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Severity of maternal HIV-1 disease is associated with adverse birth outcomes in Malawian women: a cohort study

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Abstract

Background—Compared to HIV-negative women, HIV-infected women have increased risk of low birth weight (LBW) and preterm delivery (PTD). We assessed whether severity of maternal HIV-1 disease was associated with LBW or PTD.

Methods—Secondary analysis of The Malaria and HIV in Pregnancy prospective cohort, which enrolled HIV-positive, pregnant Malawian women from 2000–2004. Included participants (n=809) were normotensive, antiretroviral treatment naïve women who delivered a live, singleton infant. Binomial regression models were used to assess the unadjusted and adjusted prevalence ratios (PRs) and 95% confidence intervals (CI) of the effect of severity of HIV-1 disease, defined by viral load and CD4-positive T-cell counts, on prevalence of LBW and PTD.

Results—In unadjusted analyses, among those with malaria (n=198), there was no association between severity of HIV-1 infection and LBW, while among women without malaria (n=611), we observed a harmful association between both increasing peripheral viral load and LBW (PR: 1.44 per one-log₁₀ increase, 95% CI: 1.12, 1.86) and placental viral load and LBW (PR: 1.24 per one-log₁₀ increase, 95% CI: 1.00, 1.53). We observed a similar association between increasing placental viral load and PTD (PR: 1.33 per one-log₁₀ increase, 95% CI: 1.04, 1.69). These associations persisted in multivariate models adjusted for residence, maternal education, primigravid status, and maternal anemia.

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Author contributions. ANT and JJK designed the study, performed the data analyses, and wrote the manuscript. WEA and ST interpreted the data and edited the manuscript. SR, SM, VM conducted the parent MHP cohort, interpreted the data, and edited the manuscript.

Conclusions—In malaria-negative women, maternal HIV-1 disease severity was significantly associated with increased prevalence of LBW and PTD. Such an association was not found in the malaria-infected women.

Keywords

Human immunodeficiency virus type 1 (HIV-1); adverse birth outcomes; low birth weight; preterm delivery; placenta; sub-Saharan Africa

INTRODUCTION

Women of child-bearing age comprise approximately half of the estimated 33.3 million people living with HIV-1. Children born to HIV-infected women have an increased risk of adverse birth outcomes, including congenital HIV-1 infection, and low birth weight (LBW)^{1–3}. Malaria is also prevalent among pregnant women, and children born to women with malaria also have an increased risk of LBW⁴. LBW is defined as “infants born weighing 2500 grams or less, regardless of gestational age or cause of LBW”, and it can be caused by preterm delivery (PTD; birth before 37 weeks’ gestation), intrauterine growth restriction (IUGR), or congenital/genetic abnormalities. LBW is a significant cause of infant morbidity and mortality⁵. In 2010, PTD was the second leading cause of child death worldwide⁶, and Malawi had the highest prevalence of PTD in the world⁷.

The placenta provides fetal nourishment and serves as a barrier to fetal infection, and deficiencies in placental function are causally linked to obstetric complications⁸. Infections that colonize the placenta, such as *Plasmodium falciparum*, are associated with adverse birth outcomes^{9, 10}. *Plasmodium falciparum* parasites can be sequestered in the intervillous space of the placenta¹¹, and placental malaria has been associated with higher HIV-1 concentration in both peripheral and placental plasma¹². Our group¹³ and others¹⁴ have shown that HIV-1 replicates in the placenta, and it has also been shown that HIV-1 infection alters the cytokine profile in the placenta^{15–17}. Thus, when evaluating risk factors for adverse birth outcomes, potential interactions between malaria and HIV-1 coinfection should be considered¹⁸. The objective of this study was to determine if the severity of HIV-1 infection is associated with LBW or PTD.

METHODS

Participant enrollment

We describe a secondary analysis of data collected through The Malaria and HIV-1 in Pregnancy (MHP) prospective cohort study. The primary purpose of the parent study was to determine the association between malaria and HIV-1 mother-to-child transmission (MTCT)¹⁹. From December 2000 until March 2004, women who presented for delivery to the Antenatal Ward at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, were screened for eligibility for MHP. Women were ineligible if they were in the active phase of labor, were participating in other research studies, lived outside Blantyre district, were less than 15 years of age, were hypertensive, had altered consciousness, delivered a stillbirth, or twins. HIV-infected women and their offspring received nevirapine according to the HIVNET 012 protocol. Only women who delivered at QECH were included in this study.

