Malaria and HIV in pregnancy, and effects on the infant in western Kenya
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The effect of dual infection with HIV and malaria on pregnancy outcome in western Kenya
Title: The effect of dual infection with HIV and malaria on pregnancy outcome in western Kenya

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Objective: To determine the effect of dual infection with HIV and malaria on birth outcome and maternal anaemia among women delivering at a large public hospital in Kisumu, western Kenya.

Subjects and methods: Data on obstetrical and neonatal characteristics, maternal and placental parasitaemia, and postpartum haemoglobin (Hb) were collected among all women enrolled in a cohort study of the interaction between malaria and HIV in pregnancy.

Results: Between 1996-1999 data were available from 2466 live singleton deliveries. The maternal HIV seroprevalence was 24.3%, and at delivery 22.0% of the women had evidence of malaria. Low birthweight (LBW), preterm delivery, intrauterine growth retardation (IUGR) and maternal anaemia (Hb<8 g/dl) occurred in 4.6%, 6.7%, 9.8% and 13.8% of deliveries respectively. Maternal HIV, in the absence of malaria, was associated with a 99 g (95% CI 52-145) reduction in mean birthweight among all gravidae. Malaria was associated with both IUGR and preterm delivery, resulting in a reduction in mean (95% CI) birthweight of 145g (82-209) among HIV-seronegative and 206g (115-298) among HIV-seropositive primigravidae, but not among multigravidae. Both HIV and malaria were significant risk factors for postpartum maternal anaemia and HIV-seropositive women with malaria were twice as likely to have Hb < 8g/dl than HIV-seronegative women with or without malaria.

Conclusion: The adverse effect of malaria on birth outcome is worsened by HIV infection. In areas with moderate or high prevalence of HIV and malaria, priority should be given to interventions that control malaria and anaemia in pregnancy for all pregnant women, as the provision of these interventions is critical to improved birth outcomes.

Keywords: Low birth weight, preterm delivery, intrauterine growth retardation, HIV, malaria, Kenya.
Introduction

Malaria in pregnancy is a major problem in sub-Saharan Africa, affecting an estimated 24 million pregnant women, especially primigravidae [1,2]. In malaria endemic areas *Plasmodium falciparum* parasitaemia in pregnancy is associated with anaemia in pregnant women, and reduced birthweight resulting from premature delivery and intrauterine growth retardation [3-5], which are important risk factors for early infant mortality and morbidity [6].

During the past two decades, HIV/AIDS has emerged as a major problem in many malaria-endemic areas of sub-Saharan Africa. It is estimated that 25.3 million people in sub-Saharan African are infected with HIV [7]. In some areas of sub-Saharan Africa, HIV infection rates as high as 25-40% have been reported among pregnant women [8-14]. Africa south of the Sahara accounts for over two-thirds of the world's HIV-infected persons, and 80% of the world's HIV-infected women [7,14]. Thus malaria and HIV infection are now common, widespread and overlapping public health problems in many areas of Africa south of the Sahara, and the potential interaction between these two important infectious diseases has been the topic of several previous investigations [9, 11, 13, 15,16]. Studies among pregnant women in sub-Saharan Africa have shown that HIV-infected women, particularly multigravidae are more likely to be infected with *P. falciparum*, and to have higher parasite densities than HIV uninfected women [9,11,13]. Thus HIV infection seems to alter the well established parity-specific pattern of malaria susceptibility in areas of stable malaria transmission, where in the absence of HIV, primigravidae and, to a lesser extent, secundigravidae are more affected than are other parities [1,3].

Although it is now well established that HIV-seropositive pregnant women are more susceptible to malaria, little is known of the adverse consequences of dual infection with maternal HIV and malaria on pregnancy outcome. We report here the effects of dual
infections on infant outcomes, including their effect on the two major etiological pathways of LBW: shortened gestation [preterm delivery (PTD)] and intrauterine growth retardation (IUGR) [i.e., small for gestational age (SGA)] [17]. Because maternal malaria and HIV infections are also associated with an increase in severity of anaemia, a known risk factor for maternal death and poor infant outcomes [18-21], we also report on the effect of dual infections on maternal anaemia at delivery. This paper forms part of a series of studies conducted in Kisumu, in western Kenya, of the interaction between HIV and malaria during pregnancy. The impact of HIV on the risk of malaria in this same study population, and that of dual infections on maternal anaemia in the third trimester will be published elsewhere [21].

