

## Effect of HIV Infection and *Plasmodium falciparum* Parasitemia on Pregnancy Outcomes in Malawi

Ella T. Nkhoma, Linda Kalilani-Phiri, Victor Mwapasa, Stephen J. Rogerson, and Steven R. Meshnick\*

Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, North Carolina; Department of Community Health, College of Medicine, University of Malawi, Blantyre, Malawi; Department of Medicine, University of Melbourne, Parkville, Australia

**Abstract.** *Plasmodium falciparum* and human immunodeficiency virus (HIV) are both risk factors for low birth weight (LBW) and maternal anemia, and they may interact to increase risk of adverse pregnancy outcomes. In 2005 and 2006, we followed 831 pregnant women attending antenatal care clinics in southern Malawi through delivery. HIV was associated with increased risk of LBW (adjusted prevalence ratio [PR<sub>adj</sub>] = 3.08, 95% confidence interval [CI] = 1.40, 6.79). Having greater than or equal to three episodes of peripheral parasitemia was also associated with increased risk of LBW (PR<sub>adj</sub> = 2.68, 95% CI = 1.06, 6.79). Among multigravidae, dual infection resulted in 9.59 (95% CI = 2.51, 36.6) times the risk of LBW compared with uninfected multigravidae. HIV infection and placental parasitemia were each associated with increased risk of anemia. Thus, HIV infection and parasitemia are important independent risk factors for adverse pregnancy outcomes. Among multigravidae, HIV infection and placental parasitemia may interact to produce an impact greater than the sum of their independent effects.

### INTRODUCTION

Malaria is a tremendous public health challenge in Malawi, with 3.6 million reported cases resulting in almost 8,000 deaths annually.<sup>1</sup> Each year in Malawi, there are approximately 500,000 pregnancies, and all are at risk for malaria.<sup>2</sup> For these women, infection with *Plasmodium falciparum* parasites may result in adverse pregnancy outcomes, including malaria-associated infant deaths.<sup>3,4</sup> These adverse outcomes may be mediated through placental malaria, a condition associated with spontaneous abortion, stillbirth, intrauterine growth retardation, pre-maturity, and severe maternal anemia.<sup>5,6</sup> The latter three conditions can lead to delivery of an infant with low birth weight (LBW), one of the most important risk factors for neonatal mortality and developmental impairment.<sup>7,8</sup>

The prevalence of human immunodeficiency virus (HIV) infection in Malawi is also high, with an estimated 12% of antenatal care clinic attendees affected.<sup>2</sup> HIV infection has also been found to be associated with adverse pregnancy outcomes such as infant mortality and LBW.<sup>9,10</sup> The increased risk of malaria coupled with HIV infection may interact to result in adverse pregnancy outcomes more pronounced than would be expected with HIV infection or malaria alone.<sup>11</sup> Studies conducted in Malawi, Tanzania, and Kenya have reported increased risk of neonatal mortality, maternal anemia, and LBW in the presence of dual infection by malaria parasites and HIV versus malaria parasitemia or HIV alone.<sup>12–14</sup> However, previous studies have relied on a cross-sectional design and determined malaria parasitemia status at single points during pregnancy. In this study, we evaluate the effects of HIV and malaria parasitemia during pregnancy on maternal anemia and LBW among a cohort of pregnant women in Malawi followed from the second trimester until delivery.

### MATERIALS AND METHODS

**Study population and data collection procedures.** This was an observational study of pregnant women receiving routine antenatal care at the Mpemba and Madziabango Health Centers in a rural area of Blantyre District, Malawi. Between March of 2005 and February of 2006, healthy pregnant women in their second trimester attending the antenatal care clinics at the study sites were invited to participate in the study. At enrollment, study nurses collected information on basic demographic characteristics, socioeconomic data, and malaria prevention activities from participating women. Women agreeing to voluntary counseling and testing (VCT) were tested for HIV on site in addition to receiving pre- and post-test counseling. HIV-infected women and their infants were given nevirapine according to national guidelines. Additionally, women found to be infected with HIV were referred to an antiretroviral treatment program. Women attending the antenatal care clinics were administered sulfadoxine-pyrimethamine (SP) for intermittent presumptive therapy in pregnancy (IPTp) and treated for clinical malaria also according to national guidelines.

