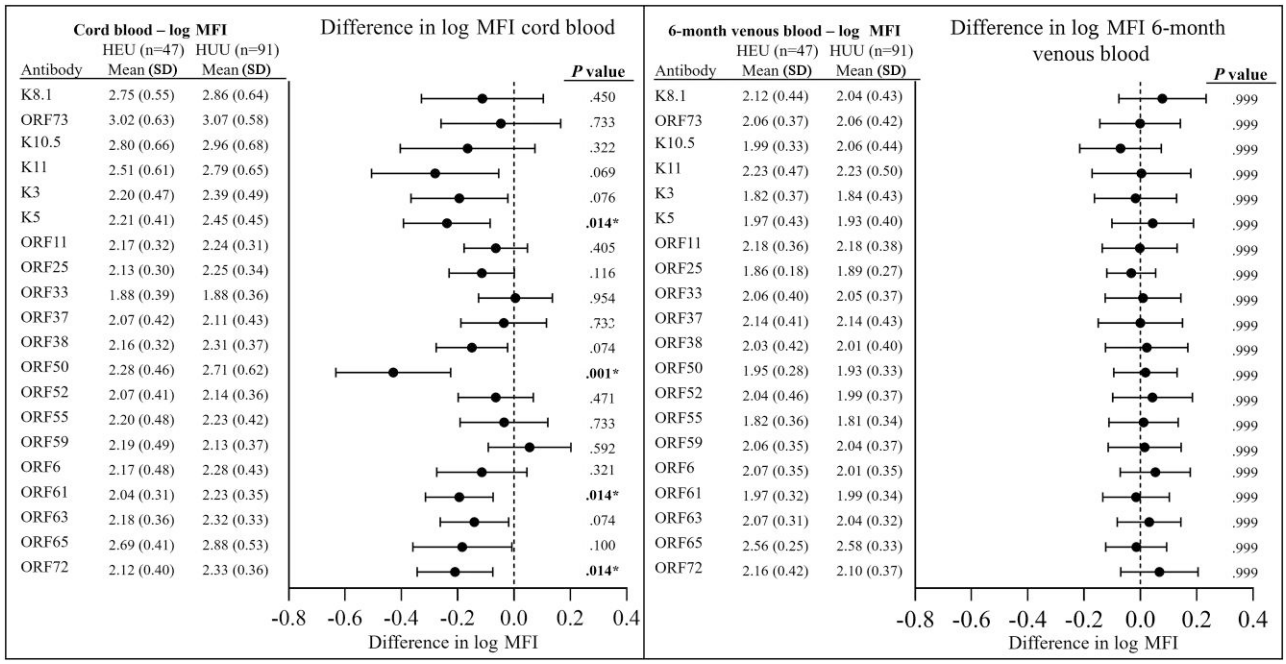

Figure 3. Comparison of anti-KSHV antibody levels in cord blood and in child venous blood at 6 months of age for HEU compared to HUU children with paired samples (n = 138). All *P* values are multiple comparison adjusted using false discovery rate. $^*P < .05$ considered statistically significant. Abbreviations: HEU, HIV exposed uninfected; HIV, human immunodeficiency virus; HUU, HIV unexposed uninfected; KSHV, Kaposi sarcoma-associated herpesvirus; MFI, median fluorescent intensity.

differences in overall anti-KSHV antibody levels by 6 months of age, and there was no difference in the time to KSHV seroconversion. Our results contrast a Ugandan study, which reported moderate increases in children being KSHV seropositive at 4–5 years of age, if their mothers were with versus without HIV [5]. However, our finding is in agreement with 2 studies of children in Zambia that found no association between maternal HIV and KSHV seroconversion in children up to 4 years old [1, 26]. Two additional studies of Zambian children also reported no association between maternal HIV infection and KSHV seropositivity in 12-month-old infants [4, 37]. Finally, a South African study of children ranging from 1.6 to 16 years old reported that maternal HIV infection was not associated with KSHV seropositivity after adjustment for maternal age and child HIV status [38]. All but one of these studies were conducted prior to ART rollout in the relevant country and that study had not collected data on maternal ART use. We were able to collect ART status on a subset of WHA. We did not find associations between maternal or infant anti-KSHV antibody levels nor time to a child's KSHV seroconversion based on maternal ART use, except higher levels of ORF50 were found in the cord blood of HEU born to ART-naive women, but this may be due to small sample size. However, our results are consistent with a Zambian study of children followed through age 12 months that reported no association between the maternal ART status and time to the child's KSHV seroconversion [27].