Definitions

Gestational age at delivery was self-reported. Maternal CD4+ T-cells were quantified by FACScan and HIV-1 RNA was quantified using Amplicor HIV-1 Monitor v1.5 (Roche Diagnostics). Peripheral and placental thick blood films were Giemsa-stained and examined

for *P. falciparum* parasites (malaria) by two trained microscopists. Thick smears were considered *P. falciparum* negative if parasites were not observed after counting 200 leukocytes. Full-thickness placenta sections were formalin-fixed and paraffin embedded according to standard procedures. After Giemsa staining, placenta sections were examined for malaria parasites¹⁹. Women were considered syphilis seroreactive if they were both RPR and TPHA positive, as described²⁰. Anemia was defined as hemoglobin <11 g/dL; “any malaria” was defined as a positive malaria diagnosis on peripheral blood smear or placental blood smear or histology. Infant HIV-1 status at birth was determined by real-time polymerase chain reaction¹⁹. Chorioamnionitis was assessed by histopathological examination according to published methods²¹.

Statistical analysis

Statistical analysis was performed with STATA (Version 10.1) and SAS (Version 9.2). Using binomial regression models, we assessed the unadjusted and adjusted prevalence ratios (PRs) and 95% confidence intervals (CI) of the effect of HIV-1 severity on prevalence of adverse birth outcomes. We characterized HIV-1 severity through three separate primary exposure variables: a) continuous placental HIV-1 viral load; b) continuous maternal peripheral HIV-1 viral load and c) continuous maternal CD4+ T-cell count. We characterized adverse birth outcomes through two separate, primary, dichotomously-coded outcome variables: LBW and PTD. We separately assessed each exposure-outcome relationship to produce unadjusted and adjusted PRs. We selected binomial regression for multivariable modeling because the prevalence of each outcome was greater than 10%, the cut-off value above which logistic regression is not recommended²². All three HIV-1 severity variables met the assumption of linearity in the log risk for each outcome. Multivariable models were constructed as follows. Using product-interaction terms, we evaluated whether any of the exposure-outcome associations under investigation varied by maternal malaria status or maternal anemia. A p-value for any interaction term of <0.20 was interpreted as evidence of meaningful modification. We developed a starting list of potential confounders of each exposure-outcome association using Directed Acyclic Graphs²³. Variables assessed for possible confounding included those previously associated with either HIV-1 severity or adverse birth outcomes, and included demographic characteristics (age, education, rural/urban residence), clinical factors (intermittent preventative therapy for malaria during pregnancy or IPTp, maternal syphilis, anemia, chorioamnionitis, primigravid status) and infant factors (gender). To construct final models, we used a manual, backward elimination, change-in-estimate strategy²⁴, with a 10% threshold for variable retention. To ease interpretability, any variable surviving backward elimination for any of the models was retained in all multivariable models.

Sensitivity analyses

Our primary analyses evaluated the effect of HIV-1 severity separately on prevalence of LBW and PTD. We undertook three sensitivity analyses to assess the robustness of our findings. First, because preterm infants will naturally have lower birthweight because of their shorter gestation, we repeated our LBW analyses after removing from the dataset all observations with PTD. We then reran our unadjusted and adjusted multivariable models (using the same set of adjustment variables as in the main analysis) to see whether any increase in prevalence of LBW due to increased severity of HIV-1 disease persisted in term infants. Second, because fetal HIV-1 status may be associated with adverse birth outcomes, we repeated both the LBW and PTD analyses after restricting the dataset to only children confirmed to be HIV-negative at delivery. We again reran the unadjusted and adjusted models to determine whether the associations between HIV-1 disease severity and adverse birth outcomes changed. Third, to further assess potential modification of the exposure-

outcome association by malaria status, we examined separately malaria diagnosed by peripheral blood smear versus placental blood smear versus placental histology.

Ethical approval

MHP was approved by the College of Medicine Research Committee at the University of Malawi and the Institutional Review Boards (IRB) of the University of Michigan and the University of North Carolina at Chapel Hill. The analyses presented in this manuscript also received IRB approval from the Ohio State University.