Women attended visits according to the standard antenatal care guidelines, with visits scheduled after enrollment occurring at approximately 26, 32, and 36–38 weeks of gestation. At each visit, a finger prick blood sample was collected for thick blood film examination for malaria parasites and determination of hemoglobin level. For women delivering at the health centers, placental, cord, and peripheral blood films were collected and examined for malaria parasites on site. Placenta histology was not conducted. Birth weight, measured with simple spring balances, was also recorded. For women not delivering at the health centers, an attempt was made to identify the women and record birth weight within 24 hours of delivery. It was not possible to follow-up with non-attendees at home, and home deliveries were only included when women attended the center for a post-natal visit.

**Laboratory procedures.** Peripheral malaria parasitemia was assessed through microscopic examination of thick blood smear slides on site by trained laboratory technicians after collecting

\*Address correspondence to Steven R. Meshnick, Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, CB #7435, Chapel Hill, NC 27599. E-mail: meshnick@unc.edu

blood samples from the participating women. Malaria parasites were quantified against 200 white blood cells (WBCs). Placental parasitemia was also assessed through thick blood smear. For quality control purposes, a 10% random sample of slides was reexamined by the laboratory supervisor at Ntcheu District Hospital. HIV infection was assessed using two rapid HIV-1 antibody tests: Determine and Unigold. There was 95% (95% confidence interval = 93.2–97.8%) agreement between the two tests in identifying HIV-positive women. CD4+ cell counts were not evaluated. Hemoglobin levels were estimated from the finger prick blood samples using HemoCue on site at each visit and post-partum.

**Definitions.** Parasitemia was defined as the presence of parasites in thick blood smears. Parasite density per microliter was computed assuming 6,000 WBC/ $\mu$ L blood. Among women with placental parasitemia, mild parasite density was defined as  $< 20,000$  parasites/ $\mu$ L, and severe parasitemia was defined as  $\geq 20,000$  parasites/ $\mu$ L. Peripheral parasitemia over the follow-up period was defined as the number of episodes of parasitemia over the follow-up visits. Because we could not distinguish between recrudescence and reinfection, measurements of parasitemia were assumed to be independent across visits. Malaria parasitemia at delivery was defined as peripheral and/or placental parasitemia on delivery. Fever was rarely observed and thus, was not included in the definition of parasitemia. Anemia was defined as hemoglobin  $< 11$  g/dL at delivery. HIV infection was defined as a positive result on two rapid tests: Determine and Unigold. Discordant results were excluded from analyses. Neonates were considered as having LBW if they weighed less than 2,500 g. For the sociodemographic characteristics, unsafe water sources were defined as unprotected wells, lakes, rivers, or ponds. Low housing quality was defined as housing with grass roofs and mud or grass walls with open unscreened windows.

**Statistical analysis.** Bivariate and multivariate analyses for dichotomous outcomes were conducted using binomial regression. When the binomial regression model was unstable, logistic regression was used. Continuous birth weight and hemoglobin were analyzed using general linear models.

To assess confounding for dichotomous outcomes, bivariate analyses with potential confounding variables were performed for both the outcomes and main exposures. In the case of binary variables, if the resultant relative risks were less than or equal to 0.7 or greater than or equal to 1.3, they were considered as potential confounders. The number of potential confounders included in final models was further narrowed using a change in estimate approach with a 10% cutoff. Covariate inclusion for continuous outcomes models mirrored the inclusion of the binary outcomes models. Interaction effects were assessed through the inclusion of interaction terms in the models and the use of the spreadsheet by Andersson and others<sup>15</sup> to determine the relative excess prevalence caused by interaction (REPI). All analyses were performed using SAS v9.1 (Cary, NC).

**Ethical considerations.** Informed consent was obtained from all participating women in Chichewa. The study was reviewed and approved by the Institutional Review Boards at the University of North Carolina at Chapel Hill and the University of Malawi College of Medicine.

## RESULTS

The study enrolled 1,496 women; 831 women completed follow-up until delivery, with 590 delivering at the health

centers. Descriptive characteristics of the study population are reported in Table 1. On average, participating women attended two follow-up visits after enrollment. The average age of the women was 23.4 years (standard deviation [SD] = 5.4). The average gestational age at enrollment was 23 weeks (SD = 3.6) and ranged between 14 and 28 weeks. The prevalence of HIV was 13%. The proportion of women experiencing two or more episodes of malaria over the follow-up period was 18%. The mean number of visits among women with one malaria episode over follow-up was 2.9 (SD = 1.1). The mean number of visits among women with two and three malaria episodes over follow-up was 3.1 (SD = 1.1) and 3.5 (SD = 1.0), respectively. There were no significant differences in the main exposures and socio-demographic characteristics between women who were lost to follow-up and women who completed follow-up. There was, however, a difference in maternal weight, with women not lost to follow-up being on average of 0.81 kg heavier than women lost to follow-up. There were no significant differences in the main variables among women delivering at the health centers versus women delivering elsewhere except for peripheral parasitemia, with women delivering elsewhere slightly more likely to have peripheral parasitemia than women delivering at the health centers (PR = 1.11, 95% CI = 1.06–1.17).