All WHA were provided with ART and regular care. Maternal health and access to care have been posited as potential factors associated with poorer health outcomes reported in HEU children. However, in our cohort, WHA were less likely to have a worm or *P. falciparum* infection identified during pregnancy. In addition, HEU children were less likely to have a sick visit for any reason, have a *P. falciparum* infection, or have a fever reported, suggesting they were healthier than their HUU counterparts. This may have been due to the regular care received by WHA and their children through HIV clinics at no or minimal cost in Kenya. Increased access to care may be leading to improved health in HEU and delayed KSHV seroconversion. HEU children also received co-trimoxazole prophylaxis, according to Kenyan Ministry of Health guidelines, which is effective as a malaria prophylaxis [39] and may account for the reduced number of sick visits, specifically those due to malaria, seen in this group. In crude analyses we identified an increased probability of being KSHV seropositive by 2 years of age in HUU compared to HEU children but the association disappeared after adjusting for childhood *P. falciparum* infection. It is possible that acute malaria infection leads to an artefactual KSHV serological response due to transient nonspecific increases in antibody levels, as it has been shown to do with other assays/antigens. However, if the detection of anti-KSHV antibodies is due to reactivation of class-switched B cells stimulated by copathogens or other conditions that are not KSHV, this means that KSHV infection may be occurring earlier than we

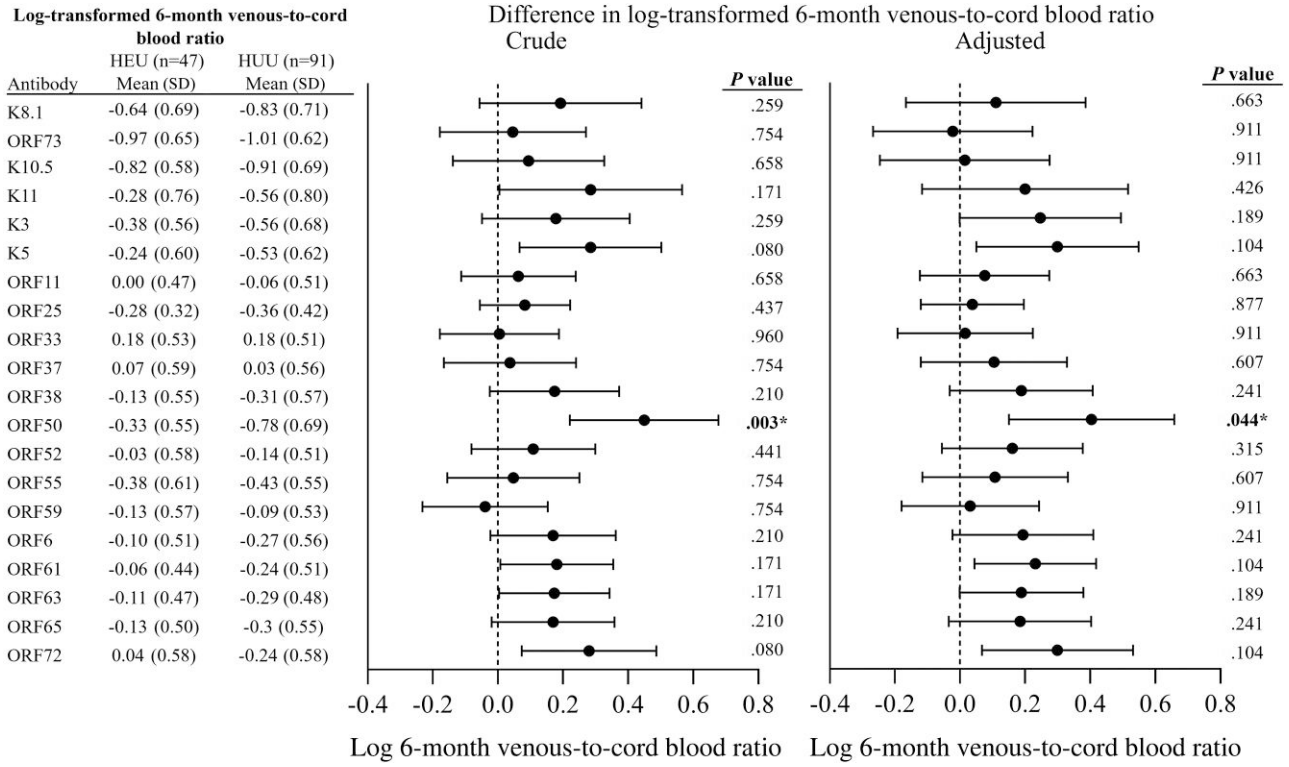

Figure 4. Comparison of changes in anti-KSHV antibodies from birth to 6 months of age, measured by child 6-month venous-to-cord blood antibody ratio for HEU compared to HUU children with paired samples (n = 138). All *P* values are multiple comparison adjusted using false discovery rate. Adjusted models include bed net use during pregnancy, any worm infection during pregnancy, any *Plasmodium falciparum* infection during pregnancy (by qPCR or blood smear), if infant ever had a fever (self-reported or measured), proportion of visits with blood samples tested for *P. falciparum* by qPCR that were *P. falciparum* positive, and proportion of total visits where child was sick. **P* < .05 considered statistically significant. Abbreviations: HEU, HIV exposed uninfected; HIV, human immunodeficiency virus; HUU, HIV unexposed uninfected; KSHV, Kaposi sarcoma-associated herpesvirus; qPCR, quantitative polymerase chain reaction.

are detecting by seroconversion. Our group has previously reported an association between malaria and KSHV seroconversion in a different cohort of Kenyan infants [2], which will require further exploration.

