The Effect of HIV Infection on the Risk, Frequency, and Intensity of *Plasmodium falciparum* Parasitemia in Primigravid and Multigravid Women in Malawi

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**Abstract.** Human immunodeficiency virus (HIV) is common in pregnant women in many malaria-endemic regions and may increase risk of placental parasitemia. Placental malaria is more common in primigravidae than multigravidae, but the relationship between HIV and malaria across gravidities is not well characterized. We recruited pregnant Malawian women during the second trimester and followed them until delivery. Parasitemia was assessed at enrollment, follow-up visits, and delivery, when placental blood was sampled. There was no difference in risk of parasitemia between HIV-positive and HIV-negative primigravidae. Among multigravidae, HIV-infected women had greater than twice the risk of parasitemia as HIV-uninfected women throughout follow-up. Human immunodeficiency virus was also associated with more frequent peripheral parasitemia in multigravidae but not primigravidae. Both HIV and primigravid status were independently associated with higher peripheral and placental parasite densities. Although risk of parasitemia is lower in multigravidae than primigravidae, the HIV effect on risk of malaria is more pronounced in multigravidae.

**INTRODUCTION**

Each year, more than 20 million women become pregnant in malaria-endemic areas of Africa.14 Infection with *Plasmodium falciparum* during pregnancy contributes to 75,000 to 200,000 infant deaths per year.3 Placental malaria is associated with spontaneous abortion and stillbirth and intrauterine growth retardation, prematurity, and severe maternal anemia, which are risk factors for low birth weight and neonatal mortality.4,5 Placental malaria occurs when pregnant women are infected by particular strains of *P. falciparum* that produce variant surface antigens on the surface of red blood cells with special affinity for receptors expressed on the surface of placental cells, specifically chondroitin sulfate A. These variants exclusively cause disease in pregnant women, so primigravidae are generally at highest risk of infection with these variants because of their lack of previous exposure. With successive pregnancies, women gradually develop immunity to these pregnancy-specific parasite populations; thus, the risk of placental malaria decreases in later pregnancies.6

Human immunodeficiency virus (HIV)-infected persons are at increased risk of clinical malaria compared with HIV-uninfected persons and have a poorer response to antimalarial drugs in some studies.7–10 The HIV increases the risk of maternal parasitemia during pregnancy,11 and of congenital malaria in the infant.12 Although congenital malaria is uncommon, it has been associated with poor maternal antibody response to malaria antigens in HIV-positive women.13 The effect of HIV on malaria risk during pregnancy is complex, however, and appears to differ between primigravid and multigravid women. Many studies have shown an increased risk of placental parasitemia in multigravid women infected with HIV, suggesting that the relative protection from placental malaria that arises with subsequent pregnancies may be attenuated by HIV infection13–16; there is no evidence suggesting that HIV infection increases the risk of parasitemia in primigravidae.

Many of the existing studies investigating this relationship between HIV infection and malaria during pregnancy have been cross-sectional, limiting ability to detect variation in risk of malaria over the course of pregnancy. To evaluate the effect of HIV infection on risk of parasitemia and placental malaria in both primigravid and multigravid women, we conducted a longitudinal study of pregnant women in southern Malawi, where the prevalence of HIV in prenatal clinics is ~12% and malaria is endemic.17

**METHODS**

**Study population and study site.** The study population consisted of all healthy women in their second trimester of pregnancy attending the Mpemba and Madziabango Health Centers in Blantyre District in southern Malawi for antenatal care and delivery between March 2005 and February 2006. These two health centers are located in rural areas outside Blantyre, Malawi’s second largest city. Exclusion criteria included declining voluntary counseling and testing for HIV.