Subjects and methods

Study site

This study was conducted at the Nyanza Provincial General Hospital (NPGH) in Kisumu town. Kisumu, with a population of approximately 300,000, is located on the shores of Lake Victoria in western Kenya. Malaria transmission within Kisumu town is perennial and *P. falciparum* is the predominant species, accounting for 98% of malaria cases. The remaining 2% are caused by *P. malariae* and *P. ovale*. Malaria prevalence in town however, is markedly lower than that reported from the surrounding rural areas [22, 23]. Chloroquine resistance is prevalent in the area with 75-80% of *P. falciparum* strains showing RII/RIII resistance pattern [24]. The prevalence of HIV infection among pregnant women in this study population is approximately 25% [11, 25]. More than 50% of the pregnant women in the study area deliver at home (M. Parise, B. Nahlen and J. Ayisi, unpublished data). NPGH is a large government referral hospital with 400 beds, which provides health care mostly to the low-income population in this area. On average, about 100 pregnant women present each day to the antenatal care (ANC) clinic, 30 of whom do so for their first visit. As part of the
ANC package for this hospital, women making their first ANC visit are tested for syphilis using the rapid plasma reagin (RPR) card test, (Becton Dickinson microbiological systems, Cockeysville, USA), and those seroreactive for syphilis are treated with 2.4mU intramuscular benzamine penicillin, according to guidelines established by the Kenyan government, Ministry of Health [26]. The current National Malaria Control Policy for malaria and anaemia in pregnancy includes protective intermittent treatment of malaria with sulfadoxine-pyrimethamine in the second and third trimesters of pregnancy. These guidelines were implemented as part of the standard antenatal care package in the NPGH on the 15th of March 1999. Only women who delivered before this date were included in the current analysis. All study women were offered routine haematinic supplementation of 200mg of ferrous sulfate and 5mg of folic acid daily until delivery.

Enrollment Procedures

Screening procedures and characteristics of the women, HIV prevalence, and risk factors have been described elsewhere [25]. All study participants gave informed consent and all agreed to be HIV tested. Briefly, women were eligible for participation if they had an uncomplicated singleton pregnancy of ≥32 weeks gestation (based on the fundal height estimation by the clinic nurse/midwife), if they resided within the Kisumu municipality, and if they had no known underlying chronic illness. After written informed consent was obtained, an interviewer-administered structured questionnaire was given in the local or the national languages (Dholuo or Kiswahili) to all women who had undergone routine antenatal care to obtain information on sociodemographic, health, and obstetric factors.

Upon completion of the questionnaire, each woman was counselled by a trained HIV counsellor in the local languages, and an appointment for posttest counselling was made. After pretest counselling, a blood sample was taken for HIV antibody testing,
hemoglobin (Hb), and malaria thick blood smear. All screened women were encouraged to deliver in NPGH.

At delivery, information was collected on the mode and outcome of delivery and on the occurrence of any episode of illnesses and its treatment in the two-week period before delivery. Within 24 hours of birth, infants were weighed to the nearest one gram on an electronic balance (Ohaus Florham Park, NJ, USA), and their gestational age was assessed by trained study assistants using a standardized Ballard method [27]. Maternal, placental and cord blood malaria smears and maternal haemoglobin were collected soon after delivery.

**Ethical review**

The study protocol was approved by the institutional review boards of the Kenya Medical Research Institute (KEMRI), the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia (USA) and the Academic Medical Center (AMC), University of Amsterdam, Amsterdam, The Netherlands.

**Laboratory Procedures**

Peripheral, placental and cord blood smears were stained with 10% Giemsa for 10 minutes and examined under oil immersion for malaria parasites. A thick smear was considered negative if 100 microscopic fields revealed no parasites. Haemoglobin was measured using a Hemocue® machine (Mission Viejo, CA, USA.). HIV testing was done using two rapid tests: an initial Serostrip HIV-1/2 [Saliva Diagnostic Systems (Singapore) Pte Ltd] and a confirmatory Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Wicklow, Ireland Ltd) on all samples that tested positive by Serostrip. Western blot was performed on discordant samples.
Definitions

An uncomplicated pregnancy was defined as a pregnancy without the presence of hypertension, pre-eclampsia, polyhydramnios, an abnormal presentation of the foetus, a history of a previous caesarean section, haemorrhage, or repeated spontaneous abortions (>2).

Because we were interested in the overall effect of malaria in pregnancy, *malaria infection at delivery* was defined as any evidence of current malarial infection (post-delivery maternal peripheral parasitaemia or placental parasitemia, either in the presence of asexual stages or malaria pigment), detected on a thick blood smear. *Anaemia* was defined as haemoglobin (Hb) <11g/dl of blood [28], and moderate to severe anaemia was defined as Hb <8g/dl of blood [29]. *HIV-seropositivity* was defined as a positive result on both rapid tests; women not reactive with the initial Serostrip HIV-1/2 test were considered HIV-seronegative.

Women whose serostatus could not be determined (i.e., those with discordant results on the two rapid tests and an indeterminate status with Western blot) were excluded from analysis. Women who were HIV-seronegative and had no malaria at delivery were considered to be uninfected. Women who had malaria at delivery but were HIV-seronegative were considered to be infected with *malaria-alone*. Women who were HIV-seropositive but had no malaria at delivery were considered to be infected with *HIV-alone*. Women were considered to have *dual infection* if they were HIV-seropositive and had malaria at delivery.