Regardless of maternal HIV status, HEU and HUU children may have had similar probabilities of KSHV seroconversion because they have similar likelihoods of exposure to KSHV. Maternal KSHV serostatus has been found to be predictive of child's seropositivity, suggesting mothers are an important source of household KSHV transmission [5, 6, 40, 41]. We found no difference in maternal KSHV seropositivity, and among KSHV-seropositive mothers, no difference in KSHV oral shedding by maternal HIV status. This contrasts with previous studies that reported that KSHV DNA was more commonly found in the oral fluid of individuals with HIV [42, 43] but is consistent with others reporting no relationship between HIV status and KSHV shedding [44–46]. KSHV shedding results from viral reactivation and, although the exact mechanisms are unknown, overall immune competency and health are believed to be predictive factors. Therefore, improved access to care and access to ART leads to better overall health in WHA, thus reducing the likelihood of KSHV oral shedding and so creating a similar likelihood of KSHV exposure in children of women with and without HIV infection. It is also possible that the effects of reduced KSHV antibody transfer due to maternal HIV infection has no residual effects on a child's immune response to KSHV.

We were able to leverage the longitudinal design of this mother-child cohort to identify the effects of maternal HIV infection on early susceptibility to KSHV infection. However, detection of KSHV DNA in PBMCs was not performed and so we were unable to compare viral detection directly to serological responses. We did not collect information on maternal CD4 counts, HIV viral loads, or factors relating to breastfeeding, although mothers are encouraged to exclusively breastfeed for the first 6 months, according to Kenyan Ministry of Health policy. Breast milk is an unlikely source of KSHV transmission [40, 44] but may confer protection against KSHV seroconversion that should be examined in future studies.

In rural Kenya, KSHV seroconversion is occurring early in childhood. In a region with high KSHV seroprevalence and HIV incidence it is paramount that we identify the factors promoting early KSHV infection. Although maternal HIV appears to affect passive humoral immunity against KSHV, it would

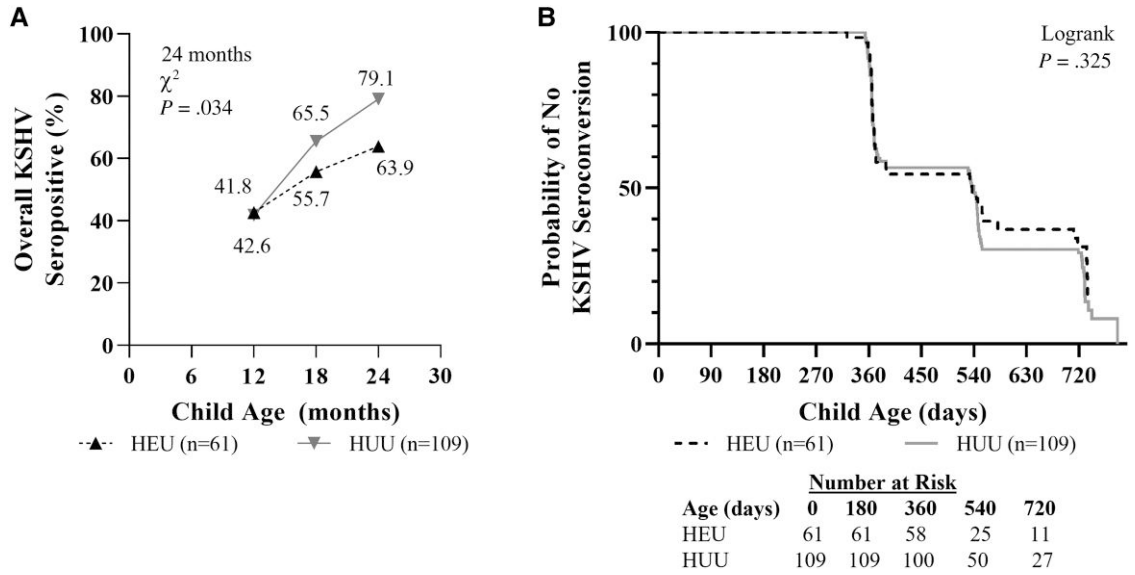

Figure 5. *A*, Child KSHV seropositivity from 12 to 24 months of age by whether the child was HEU vs HUU. *B*, Months to child KSHV seroconversion (in months from birth) for HEU compared to HUU children measured as the probability that a child would not KSHV seroconvert (n = 170). **P* < .05 considered statistically significant. Abbreviations: HEU, HIV-exposed uninfected; HIV, human immunodeficiency virus; HUU, HIV-unexposed uninfected; KSHV, Kaposi sarcoma-associated herpesvirus.

seem that HIV prevention and care programs are working in Kenya and as a result are potentially providing secondary protection against KSHV infection. Future work should confirm whether our findings hold true in other sub-Saharan African countries. In addition, the biological and environmental factors associated with KSHV infection need to be identified in more detail for the development of preventive measures.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Data availability. The data from the parent study is still under analysis and will be released publicly when analyses have been completed.

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