Journal of Public Health and Epidemiology Vol. 2(9), pp. 224-237, December 2010
Available online at http://www.academicjournals.org/jphe
ISSN 2141-2316 ©2010 Academic Journals

Full Length Research Paper

Preterm delivery and low birth weight among neonates born to HIV-positive and HIV-negative Ghanaian women

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Accepted 3 November, 2010

In sub-Saharan Africa, several hundreds of pregnancies are exposed to both malaria and HIV infections annually. Adverse perinatal outcomes as a result of these infections include preterm delivery (PTD), and low birth weight (LBW). These are not well characterized in Ghana. We determined whether malaria and HIV infections during pregnancy increase the risk of delivering a preterm or a LBW neonate. We enrolled 1,154 women at their first antenatal visit (443 HIV-positive and 711 HIV-negative), and prospectively collected data at delivery on 761 mother-infant pairs. Malaria parasitemia status, HIV status, hemoglobin concentration, and CD4+ cell count were determined using standard methods. We observed a significantly increased risk of LBW among HIV-positive women with malaria at recruitment, odds ratio (OR) = 4.4, 95% Confidence Interval [CI] (2.3 to 8.4), at delivery, OR = 2.5, 95% CI (1.1 to 3.7). The risk among those who were dually-infected at recruitment and at delivery was more pronounced; OR = 11.3; 95% CI (4.6 to 27.4). Dual infection was also associated with a 4-fold risk of delivering preterm; OR = 3.96; 95% CI (1.8 to 8.5). These findings demonstrate that neonates of HIV-positive women with multiple malaria infections are at particular risk of PTD and LBW in Ghana.

Key words: HIV, malaria, preterm delivery, low birth weight, adverse perinatal outcomes, Ghana.

INTRODUCTION

Neonates of women living in malaria endemic areas where HIV is also a major public health problem may experience a variety of adverse consequences from these infections including low birth weight (LBW), and preterm delivery (PTD). It is estimated that 40% of the world's pregnant women are exposed to malaria infection during pregnancy. This infection according to Shulman and Dorman is one of the most important preventable causes of LBW and PTD (Shulman and Dorman, 2003). Malaria is thought to reduce birth weight through a combination of systemic and local effects (Menendez et al., 2000). That is through malaria-induced anaemia, and or, the effects of placental infection (Ibhanesebhor and Okolo, 1992; Kassam et al., 2006; Okoko et al., 2002). It is also thought that a high density parasitemia, chronic infection in the placental blood and the associated cellular immune response may result in the consumption of glucose and oxygen that would have gone to the fetus. Histopathologic studies of infected placentas have also found thickening of the cytotrophoblastic membranes which may interfere with nutrient transport to the fetus, subsequently leading to LBW (Guyatt and Snow, 2004; Ismail et al., 2000). With respect to PTD, the precise effect of malaria infection on PTD is uncertain, however, malaria-infected placentas have been shown to frequently carry antibodies, cytokines and macrophages which are indicative of active immune-response, and this response may stimulate early labor (Guyatt and Snow, 2004; Ismail et al., 2000).

In addition to malaria, maternal HIV infection has been shown to be associated with a number of adverse

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perinatal outcomes including PTD and LBW. One of the earlier systematic reviews by Brocklehurst and French reported that, adverse perinatal outcomes related to maternal HIV infection included, intrauterine growth retardation, low birth weight, and preterm delivery (Brocklehurst and French, 1998). In a later review by ter Kuile, several studies were identified that examined the effect of dual infection with malaria and HIV on birth outcomes (Ayisi et al., 2003; Leroy et al., 1998; Steketee et al., 1996; Ticconi et al., 2003; Verhoeff et al., 1999; Weng et al., 1998). Although, differences in study design limited direct comparisons between the studies, the studies showed an increased risk of poor birth outcome in terms of low birth weight, preterm birth, and IUGR with both HIV and malaria, with the greatest risk in women with dual infection (Ter Kuile et al., 2004).