RESULTS

Characteristics of HIV-positive participants who delivered live, singleton infants in the Malaria and HIV-1 in Pregnancy study, Malawi

Among the HIV-infected women in the parent Malaria and HIV-1 in Pregnancy (MHP) cohort, 809 live, singleton deliveries occurred, 80% via vaginal delivery. Participants' median age at enrollment was 24 (interquartile range (IQR): 21 to 28 years) and the median gravidity was two (IQR: 1 to 4 pregnancies) (Table 1). A majority of the participants had attended primary school (91%), were unemployed (75%) and lived in an urban setting (76%). At the time of delivery, 56% of the participants had anemia, 26% had histological evidence of chorioamnionitis, and seven percent were syphilis seropositive. One-quarter of the participants were primigravid. Most (86%) participants took iron-folate supplements during the pregnancy and 93% received at least one dose of IPTp for malaria (Table 1). With regard to malaria, ten percent of the women were peripheral blood smear positive, eight percent were placental blood smear positive, and 18% had histological evidence of malaria in a placental biopsy. Because all HIV-positive women in the MHP study received their HIV-1 diagnosis at enrollment, all participants were antiretroviral treatment naïve; 95% of the HIV-infected participants received single-dose nevirapine to prevent HIV-1 mother-to-child transmission.

Low birth weight and preterm delivery are common in Malawi

In this cohort of HIV-infected women, 21% of participants delivered a LBW infant and 16% delivered preterm. Age, malaria status, and gravidity were not associated with prevalence of LBW or PTD (Table 2). Anemia was more common among women with infants with LBW (68%) and PTD (68%) compared to women with normal weight, term infants (52%). Chorioamnionitis was also more commonly observed in the LBW (33%) and PTD (37%) groups compared to the normal weight, term delivery group (23%). Female sex was more prevalent among LBW infants (57%) compared to normal weight, term infants (46%). Finally, syphilis seropositivity was more common among mothers of PTD infants (11%) than normal weight, term infants (6%) (Table 2).

Placental viral load correlates with birth weight and gestational age

Maternal peripheral HIV-1 viral load was inversely correlated with continuous infant birthweight (correlation coefficient = -0.08 , $p=0.06$), but not with continuous duration of gestation (correlation coefficient = -0.05 , $p=0.5$) (Figures 1A & 1C). HIV-1 concentration in the placenta was inversely correlated with both birth weight (correlation coefficient = -0.15 , $p=0.01$) and duration of gestation (correlation coefficient = -0.20 , $p=0.0008$) (Figures 1B & 1D).

Low birthweight

The effect of HIV-1 disease severity on prevalence of LBW differed meaningfully by maternal malaria status. Residence, education level, primigravid status and anemia were

retained as confounders in adjusted multivariable models. Among women with malaria, we observed no significant association between any measure of HIV-1 disease severity and prevalence of LBW in unadjusted or adjusted analyses (Table 3). Among malaria-negative women, for all measures of HIV-1 disease severity, more severe HIV-1 disease was significantly associated with increased prevalence of LBW in both unadjusted and adjusted models. The adjusted PR for a 1-log increase in placental HIV-1 viral load was 1.22 (95% CI: 1.00, 1.48); for a 1-log increase in peripheral HIV-1 viral load was 1.38 (95% CI: 1.08, 1.77); and for a 100-cell/ μ l decrease in CD4+ T-cells was 1.12 (95% CI: 1.05, 1.21) (Table 3).

Preterm delivery

The effect of HIV-1 disease severity on prevalence of PTD also differed by maternal malaria status (Table 4). Among malaria-positive women, neither placental viral load nor CD4+ T-cell count were significantly associated with PTD in unadjusted or adjusted analyses, but a 1-log increase in peripheral viral load was significantly *protective* against PTD in adjusted analyses (PR: 0.56, 95% CI: 0.47, 0.85). Among women without malaria, higher placental viral load and lower CD4+ T-cell count were both significantly associated with increased prevalence of PTD (adjusted PR for a 1-log increase in placental viral load: 1.29, 95% CI: 1.02, 1.63; adjusted PR for 100-cell/ μ l decrease in CD4+ T-cells: 1.16, 95% CI: 1.05, 1.28). Increases in peripheral HIV-1 viral load did not significantly increase prevalence of PTD in malaria-negative women (Table 4).