Newborns were classified as *normal birthweight* if they weighed ≥2500g regardless of gestational age and *low birthweight (LBW)* if they weighed <2500g [30]. *Preterm delivery (PTD)* was defined as any delivery that occurred before 37 completed weeks of gestation.

*Small for gestational age (SGA)* was defined as a sex-specific birthweight at or below the 10th percentile for weight-for-gestational age of an international reference population [31]. For the purpose of this analysis normal weight-for-gestational age were children with a birthweights >10th percentile of the weight-for-gestational age reference population. *Rainy*
season included the months April, May and June (long rains) and October and November (short rains).

Data analysis and statistical methods

Women screened in the ANC who delivered at the NPGH from June 1996 through March 1999 were included in this analysis. Differences between women who did and did not deliver at NPGH were assessed using data collected for all pregnant women at screening in the ANC. The relationship between malaria at delivery, maternal HIV-infection and demographic and obstetric factors on adverse birth outcome (defined as a LBW, PTD or SGA infant, or moderate to severe maternal anaemia at delivery) were investigated by bivariate analysis. The Chi square test was used to test for significant differences in proportions. Risk ratios (RR) were computed with 95% confidence intervals (CI) to measure the strength of the associations. Normally distributed continuous data were compared by the Student’s t-test and one-way analysis of variance (ANOVA). Multiple linear regression analysis [32] was used to assess the relative impact of HIV, malaria, and dual infection on birth weight, gestational age and maternal haemoglobin levels. Adjusted risk ratios for LBW, PTD, SGA and moderate to severe anaemia associated with maternal HIV, malaria, and dual infection were computed using Poisson regression analysis [33]. We first looked at all models including HIV, malaria and the covariates for birth weight, gestational age, maternal haemoglobin levels, LBW, PTD, SGA and moderate to severe anaemia, and assessed the possible statistical interaction (effect measure of modification) [34] between malaria and HIV; no evidence for a statistical interaction was seen and thus the interaction term was not included in any of the models. We then repeated the analyses using the infection categories: no infection, HIV-alone, malaria alone or dual infection. All four models for LBW, SGA, PTD and moderate to severe anaemia at delivery were adjusted for the potential confounding effect of maternal weight (<
25 percentile vs ≥25 percentile, kg), maternal age (<20 vs ≥20 years), years of education (<8 vs ≥8 years), ethnicity (Luo vs non Luo), season of delivery (rainy vs dry), hospitalization in current pregnancy, and gender of the child. The model for postpartum anaemia was also adjusted for type of delivery (i.e., caesarean section or assisted delivery vs normal delivery) and any use of haematinics. The covariates were included in the models if they were significantly associated with any of the four outcomes measures in the bivariate analyses or if they were known to be associated with these outcome measures based on prior studies.

Analysis was done using EPI INFO 6.01 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SAS (Version 6.12, SAS Institute, Cary, North Carolina, USA).

Results

Study population

During the study period, 5168 women were screened of whom 75 (1.5%) had no complete laboratory results available, and hence were excluded from analysis. Of the remaining 5093 women, and consistent with patterns of home versus health facility delivery, 2539 (49.9%) delivered at the study hospital (Figure 1). Data on birth outcome were not available for the remaining 2554 (50.1%) women who delivered elsewhere. Of the 2539 hospital deliveries, 34 twin and 2 triplet deliveries; and 37 women without malaria smear results were excluded from further analysis (Figure 1). The maternal characteristics of the women excluded and included in the analysis are shown in Table 1. Compared to women who were included in the analysis, those who were excluded were more likely to be older, to have less than complete primary school education, to be married, to be a multigravidae, to be of Luo ethnicity, to reside in the peri-urban rather than urban area of Kisumu and to have moderate to severe anaemia in the third trimester. The HIV and syphilis seroprevalence did not differ between the two groups. Among the
1297 multigravidae who were included in the analyses, 88 women (6.8%) had a history of a stillbirth or abortion as the result of the most recent pregnancy, whereas 93 women (5.4%) had this outcome among the 1711 excluded multigravidae ($P = 0.1$). Of the 5093 women seen in the third trimester, 1024 (20.1%) were parasitaemic, and the prevalence did not differ between women included and excluded from the analyses (Table 1).

Of the 2466 women included in the analysis, 1169 (47.4%) were primigravidae. Compared to multigravidae, primigravidae women were more likely to be younger [mean age, standard deviation (SD) 18.8 (2.5) versus 24.3 (4.7) years; $P<0.001$]. Among the 2466 women, 599 (24.3%) women were HIV-seropositive and 543 (22.0%) had evidence of malaria infection at delivery. Malaria infection at delivery occurred in 179 of 599 HIV-seropositive women (29.9%) compared to 364 of 1867 HIV-seronegative women (19.5%) [risk ratio (RR), 1.5; 95% Confidence Interval (CI), 1.3-1.8; $P < 0.001$], and was more common among primigravidae in whom 323 out of 1169 (27.6%) had malaria at delivery compared to 220 out of 1297 (17.0%) among multigravidae (RR, 1.6; 95% CI, 1.4-1.9; $P < 0.001$). Of the 543 women with malaria infection at delivery, 245 (45.1%) also had malaria infection in the third trimester, whereas of the 1923 women who had no malaria at delivery, 227 (11.8%) had malaria in the third trimester (RR, 3.8; 95% CI, 3.3-4.5, $P< 0.001$).