**LBW.** Information on birth weight was available for 585 women. Among these women, the incidence of LBW was 9%, and mean birth weight was 3,055 g (SD = 478). In bivariate analyses, HIV infection, placental parasite density  $\geq 20,000/\mu$ L, and greater than or equal to three episodes of peripheral parasitemia over follow-up were strongly associated with LBW (Table 2). Placental parasitemia (any density) was marginally associated with LBW in both bivariate and multivariate analyses. Furthermore, only HIV infection and greater than or equal to three episodes of peripheral parasitemia over follow-up were strongly associated with LBW after adjusting for potential confounders (Table 2). Additionally, the risk of LBW among primigravidae was nearly two times the risk among multigravidae after adjusting for maternal weight, unsafe water source, gravidity, and HIV status (Table 2).

Multivariate analyses of mean birth weight revealed a 138-g reduction in birth weight by HIV infection (Table 2). Placental parasitemia at delivery did not result in a notable reduction in birth weight; however, the mean difference for experiencing peripheral parasitemia greater than or equal to three times over follow-up was large ( $-163$ , 95% CI =  $-351$  to  $26$ ). Decreasing numbers of SP IPTp doses received were associated with decreasing birth weight ( $P < 0.01$ ) (Table 2). Being primigravid was associated with a 208-g decrease in mean birth weight (Table 2).

**Maternal anemia.** Hemoglobin measurements at delivery were available for 732 women. The prevalence of anemia ( $< 11$  g Hb/dL) was similar to the prevalence of LBW at 9%. HIV infection and placental and peripheral parasitemia were associated with an increased risk of maternal anemia and reductions in mean hemoglobin, with placental parasitemia having the strongest effect (Table 3). Maternal anemia risk varied with the degree of parasite density, with a parasite density  $\geq 20,000$  parasites/ $\mu$ L resulting in the highest increased risk and a higher reduction in mean hemoglobin. Maternal anemia risk was also associated with number of

TABLE 1  
Characteristics of the study population

	All enrolled women		All women followed until delivery		Women delivering at the health centers	
	N*	Percent	N	Percent	N	Percent
Overall	1,496	100	831	100	590	100
Age (years)						
15–19	368	24.6	227	27.3	162	27.5
20–24	538	36.0	286	34.4	200	33.9
25	590	39.4	318	38.3	228	38.7
Primigravid	370	24.8	235	28.4	170	28.9
Education = 8 years	1,290	86.3	686	82.7	491	83.2
Married	1,371	91.9	757	91.3	535	90.8
Unemployed	789	52.8	433	52.1	316	53.6
Unsafe water source	443	29.6	197	23.7	150	25.4
Low housing quality	345	23.1	199	24.0	132	22.4
Report always using a bed net	344	23.0	203	24.4	139	23.6
Report insecticide impregnation of the bed net in the past 6 months	297	19.9	182	21.9	123	20.9
Weight (kg; mean ± SD)	1,488	54.7 ± 6.8	828	55.1 ± 6.9	588	55.2 ± 7.0
HIV seropositive	185	13.5	102	13.5	66	12.3
Placental parasitemia	–	–	111	13.4	93	15.9
Peripheral parasitemia	476	31.8	112	13.5	95	16.2
Number of times parasitemic over pregnancy						
0	838	56	444	53.4	311	52.7
1	438	29.3	237	28.5	166	28.1
2	136	9.1	84	10.1	61	10.3
3	84	5.6	66	7.9	52	8.8
SP doses received						
0	85	5.7	34	4.1	26	4.4
1	388	25.9	156	18.8	108	18.3
2	561	37.5	329	39.6	238	40.3
3	462	30.9	312	37.6	218	37.0
Hemoglobin (g/dL; mean ± SD)	1,486	11.0 ± 1.6	732	12.3 ± 1.7	541	12.3 ± 1.7
Hemoglobin < 11 g/dL	345	23.2	65	8.9	47	8.7
Low birth weight (< 2,500 g)	–	–	–	–	49	8.7
Birth weight (g; mean ± SD)	–	–	–	–	590	3,057 ± 478

\*Sums may not add up to total because of missing values. All measurements were taken at enrollment.  
SD = standard deviation.

parasitemia episodes, with having two or more episodes being associated with increased anemia risk and significant reductions in mean hemoglobin. Compared with women who received greater than or equal to three doses of SP IPTp, receiving only one dose was associated with an elevated risk of maternal anemia before adjustment. There were no significant differences in anemia risk according to doses of SP received after multivariate adjustment (Table 3).