The association between HIV-1 and LBW remains after exclusion of PTD and HIV-infected infants

When the dataset was restricted to include only infants born after 37 weeks' gestation (n=662), the associations between HIV-1 severity and prevalence of LBW among women with malaria were strengthened, but remained not statistically significant: the adjusted PR for a 1-log₁₀ increase in placental viral load was 1.64 (95% CI: 0.98, 2.74), compared to 1.26 (95% CI: 0.87, 1.83) in the primary analysis (Supplemental Table 1). The PR for LBW for a 1-log₁₀ increase in peripheral HIV-1 viral load was 1.66 (95% CI: 0.80, 3.47), compared to 0.88 (95% CI: 0.57, 1.35) in the primary analysis. However, excluding these observations had almost no effect on the magnitude of the associations between HIV-1 severity and prevalence of LBW in women without malaria. Given the reduced sample size, many of the PRs are no longer statistically significant, but the strength of the associations was essentially unchanged (Supplemental Table 1).

When the dataset was restricted only to observations with infants known to be HIV-negative at birth (n=686), we observed no meaningful changes in the associations between HIV-1 severity and prevalence of LBW or PTD, for malaria-positive or malaria-negative women (Supplemental Tables 2, 3). Thus, the observed associations in our primary analysis between HIV-1 severity and LBW or PTD are unlikely to be explained by infant HIV-1 status at birth.

Our primary analysis established that most associations between HIV-1 severity and birth outcomes were meaningfully different by malaria status. Our final sensitivity analysis assessed whether our findings were robust to the method of malaria diagnosis; in other words, was the same pattern observed for women with malaria diagnosed by peripheral blood smear, placental blood smear, and placental histology? For both LBW and PTD, the patterns observed in the primary analysis were repeated in the sensitivity analysis, with no meaningful differences by method of malaria diagnosis (Supplemental Tables 4,5).

DISCUSSION

In this secondary analysis of a large cohort of HIV-infected pregnant women who delivered a live, singleton infant in Malawi between 2000 and 2004, we tested the hypothesis that severity of HIV-1 infection is associated with risk of LBW or PTD. This cohort is unique because all women were infected with HIV-1 subtype C, were antiretroviral treatment naïve, were rigorously tested for malaria, and were normotensive. This study was not designed to comprehensively determine significant predictors of LBW or PTD in HIV-infected women; rather, it was designed to characterize the independent associations between three specific measures of HIV-1 disease severity, LBW and PTD, adjusting for factors that confounded or modified those associations. Although our malaria-positive sample size was small, HIV-1 severity in this group appeared not to be associated with adverse birth outcomes. However in malaria-negative women, maternal HIV-1 disease severity was significantly associated with increased prevalence of LBW and PTD. This association persisted in multivariate models adjusted for residence, primigravid status, maternal education, and maternal anemia, and the result was also robust to several sensitivity analyses, including exclusion of PTD, exclusion of infants who became HIV-infected in utero, and stratification by mode of malaria diagnosis.

The average peripheral viral load of participants in this study, all of whom were antiretroviral treatment naïve, was approximately 32,000 copies/mL. The adjusted PR for LBW corresponding to a three-log increase in viral load among women without malaria is 2.82 (95% CI: 1.32 to 6.07), meaning that women with average viral loads have three times the prevalence of LBW as women with an undetectable viral load. Administration of antiretroviral therapy (ART) that reduces viral load to an undetectable limit could lead to extremely significant relative reductions in LBW among malaria-negative Malawian women.

An association between maternal HIV-1 infection and LBW has been previously observed^{1, 2}, including several studies in Malawi^{3, 18}. In a meta analysis of six cohorts of HIV-infected Malawian women, 13% of infants born to HIV-infected women had LBW, although malaria status was not reported³. In this study, 21% of the infants born to HIV-infected women were LBW, more than double the 8% prevalence of LBW among HIV-negative women in the MHP cohort (unpublished observation). Our findings are consistent with the results of a study from Lusaka, Zambia, which quantified the association between maternal viral load and infant birth outcomes, and found that greater HIV-1 disease severity, characterized as peripheral viral load above 100,000 copies/mL, was associated with decreased infant weight and increased infant mortality and morbidity²⁵. Several US-based studies have also quantified the association between HIV-1 disease severity and LBW (or IUGR). Lambert et al. (2000) found neither HIV-1 RNA copy number nor CD4+ T-cell counts were associated with LBW, but increasing HIV-1 culture titer was associated with LBW²⁶; these authors also observed that timing of ART initiation – before or during pregnancy – was not associated with LBW. Tuomala et al (2002) compared women who received ART to ART-naïve women and found no difference in the prevalence of LBW between the treated and untreated women²⁷. Although the authors did not report viral loads, presumably the women on ART had lower viral loads than the untreated women, and thus it can be inferred that viral load was not associated with LBW.