The presentation of the infant was cephalic for 2436 out of 2466 women (98.8%), and thirty women had a breech or transverse presentation (1.2%). Assisted deliveries (forceps/vacuum extraction) occurred in 0.4% (10 out of 2465) and 4.2% (104 out of 2465) of the women had a caesarean section. Intrapartum medical complications such as placental abruption, placenta previa and pre-eclampsia all combined were uncommon, occurring in only 7 of the 2463 women examined (0.3%). In addition, 8 out of 2461 women examined (0.3%) had post partum haemorrhage. Of the 34 stillbirths, 17 (1.1%)
were recorded among 1503 women without HIV and malaria infection, 4 (1.0%) among 420 women infected with HIV-alone, 9 (2.5%) among 364 women with malaria-alone, and 4 (2.2%) among 179 women with dual infection. Compared to women without malaria, stillbirths were more common in women with malaria infection at delivery, irrespective of HIV status [1.1% (21 of 1923) versus 2.4% (13 of 543); \( P = 0.02 \)]; stillbirth rates were similar in women with and without HIV infection regardless of malaria infection [1.3% (8 of 599) versus 1.4% (26 of 1867); \( P = 1.00 \)]. All stillborn infants were removed from the further analysis, leaving 2432 live singleton infants with maternal HIV and malarial infection status available for analysis (Figure 1). Cigarette smoking, a well-known risk factor for low birthweight, was not common in this population (reported by less than 1% of the women) and was not included in the analysis.

Adverse birth outcome in relation to maternal HIV and malaria infection

Comparison of prevalence and multivariable analyses

Birthweight:

Of the 2432 live singleton infants, birthweight was recorded for 2431 (99.9%).

The overall mean (SD) birthweight was 3235 g (450), and 113 of 2431 babies (4.6%) had low birthweight (< 2500 g), with an increase in prevalence seen among firstborns whose mothers were infected with HIV-alone, malaria-alone, or dual infection compared to those with neither of these infections (\( P = 0.09, 0.006 \) and <0.001) respectively (Table 2). However, no difference was seen in prevalence of low birthweight and maternal infection status among multigravidae (\( P = 0.3, P = 0.4 \), and \( P = 1.0 \), respectively). In a multiple linear regression model, maternal HIV infection was associated with a 99 g (95% CI, 52-145) reduction in mean birthweight among all gravidae (\( P < 0.001 \)). Among primigravidae, the mean (SD) birthweight of infants of uninfected women was 3186 g
After adjusting for other covariates in the linear regression model, this mean (95% CI) birth weight was 40g (-32 to 112, \( P = 0.3 \)) lower in primigravidae women with HIV-alone, 145g (82-209; \( P = 0.001 \)) lower in primigravidae women with malaria-alone, and 206g (115-298; \( P = 0.001 \)) lower in women with dual infection. The mean (SD) birth weight of infants of uninfected multigravidae women was 3373g (447) (Figure 2). Maternal HIV-alone and dual infection lowered this mean birth weight by 138g (78-199) and 161g (63-259), respectively (\( P < 0.001 \) for each), but malaria infection alone had no effect (8g, 95% CI -71 to 88; \( P = 0.80 \)). Women who had parasitaemia in the third trimester and also had malaria at delivery were at a higher risk of delivery of LBW baby compared to a parasitaemic women in the third trimester who had malaria at delivery [10.5% (25 out of 239) versus 5.5% (16 out of 291), RR, 1.9; 95% C, 1.0-3.5; \( P = 0.04 \)].

In a Poisson regression model, malaria-alone and dual infection were associated with a 2-fold and a 3-fold increased risk of LBW (\( P = 0.008 \) and \( P < 0.001 \)) respectively among the primigravidae, with no significant effect seen among the multigravidae (\( P = 0.7 \) for both groups; Table 3).

**Gestational age:**

Gestational age was determined for 2397 singleton live born infants (98.6% of the life born singletons). The mean (SD) gestational age was 38.6 weeks (1.3 weeks) and 160 infants were delivered preterm (6.7%). Higher rates of preterm delivery were seen with maternal malaria infection (\( P = 0.027 \)) or dual HIV/malaria infection (\( P < 0.0001 \)), but only in the primigravidae (Table 2). The mean (SD) gestational age of infants of uninfected primigravidae women was 38.6 (1.3) weeks. After adjusting for other covariates in the linear regression model, HIV infection alone had no effect on the mean gestational age among primigravidae, mean (95% CI) reduction 0.02 (-0.02 to 0.2) weeks, \( P = 0.9 \). However, the
mean (95% CI) gestational age was 0.4 weeks (0.1-0.6) lower in primigravidae with malaria-alone \( (P<0.001) \) and 0.4 weeks (0.1-0.7) \( (P=0.004) \) in dually infected primigravidae. In a multiple linear regression analysis among multigravidae, HIV infection was not associated with gestational age, mean reduction 0.04 (-0.2 to 0.1) weeks, \( (P=0.7) \). Further analysis revealed that malaria and dual infection were associated with a significant increase in the mean gestational age of 0.3 (0.1-0.5) weeks \( (P=0.02) \) and 0.3 (0.0-0.6) weeks \( (P=0.04) \) respectively.