**Interaction between HIV and parasitemia effects.** No significant interaction effects between HIV and peripheral or placental parasitemia were found during multivariate analyses for both LBW and maternal anemia when all gravidities were analyzed together. The overall REPI for LBW was 6.72 (95% CI = –3.65 to 17.08). The REPI indicates the amount of observed prevalence that is beyond the sum of the independent effects of HIV and placental parasitemia. However, after stratifying by gravidity, the odds of LBW among multigravidae with both HIV infection and placental parasitemia were 9.6 (95% CI = 2.5–36.6) times as high as the odds among women with neither infection (Table 4).

Even after stratifying by gravidity, there was no evidence of interaction for the joint effects of HIV infection and placental parasitemia on maternal anemia risk (Table 4). However, the absolute significant reduction in mean hemoglobin compared with uninfected women was highest among dually infected multigravidae. Among primigravidae, women with dual infec-

tion had the highest absolute reduction in hemoglobin levels, but the estimated difference was imprecise (Table 4).

## DISCUSSION

In this cohort of pregnant women, HIV infection was associated with LBW and maternal anemia. Both peripheral and placental parasitemia at delivery were associated with maternal anemia and LBW, with a stronger effect observed on maternal anemia. Additionally, having greater than or equal to three episodes of parasitemia over follow-up was associated with increased LBW risk. Among multigravidae, there was some evidence of superadditive interaction between HIV infection and placental parasitemia at delivery in their joint effects on LBW. No interaction effects were observed for maternal anemia.

**LBW.** Our finding that HIV infection was associated with an increased risk of LBW is consistent with many previous studies (reviewed in ref. 16). The association of placental parasitemia with LBW risk is also consistent with many previous studies (reviewed in ref. 2). Although we observed no effect of peripheral parasitemia at delivery, having greater than or equal to three episodes of peripheral parasitemia over follow-up increased LBW risk. This finding is consistent with the study conducted in the Democratic Republic of Congo by Landis and others,<sup>17</sup> in which having greater than

TABLE 2  
Predictors of risk of LBW and mean birth weight

Variable	<2,500 g n (%; N = 49)	≥2,500 g n (%; N = 517)	Crude prevalence ratio (95% CI)	Adjusted prevalence ratio* (95% CI)	Adjusted mean birth weight (g) difference† (95% CI)
HIV‡	12 (26.7)	54 (11.5)	2.32 (1.34–4.00)	3.08 (1.40–6.79)	–138 (–250 to –26)
Placental parasitemia at delivery	12 (24.5)	75 (14.7)	1.67 (0.98–2.85)	1.79 (0.83–3.84)	–36 (–137 to 66)
Peripheral parasitemia at delivery	12 (24.5)	77 (15.0)	1.63 (0.96–2.77)	0.58 (0.27–1.25)	–59 (–42 to 159)
Number of times parasitemic over follow-up					
0	23 (46.9)	276 (53.4)	Reference	Reference	Reference
1	13 (26.5)	151 (29.2)	1.03 (0.51–2.10)	0.90 (0.41–1.98)	6 (–110 to 122)
2	2 (4.1)	53 (10.3)	0.45 (0.10–1.98)	0.34 (0.07–1.59)	35 (–136 to 205)
3+	11 (22.5)	37 (7.2)	3.57 (1.61–7.91)	2.68 (1.06–6.79)	–163 (–351 to 26)
Parasite density at delivery (parasites/μL)					
0	37 (75.5)	437 (85.4)	Reference	Reference	Reference
1–20,000	9 (18.4)	69 (13.5)	1.54 (0.71–3.33)	1.53 (0.66–3.56)	–53 (–183 to 78)
≥20,000	3 (6.1)	6 (1.2)	5.91 (1.42–24.6)	3.51 (0.76–6.28)	–220 (–727 to 287)
SP doses received					
0	4 (8.2)	21 (4.1)	2.60 (0.78–8.62)	2.00 (0.55–7.22)	–242 (–498 to 14)
1	10 (20.4)	93 (18.0)	1.47 (0.63–3.43)	0.95 (0.36–2.54)	–135 (–281 to 10)
2	21 (42.9)	212 (41.0)	1.35 (0.67–2.73)	1.27 (0.60–2.67)	–46 (–162 to 70)
3+	14 (28.6)	191 (36.9)	Reference	Reference	Reference
Primigravid	20 (41.7)	140 (27.1)	1.54 (1.07–2.21)	1.98 (1.01–3.88)	–208 (–290 to –126)

\* All parasitemia outcomes and SP doses were entered in separate models. All models were adjusted for material weight, water source, gravidity, and HIV status.