The relationship between severity of HIV-1 infection and PTD differed slightly from the LBW results. Among women without malaria, decreasing CD4 T-cell count and increasing placental viral load, but not increasing peripheral viral load, was associated with increased prevalence of preterm delivery. Unexpectedly, among women with malaria, increasing peripheral viral load was protective against PTD. While the basis for this observed

association is currently unknown, one study suggests that HIV-1 infection can suppress the innate immune response to *P. falciparum*²⁸, which has the potential to modulate inflammation and onset of delivery^{29 30}. Additional analyses are required to determine if this observation is biologically relevant or if it reflects an uncharacterized bias.

There are several limitations to our findings. We cannot determine if the observed associations between severity of HIV-1 infection and LBW are a direct effect of HIV-1, or if the association is secondary to HIV-associated nutritional deficiency; the sample size for the malaria-positive stratum was smaller than the sample size for the malaria-negative stratum; neither prepregnancy body mass index nor other measures of nutritional status were available for analysis; gestational ages were self-reported, and no other measures of gestational age (e.g. e.g., ultrasonographic fetal measurements³¹) were available; the results are generalizable only to ART-naïve HIV-infected women, with normal blood pressure, who delivered in a hospital. Finally, cigarette smoking, a known LBW and PTD risk factor, was not assessed; however, estimates suggest that less than 10% of the adult population in Malawi smoke cigarettes³².

Normal fetal growth represents a finely tuned immune state requiring a temporary immune tolerance to the fetal-placental unit, while still enabling fetal protection from pathogens. Placental inflammation, which could result from a co-infection, can disrupt this tenuous immunological balance and cause LBW. However, clinical data from the MHP cohort suggest that co-infection with malaria or syphilis is not driving HIV-associated LBW. Further, although chorioamnionitis was associated with PTD and LBW, it was not a confounder of the HIV-1 severity-associations: to be a confounder, a variable must be associated with both the exposure (HIV-1 severity) and the outcome (LBW or PTD). The mean placental viral load among women with chorioamnionitis was 3.84 log₁₀ copies/mL compared to 3.73 log₁₀ copies/mL among women without chorioamnionitis (p=0.5); the mean log-transformed peripheral viral load among women with chorioamnionitis was 4.43 log₁₀ copies/mL compared to 4.39 log₁₀ copies/mL among women without chorioamnionitis (p=0.6), and the mean CD4+ T-cell count among women with chorioamnionitis was 380 cells/μL compared to 386 cells/μL among women without chorioamnionitis (p=0.9). Additional studies to determine the prevalence of other pathogenic microbes in this cohort would address the possibility that these results are confounded by a heretofore undetected co-infection.

Following the paradigm of HIV-associated disruption of the blood-brain barrier³³ and HIV-mediated impairment of the mucosal epithelial barrier³⁴, a working hypothesis posits that HIV-1 replication impairs placental function and causes LBW. HIV-1 can replicate in the placenta and it has been detected in placental cells *in vivo*³⁵, and data from several groups indicate that HIV-1 can infect *ex vivo* placental explant cultures^{36, 37}. Our previous work has shown that the placenta represents a unique and hospitable niche for HIV-1 replication; specifically, we observed that the concentration of HIV-1 in the placenta was different than the concentration of HIV-1 in peripheral blood¹³. In addition to the pathogenic effects on HIV-infected cells, it is also plausible that cellular exposure to HIV-1 could be pathogenic and disrupt placental function.

In summary, indicators of severe maternal HIV-1 infection, defined as high placental or peripheral viral load or a low CD4+ T-cell count, were associated in a dose-dependent manner with increased prevalence of LBW. This association was only observed in malaria-negative women, and it was not a secondary effect of PTD or in utero HIV-1 infection. The mechanism of HIV-associated LBW remains unknown.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Correlation between viral load and continuous birth weight or gestational age Birthweight is inversely correlated with HIV-1 concentration in peripheral plasma (panel A), placental plasma (panel B). Gestational age does not correlate with HIV-1 concentration in peripheral plasma (panel C), but is inversely correlated with HIV-1 concentration in placental plasma (panel D). P-values calculated by pairwise correlation. Horizontal, black dotted lines indicate the cutoff for low birthweight (<2500 grams) or preterm birth (<37 weeks).