**Data collection.** Participants were administered a questionnaire by the study nurses at enrollment about demographic characteristics, socio-economic factors, and malaria prevention behaviors. Participating women were encouraged to attend visits at 26, 32, and 36–38 weeks gestation according to standard antenatal care guidelines. Women were administered sulfadoxine-pyrimethamine (SP) for intermittent preventive therapy in pregnancy (IPTp) or treated for clinical malaria according to national guidelines. The HIV-infected women were given nevirapine during labor and their infants were treated with nevirapine after delivery according to Malawi’s national guidelines at the time. All HIV-infected women were referred to an antiretroviral treatment program.

**Laboratory procedures.** Participants were screened for HIV infection at enrollment with two rapid HIV-1 antibody tests: Determine (Inverness Medical Innovations, Inc., Waltham, MA) and Unigold (Trinity Biotech, Bray, Ireland). The HIV infection was defined as a positive result by both rapid tests: Determine sulfadoxine-pyrimethamine (SP) for intermittent preventive therapy in pregnancy (IPTp) or treated for clinical malaria according to national guidelines. The HIV-infected women were given nevirapine during labor and their infants were treated with nevirapine after delivery according to Malawi’s national guidelines at the time. All HIV-infected women were referred to an antiretroviral treatment program.

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preparation of thick blood smears on slides. Peripheral malaria parasitemia was assessed through microscopic examination of stained thick blood smear slides on site by trained laboratory technicians. At delivery, placental, cord, and peripheral blood samples were collected, and thick blood smears were prepared and examined as described previously. For quality control, 10% of randomly selected slides were re-examined by the laboratory supervisor at Ntcheu District Hospital.

Outcomes. Parasitemia was defined as the presence of parasites in thick blood smears. Malaria parasites were quantified against 200 white blood cells (WBCs). Parasite density was calculated assuming 6,000 WBCs/μL of blood. Frequency of peripheral parasitemia over follow-up was defined as the number of episodes of parasitemia during follow-up visits. Because we could not distinguish between recrudescence and reinfec tion, episodes of parasitemia were assumed to be independent across visits. Parasitemia was analyzed in four ways: 1) the presence or absence of parasitemia at enrollment, delivery, and anytime during the follow-up period; 2) average longitudinal risk of parasitemia during follow-up, which is the marginal probability of developing parasitemia over follow-up taking into account clustering, i.e., the possibility of more than one parasitemia episode per woman; 3) number of episodes of parasitemia during follow-up; and 4) peripheral and placental parasite density at delivery.

Statistical analysis. Binomial regression was used to estimate prevalence and risk ratios for parasitemia. Parasitemia risk over visits was analyzed using weighted generalized estimating equations (wGEE) to account for the possibility that data were missing at random. In these models, each individual response is weighted by the inverse probability of a missing response given the other responses, i.e., the probability of a missing measurement given the other measurements for a given subject. We used the following model of covariates to estimate the weight:

\[
\text{Logit}(\theta_{hi}) = \alpha + \sum_{k=1}^{7} \beta_k x_{hik},
\]

where \(\theta_{hi} = \Pr[\text{missing response at visit } h | \text{ non-missing response at visit } h-1].\) In the above model, \(h\) indexes visits, \(i\) indexes subjects, and \(k\) indexes covariates. The set of covariates used in the dropout model included continuous maternal weight in kg, maternal age at enrollment, indicators for access to an unsafe water source, < 8 years of education, primigravidity, husbands’ occupation, and low housing quality. The polynomic outcome, number of parasitemia episodes, was analyzed using generalized logistic regression. Parasite density at delivery was analyzed using zero-inflated negative binomial (ZINB) regression. The advantage of ZINB regression is that it takes into account the semi-continuous nature (excess zeros) of parasite density and allows for overdispersion in nonzero values of parasite density. This is preferable to comparing geometric mean parasite density using Student’s \(t\) test and analysis of variance or to performing analyses on log-transformed parasite density for two reasons. First, because of the high degree of skew in parasite density, log-transformation may not result in a normal distribution of transformed values precluding use of tests with a normality assumption; additionally, the optimal Box-Cox transformation that would result in a normal distribution may vary from population to population, given factors such as seasonality and transmission intensity that tend to differ across populations. Second, back-transformation of transformed values may not result in sensible results. The ZINB regression appropriately takes into account the distribution of parasite density and allows for the estimation of a predicted mean change in parasite density, which has use in determining the impact of the risk/protective factors of interest on parasite burden.