† All models were adjusted for material weight, housing quality, gravidity, and HIV status (only women with concordant results on the two HIV tests were included in analyses).

‡ N = 45 and 496, respectively.

CI = confidence interval.

or equal to three infections was associated with an increased risk of intrauterine growth retardation. Additionally, another study conducted in Malawi among a similar population reported a similar estimate caused by greater than or equal to two parasitemia episodes on the risk of LBW and maternal anemia.<sup>18</sup> In this latter study, parasitemia at delivery was included in the number of episodes detected, whereas in the current study, number of episodes excludes parasitemia at delivery. These results suggest that parasitemia throughout the gestation period and not only at delivery should be considered when assessing effect on LBW risk.

In the study conducted in Zimbabwe by Ticconi and others,<sup>19</sup> the risk of LBW among pregnant women with clinical malaria (defined as the presence of malaria parasitemia

and symptoms) was 10 (95% CI = 6.50–15.65) times the risk of women without malaria. In our study, high parasite density, defined as over 20,000 parasites/μL, was associated with a 3.5-fold increase in the risk of LBW, but this increase was not significant. The smaller effect seen in our study may be because of the fact that high parasite density was rarely accompanied by fever. Nevertheless, the two studies suggest that the risk of LBW may be influenced by the severity of malaria infection.

Most previous studies of HIV and malaria during pregnancy did not report any interaction between HIV infection and placental parasitemia in the effect on LBW.<sup>3–5,19</sup> One study, however, found that HIV-positive parasitemic women had 2.5 (95% CI = 1.0–5.9) times the risk of LBW compared

TABLE 3  
Predictors of risk of maternal anemia and mean hemoglobin

Variable	Hb < 11 g/dL n (%; N = 65)	Hb ≥ 11 g/dL n (%; N = 667)	Crude prevalence ratio (95% CI)	Adjusted prevalence ratio (95% CI)	Adjusted mean hemoglobin (g/dl) difference (95% CI)
HIV*	17 (27.4)	70 (11.6)	2.36 (1.49–3.74)	2.12 (1.09–4.14)	–0.74 (–1.13 to –0.36)
Placental parasitemia at delivery	21 (32.3)	84 (12.6)	2.57 (1.71–3.85)	3.60 (1.95–6.64)	–1.07 (–1.44 to –0.70)
Peripheral parasitemia at delivery*	19 (29.2)	90 (13.5)	2.17 (1.42–3.31)	2.84 (1.53–5.28)	–1.08 (–1.45 to –0.72)
Parasites density at delivery (parasites/μL)					
0	44 (67.7)	583 (87.4)	Reference	Reference	Reference
0–20,000	17 (26.2)	75 (11.2)	3.00 (1.63–5.52)	3.30 (1.73–6.28)	–1.04 (–1.43 to –0.65)
≥20,000	4 (6.2)	9 (1.4)	5.89 (1.74–19.9)	5.97 (1.58–22.52)	–1.26 (–2.21 to –0.32)
Number of times parasitemic over follow-up†					
0	30 (46.2)	366 (54.9)	Reference	Reference	Reference
1	16 (24.6)	187 (28.0)	1.04 (0.56–1.90)	1.07 (0.54–2.13)	–0.30 (–0.72 to 0.11)
2	10 (15.4)	66 (9.9)	1.85 (0.86–3.96)	2.27 (1.00–5.16)	–0.74 (–1.34 to –0.12)
3+	9 (13.9)	48 (7.2)	2.29 (1.02–5.11)	2.34 (0.96–5.72)	–0.96 (–1.66 to –0.26)
SP doses received†					
0	4 (6.2)	29 (4.4)	1.74 (0.56–5.45)	1.44 (0.45–4.63)	–0.04 (–0.89 to 0.81)
1	19 (29.2)	118 (17.7)	2.03 (1.04–3.95)	1.50 (0.73–3.07)	–0.40 (–0.91 to 0.10)
2	22 (33.9)	268 (40.2)	1.03 (0.55–1.94)	0.99 (0.52–1.90)	–0.12 (–0.52 to 0.29)
3+	20 (30.8)	252 (37.8)	Reference	Reference	Reference
Primigravid	17 (26.2)	179 (27.0)	0.97 (0.63–1.49)	0.88 (0.47–1.67)	0.04 (–0.25 to 0.34)

\* Only women with two concordant HIV tests (N = 62 and 602, respectively).