Table I

Characteristics of HIV-positive participants who delivered live, singleton infants in the Malaria and HIV-1 in Pregnancy study, Malawi, 2000–2004.

CHARACTERISTIC	N= 809
	Median (IQR), n
Age (years)	24 (21, 28), 638
Blood pressure	
Systolic	110 (100, 120), 809
Diastolic	70 (60, 70), 809
Body mass index at delivery	24 (22, 26), 788
CD4+ T-cell count, cells/μL	343 (198, 516), 755
Gestational age, weeks	39 (38, 40), 772
Gravidity	2 (1, 4), 809
Hemoglobin concentration [g/dL]	11 (9, 12), 805
Infant birth weight, kilograms	2.9 (2.5, 3.2), 808
Placental weight, grams	520 (440, 600), 770
Peripheral HIV-1 concentration, log₁₀ copies/mL	4.5 (4.0, 5.0), 539
Placental HIV-1 concentration, log₁₀ copies/mL	3.7 (2.8, 4.4), 275
	n (%)
Anemia (hemoglobin < 11 g/dL)	
No	353 (44)
Yes	452 (56)
Missing	4 (1)
Chorioamnionitis	
No	469 (57)
Yes	207 (26)
Missing	143 (17)
Education level (maternal)	
None	70 (9)
Primary school	477 (59)
Secondary school	260 (32)
University	1 (0)
Missing	1 (0)
Employment	
No	603 (75)
Yes	186 (23)
Missing	20 (2)
Infant gender	
Female	395 (49)
Male	414 (51)
Iron-folate tablets (during antenatal period)	

CHARACTERISTIC	N= 809
	Median (IQR), n
No	111 (14)
Yes	698 (86)
Missing	0 (0)
Low birth weight (<2.5 kilograms)	
No	637 (79)
Yes	171 (21)
Missing	1 (0)
Malaria	
Peripheral blood smear positive	
No	728 (90)
Yes	80 (10)
Missing	1 (0)
Placental blood smear positive	
No	710 (88)
Yes	62 (8)
Missing	37 (5)
Placental histology positive	
No	580 (72)
Yes	147 (18)
Missing	82 (10)
Any malaria	
No	611 (76)
Yes	198 (24)
Missing	0 (0)
Intermittent Presumptive Treatment	
None	55 (7)
1 Dose	261 (32)
2 Doses	314 (39)
3 Doses	175 (22)
Missing	4 (0)
Mode of delivery	
Elective cesarean section	21 (3)
Emergency cesarean section	137 (17)
Vaginal, breech	12 (1)
Vaginal, vacuum extraction	38 (5)
Vaginal, vertex	601 (74)
Missing	0 (0)
Preterm delivery (<37 weeks)	
No	648 (80)
Yes	124 (16)

CHARACTERISTIC	N= 809
	Median (IQR), n
Missing	37 (5)
Residence	
Urban	612 (76)
Periurban	94 (12)
Rural	88 (11)
Missing	15 (2)
Syphilis seropositivity	
No	746 (92)
Yes	60 (7)
Missing	3 (0)

Definitions: n/a= not available; any malaria= a positive malaria diagnosis on peripheral blood smear or placental blood smear or placental histology.

Table 2

Characteristics of HIV-infected women, by adverse birth outcome (n=809).