In a Poisson regression model, primigravidae women infected with malaria-alone had increased risk of PTD when compared with uninfected women, but this risk was only marginally significant \( (P=0.06) \), while dual-infection in the same group increased the risk of PTD by 3-fold \( (P<0.001) \); none of the infections had any effect on PTD among the multigravidae (HIV-alone \( P=0.5 \), malaria-alone \( P=0.3 \) and dual-infection \( P=0.2 \), Table 3).

Overall, the prevalence of small-for-gestational age (SGA) was 9.8% (236 out of 2397) and like PTD, the prevalence of SGA was higher among primigravidae with malaria-alone \( (P=0.008) \) or dual-infection \( (P=0.02) \), and was higher in multigravidae with dual infection \( (P=0.01) \) (Table 2). As was the case with LBW, women who had parasitaemia in the third trimester; and also at delivery were at higher risk of delivery of SGA infant compared to aparasitaemic women in the third trimester who had malaria at delivery [18.2% (43 out of 236) \textit{versus} 11.4% (33 out of 290), RR, 1.6; 95% CI, 1.1-2.4; \( P=0.03 \)], but no effect was observed on prematurity [8.5% (20 out of 236) \textit{versus} 9.0% (26 out of 290), RR, 0.9; 95% CI, 0.5-1.7; \( P=0.8 \)]. In a Poisson regression model, primigravidae women infected with malaria-alone or dual infection were significantly at increased risk of delivery of a SGA infant when compared with uninfected women \( (P= \)
0.01 and \( P = 0.03 \) respectively. Multigravidae with dual infection had a borderline significant increase in the risk of SGA \( (P = 0.06) \).

**Maternal anaemia:**

Hemoglobin levels taken within 12 hours of delivery were available for 2173 out of 2432 women (89.4%) who delivered singleton live-born infants. The mean (SD) hemoglobin was 10.5 (2.4) g/dl and 1243 out of 2173 women (57.2%) had anaemia (Hb < 11 g/dl), and 300 women (13.8%) had moderate to severe anaemia (Hb < 8 g/dl). A higher prevalence of moderate to severe anaemia was seen among primigravidae with HIV-alone \( (P = 0.008) \) or in both gravidae women with dual infection \( (P = 0.003 \) for primigravidae) and \( (P < 0.001 \) for multigravidae) (Table 2). Mean hemoglobin levels, stratified by maternal infection status and gravidity are shown in Figure 2. The mean (SD) haemoglobin of uninfected primigravidae was 10.8 (2.4) g/dl. After adjusting for other covariates in the multiple linear regression model, this mean (95% CI) haemoglobin was 0.9 (0.4–1.3) g/dl lower in primigravidae women with HIV-alone \( (P < 0.001) \), 0.8 (0.4–1.2) g/dl lower in primigravidae women with malaria-alone \( (P < 0.001) \), and 1.5 (0.9–2.0) g/dl lower in women with dual infection \( (P < 0.001) \) (Figure 2). Among uninfected multigravidae women, the mean (SD) haemoglobin was 10.7 (2.4) g/dl. Among the multiple women, multiple linear regression showed that infection with HIV-alone lowered the mean (95% CI) Hb levels by 0.4 (0.05–0.7) g/dl \( (P = 0.03) \), but malaria-alone had no effect, mean reduction 0.4 (0.0–0.8) \( (P = 0.1) \). Dual infection significantly lowered the mean (95% CI) Hb by 1.3 (0.7–1.8) g/dl \( (P < 0.001) \) (Figure 2).

In a Poisson regression model, primigravidae with HIV-alone had a 1.8-fold increased risk of postpartum moderate to severe anaemia \( (P = 0.01; \) Table 3), and this
risk was more than 2-fold among dually infected women in both primigravidae and multigravidae ($P = 0.002$ and $P < 0.001$ respectively).

**Adverse birth outcome in relation to single versus dual infection**

Compared to primigravidae women infected with HIV-alone, those with dual infection had a borderline significant increase in the risk of LBW ($P = 0.06$), and an increased risk of PTD ($P = 0.03$); and among the multigravidae, there was an increased risk of moderate to severe anaemia ($P = 0.002$; Table 3). Dually infected primigravidae women had a borderline increased risk of moderate to severe anaemia when compared to women infected with malaria-alone ($P = 0.05$), and this effect was significant among the multigravidae ($P = 0.03$; Table 3).