† All parasitemia outcomes and SP doses were entered in separate models. All models were adjusted for maternal weight, water source, gravidity, and HIV status.

CI = confidence interval; Hb = hemoglobin.



TABLE 4  
HIV and placental parasitemia interaction effects

Factor	Adjusted prevalence ratio (95% CI)*		Adjusted mean differences (95% CI)*	
	Primigravida, low birth weight (< 2,500 g)	Multigravida, low birth weight (< 2,500 g)	Primigravida, birth weight (g)	Multigravida, birth weight (g)
Neither infection	Reference	Reference	Reference	Reference
HIV only	2.52 (0.44–14.47)	2.02 (0.62–6.57)	–220 (–614 to 174)	–99 (–305 to 106)
Parasitemia only†	1.65 (0.51–5.35)	0.59 (0.08–4.66)	–162 (–377 to 53)	127 (–95 to 349)
Dual infection	6.37 (0.46–88.5)	9.59 (2.51–36.6)	–174 (–843 to 495)	–216 (–559 to 126)
Interaction <i>P</i> value	0.8	0.1	0.4	0.2
	Primigravida, Anemia (Hb < 11 g/L)	Multigravida, Anemia (Hb < 11 g/L)	Primigravida, Hemoglobin (g/dL)	Multigravida, Hemoglobin (g/dL)
Neither infection	Reference	Reference	Reference	Reference
HIV only	5.42 (1.79–16.40)	3.73 (1.47–9.47)	–0.14 (–1.80 to 1.52)	–0.89 (–1.51 to –0.28)
Parasitemia only	2.56 (0.27–24.01)	2.87 (1.25–6.60)	–1.02 (–1.86 to –0.17)	–1.16 (–1.92 to –0.40)
Dual infection	9.03 (0.68–120.09)	5.21 (1.53–17.7)	–1.72 (–4.16 to 0.72)	–1.68 (–2.82 to –0.54)
Interaction <i>P</i> value	0.8	0.4	0.6	0.5

\* All models were adjusted for maternal weight, water source, gravidity, and HIV status.

† Placental parasitemia.  
CI = confidence interval.

with aparasitemic HIV-infected women.<sup>13</sup> In our study, among primigravid women, neither infection increased the risk of LBW in the presence of the other. It should be noted, however, that the small number of primigravid women may not have afforded the analysis sufficient power to detect an effect. Among multigravid women with placental parasitemia, HIV-infected women had almost 10 times the risk of LBW compared with HIV-uninfected parasitemic women. Although the confidence limit ratio of this estimate was 15, indicating imprecision, the magnitude of the estimate is strongly indicative of superadditive interaction. Furthermore, at the specified  $\alpha$ -level of 0.10, the interaction term among multigravidae reaches statistical significance, further adding to the evidence of interaction.

**Maternal anemia.** The result that HIV and peripheral and placental parasitemia were strong independent risk factors for maternal anemia both at delivery and over follow-up corroborates previous literature. In the study by Van Eijk and others,<sup>20</sup> malaria and HIV were associated with a slightly elevated risk of anemia among all gravidities. In the study conducted in Malawi by Rogerson and others,<sup>21</sup> both placental and peripheral parasitemia were associated with anemia (peripheral parasitemia: odds ratio = 1.85, 95% CI = 1.45–2.36; placental parasitemia: odds ratio = 2.0, 95% CI = 1.5–2.7), where placental parasitemia was determined through histological examination. Additionally, the finding that greater than or equal to two episodes of parasitemia are associated with a doubling of maternal anemia risk has also been reported in this population.<sup>18</sup> In the study conducted in Kenya by Ayisi and others,<sup>13</sup> however, among primigravidae, only HIV infection was independently associated with anemia (defined as Hb < 8 g/dL). Among multigravidae, no independent effects of HIV and parasitemia were detected; however, coinfection was strongly associated with increased risk of anemia.<sup>13</sup> These differences in results may be caused by the differing definitions of maternal anemia.