	None N=570	Low birth weight N=171	Preterm delivery N=124
CHARACTERISTIC	Median (IQR), n	Median (IQR), n	Median (IQR), n
Age, years	24 (21,27), 457	24 (20, 28), 128	24 (20, 29), 99
Body mass index at delivery	24 (23, 26), 554	22 (21, 24), 167	23 (21, 25), 124
Gravidity	2 (1, 4), 570	2 (2, 4), 171	3 (2, 4), 124
[HIV-1], (periphery)*	4.5 (3.8, 5.0), 387	4.6 (4.2, 5.2), 110	4.6 (4.0, 5.1), 86
[HIV-1] (placenta)*	3.5 (2.7, 4.3), 187	4.1 (2.9, 4.8), 65	4.0 (3.2, 4.7), 54
Placental weight, grams	540 (470, 620), 548	420 (370, 500), 162	450 (390, 520), 119
	n (%)	n (%)	n (%)
Anemia (hemoglobin <11 g/dL)			
No	269 (47)	55 (32)	40 (32)
Yes	297 (52)	116 (68)	84 (68)
Missing	4 (1)	0 (0)	0 (0)
Chorioamnionitis			
No	337 (59)	87 (51)	65 (52)
Yes	131 (23)	57 (33)	46 (37)
Missing	102 (18)	27 (16)	13 (10)
CD4+ T-cells <200 cells/μL			
No	418 (73)	100 (59)	76 (61)
Yes	119 (21)	55 (32)	41 (33)
Missing	33 (6)	16 (9)	7 (6)
Infant gender			
Female	261 (46)	98 (57)	63 (51)
Male	309 (54)	73 (43)	61 (49)
Iron-folate tablets			
No	73 (13)	31 (18)	21 (17)
Yes	497 (87)	140 (82)	103 (83)
Length of Gestation (duration)			
Extremely preterm (<28 weeks)	0 (0)	0 (0)	0 (0)
Very preterm (28 – 32 weeks)	0 (0)	12 (8)	12 (10)
Moderately preterm (33 – 34 weeks)	0 (0)	22 (13)	33 (27)
Late preterm (35 – 36 weeks)	0 (0)	45 (26)	79 (64)
Term (37 weeks)	570 (100)	78 (46)	0 (0)
Missing	0 (0)	14 (8)	0 (0)
Malaria (peripheral or placental)			
No	437 (77)	125 (73)	86 (69)
Yes	133 (23)	46 (27)	38 (31)
Malaria Intermittent Preventative Therapy			

	None N=570	Low birth weight N=171	Preterm delivery N=124
CHARACTERISTIC	Median (IQR), n	Median (IQR), n	Median (IQR), n
No	33 (6)	16 (9)	13 (10)
Yes	534 (94)	155 (91)	110 (89)
Missing	3 (1)	0 (0)	1 (1)
Primigravidity			
No	425 (75)	129 (75)	95 (77)
Yes	145 (25)	42 (25)	29 (23)
Missing	0 (0)	0 (0)	0 (0)
Syphilis seroreactivity			
No	531 (93)	157 (92)	110 (89)
Yes	37 (6)	14 (8)	14 (11)
Missing	2 (0)	0 (0)	0 (0)

*
[HIV-1]= HIV-1 RNA concentration, in log₁₀ copies/mL

Unadjusted and adjusted effect of placental viral load, peripheral viral load, and CD4+ T-cell count on prevalence of low birthweight, stratified by maternal malaria status.

Table 3

METRIC	Malaria-positive				Malaria-negative			
	uPR	95% CI	aPR	95% CI	uPR	95% CI	aPR	95% CI
Placental viral load								
Per 1.0-log ₁₀ increase	1.18	0.82, 1.70	1.25	0.87, 1.82	1.24	1.00, 1.53	1.23	1.02, 1.49
Peripheral viral load								
Per 1.0- log ₁₀ increase	0.92	0.61, 1.38	0.86	0.56, 1.32	1.44	1.12, 1.86	1.41	1.10, 1.82
CD4+ T-cell count								
Per 100-cell/ μ l decrease	0.98	0.85, 1.12	0.98	0.86, 1.11	1.13	1.05, 1.23	1.12	1.04, 1.21

uPR: unadjusted prevalence ratio
aPR: adjusted prevalence ratio; all adjusted models control for residence, maternal education, primigravid status and maternal anemia
CI: confidence interval

Unadjusted and adjusted effect of placental viral load, peripheral viral load, and CD4+ T-cell count on prevalence of preterm delivery, stratified by maternal malaria status.

Table 4

METRIC	Malaria-positive				Malaria-negative			
	uPR	95% CI	aPR	95% CI	uPR	95% CI	aPR	95% CI
Placental viral load								
Per 1.0- log ₁₀ increase	1.09	0.73, 1.62	1.05	0.70, 1.56	1.33	1.04, 1.69	1.29	1.02, 1.64
Peripheral viral load								
Per 1.0- log ₁₀ increase	0.71	0.49, 1.03	0.55	0.36, 0.85	1.21	0.90, 1.62	1.16	0.86, 1.55
CD4+ T-cell count								
Per 100-cell/ μ l decrease	1.04	0.89, 1.21	1.02	0.88, 1.18	1.18	1.07, 1.30	1.16	1.05, 1.28

uPR: unadjusted prevalence ratio
aPR: adjusted prevalence ratio; all adjusted models control for residence, maternal education, primigravid status and maternal anemia
CI: confidence interval