Covariate inclusion in regression modeling was decided using a causal diagram. Malaria preventive behaviors are endogenous to socioeconomic/demographic factors so these variables were coupled together. Age and gravidity were correlated to the point of exchangeability; therefore, gravidity was chosen for analysis because it was the main confounder of interest. The wGEE were conducted using the macro developed by Molenbergh and Verbeke. All analyses except one were conducted using SAS version 9.1 for Windows (SAS Inc., Cary, NC). Zero-inflation negative binomial regression was conducted using StataEE version 10 (StataCorp., College Station, TX).

Ethical considerations. Informed consent was obtained from all participating women. The study was approved by the institutional review boards of the University of North Carolina at Chapel Hill and the University of Malawi College of Medicine.

RESULTS

One thousand four hundred ninety-six (1,496) pregnant women were approached about the study; of these, 111 women did not agree to voluntary counseling and testing for HIV, 15 had discordant HIV rapid test results, and 4 others were not available for follow-up. The remaining 1,366 women were included in the analyses, and 831 (61%) were followed until delivery. The characteristics of participating women are presented in Table 1. The HIV-infected primigravidae had higher educational attainment and higher rates of reported bed net use and bed net treatment with insecticide at regular intervals than HIV-uninfected primigravidae. A higher proportion of HIV-infected primigravidae reported receiving antimalarials for treatment of clinical malaria during pregnancy before enrollment than HIV-uninfected primigravidae. These differences were not observed among multigravidae.

During the follow-up period, 605 women (41%) experienced at least one episode of parasitemia (Table 2 and Figure 1). Of these women, 35% experienced more than one episode. For primigravidae, at enrollment, HIV-infected women had a lower prevalence of parasitemia (43.5%) than HIV-uninfected women (58.9%), and this difference diminished over time. These differences were not significant and were likely caused by differences in behaviors described previously. In contrast, among multigravidae, HIV-infected women were consistently more likely to be parasitemic than HIV-uninfected women at all time points.

Women with higher levels of education and higher bed net use were, in general, protected against parasitemia. In multivariate analysis, low educational attainment among primigravidae was associated with parasitemia at enrollment (prevalence ratio [PR] = 2.25, 95% confidence interval [CI]: 1.29, 3.91) and anytime over follow-up (risk ratio [RR] = 1.80, 95% CI: 1.01, 3.50). There was a similar but non-significant trend among multigravidae, in whom the effect estimates were 1.76 (95% CI: 0.40, 2.15) and 1.10 (95% CI: 0.64, 1.90), respectively. Reported use of a bed net was inversely associated with
HIV infection was associated with more episodes of parasitemia. The frequency of parasitemia is shown in Table 4. Among multigravidae, but not in primigravidae, the effect of HIV infection on parasitemia is presented in Table 3. After adjusting for education, maternal age, weight and bed net use, number of IPTp doses received, HIV-infected primigravidae were slightly less likely to have parasitemia at enrollment; however, this result was not significant and was imprecise with a confidence limit ratio of 6.2. Similarly, there was no difference in risk of parasitemia during follow-up or delivery between HIV-infected and HIV-uninfected primigravidae. In contrast, there was a significant difference in prevalence of parasitemia in HIV-positive and HIV-negative multigravidae at all time points, with HIV-positive women being 2.16–2.44 times as likely to be parasitemic. Results from wGEE for multigravidae were more pronounced because of zero cell counts at some visits; for multigravida women, wGEE revealed that HIV-positive women had a 2.63 times higher average risk of parasitemia than HIV-negative women. Thus, HIV infection increased the likelihood of malaria parasitemia in multigravidae but not in primigravidae.