In contrast to the study by Ayisi and others,<sup>13</sup> we did not detect any interaction between HIV and peripheral or placental parasitemia in their effect on maternal anemia at delivery among both primigravid and multigravid women. Although the risk of anemia was highest among dually

infected women, the estimate for the joint effect was not greater than the additive effects of HIV infection only and placental parasitemia only. Furthermore, among primigravid women, we found a significant reduction in hemoglobin levels only among women with placental parasitemia in the absence of HIV infection. Among multigravidae, we found no indication of interaction between HIV infection and placental parasitemia in their effect on mean hemoglobin levels. These differences in the results regarding maternal anemia risk may be caused by differing distributions of HIV infection and gravidity in the two study populations. Another possible reason is the different cutoff values used, with the study by Ayisi and others<sup>13</sup> using a lower cutoff of < 8 g/dL. However, we would still expect the differences in mean hemoglobin to corroborate previous results. The more likely explanation for the difference is the multifactorial nature of the etiology of anemia. The factors that contribute to anemia in the two populations may be different or where similar, have differing distributions, thus affecting the impact of both HIV infection and placental parasitemia on anemia risk and mean hemoglobin.

**Limitations.** The interpretation of these results is subject to some limitations. First, placental parasitemia was determined through placental blood film instead of histological examination, a more sensitive method.<sup>21–23</sup> However, this test would have resulted in misclassification that would have biased the estimates to the null. Second, we did not have information on CD4+ counts or other clinical measures of the stage of HIV infection. Given the association of HIV infection with age, this information may have influenced the effects of HIV differentially according to gravidity. Third, birth weight was available for approximately 70% of the women. Although there were no significant differences in HIV status, malaria preventive behaviors, and demographic variables between women delivering at the health centers and those women delivering elsewhere, women delivering at the health centers were more likely to have peripheral parasitemia. This finding may limit the generalizability of the results with regard to birth weight to women delivering at the health centers versus women in the catchment area of the health centers. Fourth, there is a limitation that concerns the multiple factors contributing to maternal

anemia. In this study, we did not assess nutritional status and the presence of other parasitic infections associated with anemia risk. However, we were able to adjust for variables linked to anemia, such as socioeconomic factors and maternal weight.

### CONCLUSIONS

This study confirms the deleterious effects of HIV infection and parasitemia during pregnancy. We found evidence of interaction between HIV infection and placental parasitemia in increasing risk of LBW among multigravidae. Contrasting previous results, we found no evidence of interaction between HIV infection and placental parasitemia on maternal anemia risk. These results underscore the need for continued research to understand the role of HIV in contributing to adverse pregnancy outcomes in malaria-endemic areas. Furthermore, the differential effects according to gravidity highlight the importance of targeted malaria prevention programs to ensure that the benefits of protective measures, whether through IPTp or insecticide-treated nets, accrue to all pregnant women.

Received June 17, 2011. Accepted for publication January 12, 2012.

**Acknowledgments:** We would like to thank Ebbie Chaluluka, Innocent Mofolo, and Marjorie Chabonda for their invaluable assistance in conducting the study. We would also like to acknowledge Charlie Poole and Rick Fairhurst for commenting on early drafts of the manuscript.

**Financial support:** This work was supported by Centers for Disease Control and Prevention Grant CDC/ASPH/ASTDR S1935-21/21. E.T.N. was funded through the Kenan Fellowship at the University of North Carolina at Chapel Hill.

**Authors' addresses:** Ella T. Nkhoma, Safety and Epidemiology, HealthCore, Inc., Andover, MA, E-mail: ella.nkhoma@gmail.com. Linda Kalilani-Phiri and Victor Mwapasa, Department of Community Health, College of Medicine, University of Malawi, Chichiri, Blantyre, Malawi, E-mails: lkalilani@hotmail.com and vmwapasa@medcol.mw. Stephen J. Rogerson, Department of Medicine, University of Melbourne, Royal Melbourne Hospital, Melbourne, Victoria, Australia, E-mail: sroger@unimelb.edu.au. Steven R. Meshnick, Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, E-mail: meshnick@unc.edu.