**Discussion**

The current analyses were conducted to determine the effect of HIV, malaria and dual infection on birth outcome. Several studies conducted in areas of different malaria transmission pressure in sub-Saharan Africa have reported that, compared to HIV-seronegative women, HIV-seropositive women have higher prevalences and density of both peripheral and placental parasitaemia [9,11,13]. However, the adverse consequences of malaria and HIV on birth outcome and maternal anemia have been less clear.

Consistent with findings of other studies in the region [35, 36], we found that, compared to uninfected women (with no malaria or HIV), maternal HIV infection was associated with a reduction in mean birthweight of 99 g and the effect was most evident among multigravidae seropositive for HIV-alone where there was a 138 g reduction in
mean birthweight. This differential effect of HIV by gravidity is likely due to the fact that multigravidae are older and more likely to have an HIV infection of longer duration. Indeed, in our study, women with higher gravidity had, on average, lower CD4 counts (data not shown). The HIV-associated reduction in birth weight may be attributable to the direct effects of intrauterine infection through mother-to-child transmission [37]. In our study, women infected with HIV-alone were also at greater risk of delivering a LBW baby, but the prevalence of LBW overall was low and the difference was not statically significant.

In the present study, neither prematurity nor small for gestational age was found to be associated with maternal HIV-infection alone, a finding that is consistent with the study carried out among asymptomatic HIV-seropositive women in Uganda [36], but is in contrast to a study conducted in Kinshasa that included many women with HIV-associated disease (symptomatic women) and reported a significantly higher prevalence of prematurity and LBW among infants born to symptomatic HIV-seropositive women [38]. We have no explanation for the modest, but significant increase in gestational age among multigravidae infected with malaria-alone or dual infection observed in our study.

Infection with HIV-alone nearly doubled the risk of postpartum moderate to severe anaemia in primigravidae, and was associated with a reduction in mean haemoglobin levels in multigravidae, consistent with a previous study in which HIV-seropositive multigravidae were found to be at a significantly increased risk for severe anaemia (Hb < 7g/dl) in the third trimester, independent of malaria [21]. Postpartum haemorrhage has been found to be associated with HIV infection [39] and could have lowered the postpartum Hb levels in our study, however, this pregnancy outcome was reported in less than 1% of our study population and could not be further evaluated in our study. In addition, time between delivery and taking blood for Hb could affect the
maternal postpartum Hb levels due to volume redistribution resulting from the effects of labour. The blood sample for Hb estimation in our study was taken soon after delivery in a consistent manner for all the women in the four groups. Thus the observed trend in Figure 2 is likely a true trend reflecting the direct effect of HIV and/or its co-infection with malaria on postpartum anaemia.

Women who had parasitaemia during the third trimester and also had malaria at delivery had increased risk of both LBW and SGA, when compared to women with malaria at delivery but had no detectable parasitaemic in third trimester. This is presumably because longer-standing infection is necessary to impact on foetal growth. As expected maternal malaria infection identified at delivery was associated with a reduction in mean birth weight resulting from a combination of reduced gestational age and an increased risk of intrauterine growth retardation among the primigravidas [40]. However, among multigravidas, infection with malaria-alone had no effect on birth weight, prematurity or intrauterine growth retardation.

Comparison of dual with singly infected women showed that primigravidas with dual infection had increased risk of delivery of a premature baby and a marginally increased risk of delivery of a LBW baby compared to their counterparts infected with HIV-alone. Among the multigravidas, dual infection substantially increased the risk of moderate to severe anaemia over and above that among women infected with HIV-alone or malaria-alone. No additional adverse effects on infant outcome were observed among dually infected women in comparison with women with single infections. Even though we observed lack of a statistical interaction (effect measure of modification) between malaria and HIV infection in our analysis, our data show that the excess risk of adverse birth outcome due to dual infection is equal or greater than the sum of excess risks due to individual infections, suggesting a synergistic (additive) effect of the dual infection [34]. When compared to uninfected women,
dual infection among primigravidae was associated with a 3-fold increased risk of LBW, almost a 3-fold increased risk of prematurity, and about a 2-fold increased risk of SGA. In addition, dual infection more than doubled the risk of moderate to severe anaemia (Hb < 8 g/dl) in both primigravidae and multigravidae. A recent study has shown maternal anaemia to be associated with a high risk of LBW [41], a known risk factor for infant mortality [6]. Dual infection may thus have important detrimental impact on maternal morbidity through its association with maternal anaemia [20, 21], and on infant survival through its direct and indirect association with restriction on infant growth. We are currently also investigating whether children born from women dually infected with HIV and placental malaria are more likely to be infected with HIV, due to increased transmission of HIV from mother to infant in the presence of placental infections with *P. falciparum* as was postulated in a study from Malawi [42].

Our study has several limitations and may not be fully generalizable to other populations. In our study population, only 4.6% of the infants had LBW, compared to 15.0% reported by a previous study from the same hospital [43]. This is likely to have resulted from the different entry criteria used in the current study: as opposed to the previous study that included all women attending delivery at our study hospital, women in the current study were eligible for screening only if they had no known underlying disease and, had attained a gestational age of 32 weeks or more (based on the fundal height estimation by clinic nurse/midwife), and had an uncomplicated pregnancy.