Multivariate analysis of risk factors for number of episodes of parasitemia is shown in Table 4. Among multigravidae, HIV infection was associated with more episodes of parasitemia, with a RR of 4.82 (95% CI: 2.12, 10.96) for more than three episodes. The HIV was not significantly associated with a higher frequency of parasitemia in primigravid women. Low educational level and bed net use were not associated with frequency of parasitemia. Receiving exactly two doses of SP IPTp appeared slightly protective against repeated episodes of parasitemia when compared with just one or more than three doses.

At delivery, infection with HIV was associated with higher peripheral and placental parasite densities than in HIV-uninfected women when adjusted for gravidity, maternal weight, and bed net use, with a greater effect estimate observed in the placenta (Table 5). Independent of HIV infection, primigravidity was associated with higher peripheral and placental parasite densities, an effect again more pronounced in placental samples. Although both reported bed net use and regular insecticide impregnation of the bed net were associated with lower peripheral parasite densities, only reported insecticide treatment of bed nets was associated with lower placental parasite densities.

### DISCUSSION

In this article, we report the effect of HIV infection on malaria parasitemia in a cohort of pregnant women observed from the second trimester until delivery. The HIV infection was associated with a higher prevalence of parasitemia at delivery, infection with HIV was associated with higher peripheral and placental parasite densities than in HIV-uninfected women when adjusted for gravidity, maternal weight, and bed net use, with a greater effect estimate observed in the placenta (Table 5). Independent of HIV infection, primigravidity was associated with higher peripheral and placental parasite densities, an effect again more pronounced in placental samples. Although both reported bed net use and regular insecticide impregnation of the bed net were associated with lower peripheral parasite densities, only reported insecticide treatment of bed nets was associated with lower placental parasite densities.
enrollment and with increased frequency of peripheral parasitemia during follow-up in multigravid but not primigravid women. It was associated with a higher prevalence of placental parasitemia regardless of gravidity.

We expected to see a higher prevalence of parasitemia in HIV-infected primigravid women compared with HIV-uninfected primigravidae but instead found a lower prevalence of parasitemia in women with HIV infection, though this was not significant. This lack of association might be caused by higher levels of educational attainment (negatively associated with risk of parasitemia in our cohort) and more frequent malaria preventive behaviors reported by HIV-infected primigravidae compared with HIV-uninfected primigravidae. In multivariate analyses, there was no difference in the prevalence ratio for malaria parasitemia between HIV-infected and uninfected primigravidae.

In contrast, HIV-infected multigravidae were more likely to be parasitemic than HIV-uninfected multigravidae. Among multigravidae, HIV infection doubled the risk of parasitemia at any time during follow-up; this effect was also seen when estimating the average risk of parasitemia over follow-up. Ordinarily, multigravidae are protected against malaria compared with primigravidae. In our study, HIV infection nullified this protective effect.

For most women, the enrollment visit was their first antenatal clinic visit and should have been the first visit at which they would have received IPTp; however, HIV-infected primigravidae to report receiving an IPTp dose before enrollment. The reason for this finding is not known and could be related to this group’s higher educational status, which may indicate greater access to social services such as schools and health care. Alternatively, HIV-infected primigravidae may have been more likely to experience parasitemia early in pregnancy before enrolling in the study. Consistent with this hypothesis, HIV-infected primigravidae were more than four times as likely to report having received antimalarial treatment during the pregnancy before enrollment. A similar relationship was not observed among multigravidae, but it is well established that immunity to malaria during pregnancy increases with subsequent pregnancies. Both elevated prevalence of parasitemia in HIV-positive women in the first trimester and treatment with antimalarials before initiation of IPTp has been observed in another study from Malawi. Higher risk for malaria infection for HIV-infected women in early pregnancy and consequent treatment before enrollment may have contributed to the lower prevalence of parasitemia at enrollment.