### REFERENCES

- World Health Organization, 2009. *World Malaria Report 2009*. Geneva, Switzerland: World Health Organization.
- Cabinet of the President, 2008. *Malawi HIV and AIDS Monitoring and Evaluation Report*, 2007. Lilongwe, Malawi: National Government Publication Office.
- Brabin BJ, Hakimi M, Pelletier D, 2001. An analysis of anemia and pregnancy-related maternal mortality. *J Nutr* 131: 604S–614S.
- Steketee RW, Nahlen BL, Parise ME, Menendez C, 2001. The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg* 64: 28–35.
- Okoko BJ, Enwere G, Ota MO, 2003. The epidemiology and consequences of maternal malaria: a review of immunological basis. *Acta Trop* 87: 193–205.
- Shulman CE, Dorman EK, 2003. Importance and prevention of malaria in pregnancy. *Trans R Soc Trop Med Hyg* 97: 30–35.
- McCormick MC, 1985. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* 312: 82–90.
- Guyatt HL, Snow RW, 2004. Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. *Clin Microbiol Rev* 17: 760–769.
- Brocklehurst P, French R, 1998. The association between maternal HIV infection and perinatal outcome: a systematic review of the literature and meta-analysis. *Br J Obstet Gynaecol* 105: 836–848.
- Leroy V, Ladner J, Nyiraziraje M, De Clercq A, Bazubagira A, Van de Perre P, Karita E, Dabis F, 1998. Effect of HIV-1 infection on pregnancy outcome in women in Kigali, Rwanda, 1992–1994. Pregnancy and HIV Study Group. *AIDS* 12: 643–650.
- Renia L, Potter SM, 2006. Co-infection of malaria with HIV: an immunological perspective. *Parasite Immunol* 28: 589–595.
- Villamor E, Msamanga G, Aboud S, Urassa W, Hunter DJ, Fawzi WW, 2005. Adverse perinatal outcomes of HIV-1-infected women in relation to malaria parasitemia in maternal and umbilical cord blood. *Am J Trop Med Hyg* 73: 694–697.
- Ayisi JG, van Eijk AM, ter Kuile FO, Kolczak MS, Otieno JA, Misore AO, Kager PA, Steketee RW, Nahlen BL, 2003. The effect of dual infection with HIV and malaria on pregnancy outcome in western Kenya. *AIDS* 17: 585–594.
- Bloland PB, Wirima JJ, Steketee RW, Chilima B, Hightower A, Breman JG, 1995. Maternal HIV infection and infant mortality in Malawi: evidence for increased mortality due to placental malaria infection. *AIDS* 9: 721–726.
- Andersson T, Alfredsson L, Kallberg H, Zdravkovic S, Ahlbom A, 2005. Calculating measures of biological interaction. *Eur J Epidemiol* 20: 575–579.
- ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, Rogerson SJ, Steketee RW, 2004. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa. *Am J Trop Med Hyg* 71: 41–54.
- Landis SH, Lokomba V, Ananth CV, Atibu J, Ryder RW, Hartmann KE, Thorp JM, Tshetu A, Meshnick SR, 2009. Impact of maternal malaria and under-nutrition on intra-uterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo. *Epidemiol Infect* 137: 294–304.
- Kalilani L, Mofolo I, Chabonda M, Rogerson SJ, Meshnick SR, 2010. The effect of timing and frequency of *Plasmodium falciparum* infection during pregnancy on the risk of low birth weight and maternal anemia. *Trans R Soc Trop Med Hyg* 104: 416–422.
- Ticconi C, Mapfumo M, Dorrucchi M, Naha N, Tarira E, Pietropoli A, Rezza G, 2003. Effect of maternal HIV and malaria infection on pregnancy and perinatal outcome in Zimbabwe. *J Acquir Immune Defic Syndr* 34: 289–294.
- van Eijk AM, Ayisi JG, ter Kuile FO, Misore AO, Otieno JA, Rosen DH, Kager PA, Steketee RW, Nahlen BL, 2003. HIV increases the risk of malaria in women of all gravidities in Kisumu, Kenya. *AIDS* 17: 595–603.
- Rogerson SJ, Mkundika P, Kanjala MK, 2003. Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology. *J Clin Microbiol* 41: 1370–1374.
- Mockenhaupt FP, Ulmen U, von Gaertner C, Bedu-Addo G, Bienzle U, 2002. Diagnosis of placental malaria. *J Clin Microbiol* 40: 306–308.
- Rogerson SJ, Chaluluka E, Kanjala M, Mkundika P, Mhango C, Molyneux ME, 2000. Intermittent sulfadoxine-pyrimethamine in pregnancy: effectiveness against malaria morbidity in Blantyre, Malawi, in 1997–99. *Trans R Soc Trop Med Hyg* 94: 549–553.