Therefore, our study sample is not representative of the overall ANC population in this hospital and represents a selection of otherwise “healthy” women. Because we only included women with a gestational age ≥ 32 weeks, the effects of HIV and malaria infection on early events of pregnancy outcome, such as abortions, and very premature
delivery could not be evaluated. In addition, the number of perinatal deaths in our study was small (2.0% n = 2466), likely due to the same reasons noted above.

Our study, which is one of few studies with substantial numbers to determine the effect of dual infection of HIV and malaria on pregnancy outcome confirms an additional HIV-related public health problem in malaria endemic areas of sub-Saharan Africa, where the effects of malaria (single) infection on risk of LBW and prematurity among the primigravidae are exacerbated by dual infection in comparison with women with no infection. We conclude that in areas with moderate or high prevalence of HIV and malaria, priority should be given to interventions that control malaria in pregnancy for all pregnant women, as the provision of these interventions is critical to improved birth outcomes. It is also important to assess if treatments of anaemia that have proven to be beneficial in HIV-seronegative pregnant women offer the same benefits in HIV-seropositive women.
Acknowledgements

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Figure 1. Flowchart of inclusion and exclusion of study women for analysis

5168
Screened at ANC

75
No lab results, excluded

5093
Had HIV results

2554
Delivered elsewhere, excluded

2539
Delivered at NPGH

36
Multiple deliveries, excluded

37
No PM results, excluded

2466
Singletons

34
Stillbirths, excluded

2432
Live singletons
Figure 2. Mean birth weight* by maternal HIV and malaria (Mal) infection status and by gravidity, in Kisumu, western Kenya, June 1996-March, 1999

Maternal status
HIV: human immunodeficiency virus. Error bars indicate the 95% confidence interval for mean estimates.

*Adjusted for maternal weight (<25 percentile versus ≥25 percentile, kg), maternal age (<20 vs ≥20 years), years of education (<8 vs ≥8 years), ethnicity (Luo vs non Luo), season of delivery (rainy vs dry), a hospitalization in pregnancy and gender of the child.
Figure 3. Mean haemoglobin by maternal HIV and malaria (Mal) infection status and by gravidity, in Kisumu, western Kenya, June 1996-March, 1999

HIV: human immunodeficiency virus. Error bars indicate the 95% confidence interval for mean estimates.

*Adjusted for maternal weight (<25 percentile versus ≥25 percentile, kg), maternal age (<20 vs ≥20 years), years of education (<8 vs ≥8 years), ethnicity (Luo vs non Luo), season of delivery (rainy vs dry), a hospitalization in pregnancy, gender of the child, type of delivery (caesarean section or assisted delivery vs normal delivery) and use of haematinics.
Table 1. Baseline Characteristics of the women excluded and included in the analysis, Nyanza Provincial General Hospital, Kisumu, western Kenya, June 1996-March, 1999

<table>
<thead>
<tr>
<th>Variable</th>
<th>Excluded from analysis&lt;sup&gt;1&lt;/sup&gt; (N=2627)</th>
<th>Included in analysis (N=2466)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years) ± SD</td>
<td>22.4 ± 4.9</td>
<td>21.7 ± 4.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Primigravidae</td>
<td>916/2627 (34.9%)</td>
<td>1169/2466 (47.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Completed ≥ 8 years of education</td>
<td>1555/2626 (59.2%)</td>
<td>1684/2466 (68.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unemployed</td>
<td>1960/2624 (74.7%)</td>
<td>1879/2465 (76.2%)</td>
<td>0.216</td>
</tr>
<tr>
<td>Married</td>
<td>2148/2627 (81.8%)</td>
<td>1881/2466 (76.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Luo ethnicity</td>
<td>2125/2627 (80.9%)</td>
<td>1907/2466 (77.3%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Urban residence</td>
<td>1884/2627 (71.7%)</td>
<td>2142/2466 (86.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Syphilis (RPR)+</td>
<td>58/2445 (2.4%)</td>
<td>55/2288 (2.4%)</td>
<td>1.00</td>
</tr>
<tr>
<td>HIV+</td>
<td>670/2627 (25.5%)</td>
<td>599/2466 (24.3%)</td>
<td>0.331</td>
</tr>
<tr>
<td>Maternal parasitaemia in 3&lt;sup&gt;rd&lt;/sup&gt; trimester</td>
<td>552/2627 (21.0%)</td>
<td>472/2466 (19.1%)</td>
<td>0.100</td>
</tr>
<tr>
<td>Hb &lt; 8 g/dl 3rd trimester</td>
<td>439/2578 (17.0%)</td>
<td>361/2438 (14.8%)</td>
<td>0.034</td>
</tr>
<tr>
<td>Malaria at delivery</td>
<td>NA</td>
<td>543/2466 (22.0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Cord blood parasitaemia</td>
<td>NA</td>
<td>9/2409 (0.4%)</td>
<td>NA</td>
</tr>
<tr>
<td>Hb &lt; 8g/dl Post delivery</td>
<td>NA</td>
<td>305/2197 (13.9%)</td>
<td>NA</td>
</tr>
<tr>
<td>Unassisted vaginal</td>
<td>NA</td>
<td>2351/2465 (95.4%)</td>
<td>NA</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>NA</td>
<td>122/2464 (5.0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Preterm delivery&lt;sup&gt;1&lt;/sup&gt;</td>
<td>NA</td>
<td>160/2399 (6.7%)</td>
<td>NA</td>
</tr>
<tr>
<td>Small for gestational age&lt;sup&gt;1&lt;/sup&gt;</td>
<td>NA</td>
<td>236/2399 (9.8%)</td>
<td>NA</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>NA</td>
<td>34/2466 (1.4%)</td>
<td>NA</td>
</tr>
</tbody>
</table>
Reasons for exclusion: no hospital delivery (2554), multiple delivery (36), no malaria result at delivery (37).
SD = standard deviation, NA = not applicable.
n = Number studied.
RPR = rapid plasma reagin card test.
Preterm delivery was classified as any that occurred before 37 completed weeks' gestation, using a standardized Ballard method [27].
Defined as sex-specific birth weight at or below the 10th percentile weight-for-gestational age of an international reference group [31].
Table 2. Prevalence of low birth weight (LBW), small for gestational age (SGA), preterm delivery (PTD) among infants and maternal anaemia at delivery by maternal status and gravidity, Kisumu, western Kenya, June 1996-March, 1999