As shown in Figure 1, the risk difference for parasitemia caused by HIV infection among multigravidae did not change significantly across follow-up visits, suggesting a constant effect of HIV infection on risk of parasitemia in multigravid women. In contrast, the risk difference for parasitemia caused by HIV infection in primigravid women increased across visits, suggesting that the effect of HIV infection on parasitemia risk may vary over the course of pregnancy; however,

Table 3

<table>
<thead>
<tr>
<th>Time point</th>
<th>Primigravidae</th>
<th>Multigravidae</th>
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<tbody>
<tr>
<td></td>
<td>Fraction parasitemic (%)</td>
<td>Prevalence ratio (95% CI)</td>
</tr>
<tr>
<td>Enrollment</td>
<td>10/23 (43.5)</td>
<td>186/316 (58.9)</td>
</tr>
<tr>
<td>Anytime during follow-up*</td>
<td>8/20 (40)</td>
<td>121/291 (41.6)</td>
</tr>
<tr>
<td>Delivery</td>
<td>4/14 (28.6)</td>
<td>45/202 (21.3)</td>
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<tr>
<td>Average risk over follow-up†</td>
<td>–</td>
<td>–</td>
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* Excludes enrollment visit, risk ratio.
† Primigravidae weighted generalized estimating equations (wGEE) results not reported because of sparseness of data across visits, average risk over follow-up, frequencies vary according to visit.

Models adjusted for education, maternal weight, bed net use, and number of SP doses received.
our cohort included a relatively small number of HIV-positive primigravid women, which limits our ability to draw conclusions about this relationship.

The finding that HIV infection is associated with a higher number of parasitic episodes during pregnancy corroborates previous literature. In a study conducted in Malawian non-pregnant adults, HIV infection increased the hazard of one episode 1.8 times (95% CI: 1.2, 2.7), and two episodes 2.5 times (95% CI: 1.5, 4.2)\(^2\) in this study, we report similar and more precise estimates for one and two episodes among multigravidae. We also found a modest association between HIV infection and having three or more episodes of parasitemia among multigravidae, a relationship that was not observed in primigravidae.

The elevated risk of placental parasitemia observed among primigravidae and multigravidae support findings from previous studies. An association between HIV infection and higher parasite density has been reported elsewhere.\(^14,21,22\) Consequently, the differences observed among primigravidae and multigravidae in the effect of HIV infection on parasitemia may reflect more advanced HIV disease in multigravidae, who were older. We also did not collect data on the use of cotrimoxazole by HIV-infected women, which has been shown to be protective against malaria\(^3\) and could be a confounder by lowering the risk of parasitemia among HIV-infected women taking the drug. Cotrimoxazole use was not standard of care in Malawi when this study was conducted, therefore we expect that few women received the drug. Furthermore, if HIV infection is associated with early pregnancy loss, enrollment of HIV-infected women with sufficient healthy pregnancies to reach the second trimester may have resulted in selection bias. This may be associated with other factors such as socioeconomic status and may be differential according to gravidity, because primigravidae are generally at much higher risk of adverse pregnancy outcomes than multigravidae.

There are however, some unique strengths to our study. The longitudinal study design and analytical procedures enabled examination of parasitemia risk from the second trimester until delivery. Additionally, the richness of sociodemographic information recorded from the women that allowed for a comprehensive missingness model also allowed