In Malawi, Abrams and colleagues after examining risk factors and mechanisms of PTD in malaria-exposed pregnant women reported that HIV was associated with PTD while malaria was not (Abrams et al., 2004). This finding was contrasted by that of Noble et al. where both maternal HIV infection and malaria history, among Zimbabwean women were associated with preterm delivery (Noble et al., 2005). Almost concurrently in Tanzania, Villamor et al. examined the risk of adverse perinatal outcomes in relation to maternal or umbilical cord Plasmodium falciparum parasitemia among HIV-infected women and also reported that malaria and HIV infections were associated with LBW and PTD (Villamor et al., 2005). A relatively recent review by Desai and colleagues on evidence of the clinical implications and burden of malaria in pregnancy suggest that successful prevention of these infections reduces the risk low birth weight by 43% and perinatal mortality by 27% among paucigravidae (Desai et al., 2007).

In Ghana, very few studies have looked at PTD and LBW among HIV-positive pregnant women with malaria. This paper seeks to publicize the magnitude of this problem and also identify clinical characteristics of the mother that may be associated with the incidence of PTD and LBW among HIV-positive women delivering at three public hospitals in Ghana.

METHODOLOGY

The study sites

This study was conducted at three public hospitals in Ghana. These were the Tema General Hospital in the Tema Municipality, Greater Accra region, Atua Government Hospital and St Martins de Porres Hospital both in the Manya Krobo District, Eastern region.

Study design, population, participants and summary of field procedures

This was a prospectively designed study involving pregnant women seeking antenatal care services from June 2005 to August 2008. From this population, the women who met the study inclusion criteria and consented to enrol in the study were recruited. When these women returned to deliver at their designated hospitals, follow up data on perinatal outcomes were taken.

We recruited 1,154 (443 HIV-positive and 711 HIV-negative antenatal attendees), 171 with malaria and 983 without malaria. At their first antenatal visit, the investigators with assistance from trained nurses and research assistants collected information on the background, socio-economic, socio-demographic characteristics, obstetric, and reproductive history of the study participants. Complete physical and clinical appraisals were carried out by the experienced nurses/midwives and 5ml blood samples collected by competent phlebotomists for various "hematologic and immunologic" determinations. Seven hundred and sixty-one (295 HIV-positive and 466 HIV-negative) of the 1154 women had their follow up data at delivery taken. All the women were counselled by trained nurse counsellors, who were thoroughly informed about the purpose of the study.

Inclusion criteria

A pregnant woman attending the antenatal clinic was eligible to enrol into the study if she had not previously been registered at any of the antenatal clinics with her current pregnancy, if she had an established HIV status (positive or negative) or was willing to be tested, was 18 years of age or older, was resident in the study areas or surrounding townships, was willing to accept follow up visits, planned to stay in the study area for up to 6 months post-delivery, and willingly provided informed consent to be part of the study.

Exclusion criteria

Pregnant women who had previously been registered at any of the antenatal clinics at the three sites or were unwilling to give consent, or younger than 18 years of age were excluded.

Sample size determination, sampling and follow up procedures

The sample size determination was done using the Statcalc statistical module in the Epi Info software (version 3.3.2). Epi Info is a public domain statistical software for epidemiology developed by the Centers for Disease Control and Prevention (CDC). In the absence of any empirically documented local data on the main outcomes of the study (including PTD, LBW) among HIV-positive pregnant women, findings from two Zimbabwean studies (Ticconi et al., 2003; Noble et al., 2005), one Tanzanian study (Villamor et al., 2005) and a review by ter Kuile et al. (2004) were used.

From the above studies, the prevalence of PTD among HIV-positive pregnant women with no malaria (herein referred to as the unexposed group) was 10.7%; the prevalence of PTD in the exposed group (HIV-positive pregnant women with malaria) was 25.7%. At 95% confidence level, with a statistical power of 80%, a sample size of 249 HIV-positive pregnant women was deemed adequate to test the hypothesis related to this outcome (Fliess, 1981). The minimum samples needed for the assessment of the other outcome was similarly calculated. The largest of the samples was 249. To satisfy the apriori decided 1:2 ratio of exposed (HIV-positive pregnant women with malaria) to unexposed (HIV-positive pregnant women with no malaria), 63 HIV-positive pregnant women with malaria and 166 HIV-positive women without malaria needed to be enrolled and followed up at delivery. With an anticipated participation rate of 95% , and attrition from all causes (attrition rate of 30% anticipated) , the 249 figure was upwardly adjusted and rounded up to 300.Two HIV-negative pregnant women were concurrently recruited for every HIV-positive woman as a comparison group.