<table>
<thead>
<tr>
<th>Infection Status</th>
<th>Low birth weight*</th>
<th>Preterm delivery†</th>
<th>Small for gestational age‡</th>
<th>Hemoglobin &lt; 8 g/dl postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primigravidae</td>
<td>Multigravidae</td>
<td>Primigravidae</td>
<td>Multigravidae</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>LBW</td>
<td>LBW</td>
<td>PTD</td>
<td>PTD</td>
<td>SGA</td>
</tr>
<tr>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>1153</td>
<td>1278</td>
<td>1139</td>
<td>1258</td>
<td>1139</td>
</tr>
<tr>
<td>1041</td>
<td>1132</td>
<td></td>
<td>1041</td>
<td>1132</td>
</tr>
</tbody>
</table>

*Low birthweight: a birthweight < 2500 g.

†Classified as any delivery that occurred before 37 completed weeks’ gestation, using a standardized Ballard method [27].

‡Defined as sex-specific birth weight at or below the 10th percentile weight-for-gestational age of an international reference group [31].

n: refers to number in each category

HIV, human immunodeficiency virus.
Table 3. The effect of infection with HIV-alone, malaria at delivery alone and the dual infection on birth outcome (multivariable Poisson models*), Kisumu, western Kenya, June 1996-March, 1999

<table>
<thead>
<tr>
<th>Infection status</th>
<th>Poisson regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low birth weight</td>
</tr>
<tr>
<td></td>
<td>Primigravidae</td>
</tr>
<tr>
<td></td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>Uninfected</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>1.7 (0.8-3.3)</td>
</tr>
<tr>
<td>HIV-alone</td>
<td>1.7 (0.8-3.3)</td>
</tr>
<tr>
<td>Malaria-alone</td>
<td>2.2 (1.2-3.8)</td>
</tr>
<tr>
<td>Dual-infection</td>
<td>3.1 (1.6-6.1)</td>
</tr>
<tr>
<td>HIV-alone</td>
<td>Reference</td>
</tr>
<tr>
<td>Dual-infection</td>
<td>2.2 (0.9-4.9)</td>
</tr>
<tr>
<td>Malaria-alone</td>
<td>Reference</td>
</tr>
<tr>
<td>Dual-infection</td>
<td>1.5 (0.7-3.0)</td>
</tr>
</tbody>
</table>

*All the four models for LBW (low birth weight); SGA (small for gestational age); PTD (preterm delivery) and anaemia at delivery [haemoglobin (Hb) < 8 g/dl] adjusted for maternal weight (< 25 percentile versus ≥25 percentile, kg), maternal age (< 20 vs ≥ 20 years), years of education (< 8 vs ≥ 8 years), ethnicity (Luo vs non Luo), season of delivery (rainy vs dry), a hospitalization in pregnancy and gender of the child. ¹Model for anaemia at delivery [haemoglobin (Hb) < 8 g/dl] also adjusted for type of delivery (cesarean section or assisted delivery
vs normal delivery) and use of haematinics. Abbreviations: HIV, human immunodeficiency virus, malaria-alone refers to any evidence of current malarial infection (maternal peripheral parasitaemia or placental malaria); RR, adjusted risk ratio; CI, confidence interval. Bold-faced results were statistically significant (p<0.05). *Borderline significant: P = 0.06.