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</thead>
<tbody>
<tr>
<td>HIV infection</td>
<td>0.76 (0.38–1.54)</td>
<td>1.31 (1.02–1.67)</td>
<td>1.40 (0.68–2.89)</td>
<td>2.20 (1.53–3.15)</td>
<td>1.49 (0.64–3.51)</td>
<td>4.82 (2.12–10.96)</td>
</tr>
<tr>
<td>Less than 8 years of education</td>
<td>1.08 (0.78–1.51)</td>
<td>1.06 (0.77–1.46)</td>
<td>1.77 (0.99–3.15)</td>
<td>0.86 (0.52–1.43)</td>
<td>1.98 (0.90–4.37)</td>
<td>N/A</td>
</tr>
<tr>
<td>Report always using bed net SP doses</td>
<td>0.64 (0.42–0.99)</td>
<td>0.96 (0.77–1.19)</td>
<td>0.62 (0.32–1.17)</td>
<td>0.96 (0.18–5.00)</td>
<td>1.01 (0.55–1.85)</td>
<td>0.95 (0.41–2.20)</td>
</tr>
<tr>
<td>0–1</td>
<td>1.44 (0.96–2.17)</td>
<td>1.19 (0.93–1.53)</td>
<td>1.03 (0.57–1.84)</td>
<td>0.73 (0.45–1.18)</td>
<td>0.54 (0.19–1.55)</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>0.75 (0.55–1.01)</td>
<td>0.97 (0.78–1.20)</td>
<td>0.80 (0.42–0.96)</td>
<td>0.68 (0.47–1.00)</td>
<td>0.50 (0.29–0.86)</td>
<td>0.48 (0.21–1.10)</td>
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<tr>
<td>3+</td>
<td>Reference</td>
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Multivariate analysis of risk factors for number of episodes of parasitemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Primigravigd (N = 339)</th>
<th>Multigravigd (N = 1122)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report always using bed net</td>
<td>0.75 (0.55–1.01)</td>
<td>0.97 (0.78–1.20)</td>
</tr>
<tr>
<td>SP doses received</td>
<td>Reference</td>
<td>Reference</td>
</tr>
</tbody>
</table>

HIV infection 2.59 (1.25–5.34) 3.56 (1.76–7.21)  
Primigravid 2.03 (1.11–3.73) 3.97 (2.16–7.30)  
Maternal weight, kilograms (continuous) 0.94 (0.89–1.00) 0.89 (0.85–0.92)  
Report always using bed net* 0.42 (0.20–0.87) 1.22 (0.57–2.62)  
Report insecticide impregnation in past 6 months 0.35 (0.17–0.73) 0.19 (0.04–0.94)  

Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Periphieral</th>
<th>Placental</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV infection</td>
<td>0.75 (0.55–1.01)</td>
<td>0.97 (0.78–1.20)</td>
</tr>
<tr>
<td>Primigravid</td>
<td>2.03 (1.11–3.73)</td>
<td>3.97 (2.16–7.30)</td>
</tr>
<tr>
<td>Maternal weight, kilograms (continuous)</td>
<td>0.94 (0.89–1.00)</td>
<td>0.89 (0.85–0.92)</td>
</tr>
<tr>
<td>Report always using bed net*</td>
<td>0.42 (0.20–0.87)</td>
<td>1.22 (0.57–2.62)</td>
</tr>
<tr>
<td>Report insecticide impregnation in past 6 months</td>
<td>0.35 (0.17–0.73)</td>
<td>0.19 (0.04–0.94)</td>
</tr>
</tbody>
</table>

CI = Confidence Interval, SD = Standard deviation, N = 831.

* All variables in model. Bed net model excludes insecticide impregnation variable.
for the examination of the role of other individual factors in determining parasitemia risk over the follow-up period.

With the reduced effectiveness of SP IPTp among HIV-infected women, it is important that antenatal care services promote the full repertoire of malaria prevention methods. The study underscores the necessity of targeting both primigravidae and multigravidae for malaria prevention in areas with a high prevalence of HIV infection. Although we report an elevated risk of parasitemia among multigravidae throughout the second and third trimesters, our results suggest that the effect of HIV infection on parasitemia risk may be less important for primigravidae, who are at higher risk of malaria than multigravidae. Further studies are needed to understand the effect of HIV infection on parasitemia risk over the course of pregnancy.

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REFERENCES