Anticipating a low uptake of VCT services, all antenatal clients at the three study hospitals between June 2005 and March 2008 were approached for enrolment into the study. A total of 27,002 pregnant women visited the three hospitals as new antenatal registrants throughout the period. A research nurse (running the PMTCT services) and research assistants aided the investigators to enrol all HIV-positive pregnant women who met the study's inclusion criteria. The investigators trained the nurses and research assistants on the methods, the inclusion criteria, as well as the objectives of the study. The women who consented to participate were enrolled. The information documented at recruitment included their background, socio-economic, socio-demographic characteristics, obstetric, and reproductive history. Clinical appraisals were carried out by the nurses and 5 ml blood samples collected by competent laboratory technicians for various determinations. All the above were documented on the first day of contact.

Follow-up data collection took place when the study participants returned to deliver at their designated hospitals. Of the 1,154 study participants enrolled into the study, seven hundred and ninety three (315 HIV-positive and 478 HIV-negative) turned up for delivery at the three hospitals. However, only 761 (295 HIV-positives and 466 HIV-negatives) had all the required data at delivery taken. These formed the sample for the follow-up data analysis. The details of the study profile are given in Figure 1.

Laboratory

Blood samples (5 ml) were collected from participating women at two time points. Venous blood was taken both at recruitment and at delivery; cord blood was taken only at delivery into heparinized EDTA vacutainers for analysis.

Determination of maternal HIV status at recruitment

HIV infection was determined using the Determine® HIV-1/2 rapid test kit (Abbott Laboratories Diagnostics Division, IL, USA) Kit. It is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. Blood sample is added to the sample pad. As the blood migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site.

If antibodies to HIV-1 and or HIV-2 are present in the blood sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site. If antibodies to HIV-1 and/or HIV-2 are absent, the antigen-selenium colloid flows past the patient window and no red line is formed at the patient window site.

Determination of maternal and cord malaria parasitemia

Malaria parasitemia was determined using Rapid Test Kit (Paracheck Pf, Orchid Biomedical Systems, India) that detects the presence of Plasmodium falciparum-specific protein (Pf. HRP-2) in whole blood specimen up to 14 days after the infection has been cleared. This test also utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the dipstick after placing into the clearing buffer tube, the colored anti Pf HRP-2 antisera-colloidal gold conjugate (monoclonal) complexes the Pf HRP-2 in the lysed sample. This complex moves further on the membrane to the test region, where it is immobilized by the anti Pf HRP-2 (monoclonal) antisera coated on the membrane leading to formation of a pink colored band which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result, with a control band that serves to validate the test performance.

Determination of maternal hemoglobin concentration

Maternal venous blood and infant cord blood hemoglobin concentrations were determined using an Automated Hematologic Analyzer (which measures hemoglobin by the formation of hemoglobincyanide). In addition, the analyzer also directly measures the cell count for total red blood cells, white blood cells and platelets.

Determination of maternal CD4+ count

Maternal CD4+ count was determined using the Becton Dickinson (BD) FACScount system, which measures absolute CD4 counts (Immunocytometry Systems, San Jose, CA).

Birth weight and gestation length

A research midwife weighed infants to the nearest 10 g on a standard seca scale immediately after birth. Gestational length was determined based on the recall of the last menstrual period of the study participant.

Ethical issues

The research protocol met the guidelines for research involving human subjects of the Noguchi Memorial Institute for Medical Research (NMIMR). The study protocol was first reviewed and vetted by the Proposal Review Board of the School of Public Health for appropriateness and scientific content.

An ethical clearance was afterwards sought from the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (FWA 00001824, NMIMR-IRB CPN 044/04-05, IRB 0001276).Written informed consent, for those who were literate and witnessed verbal informed consent, for the illiterate was obtained from each study participant. Subjects were informed about the objectives and methods of the study. They were also assured of strict confidentiality with regards to any information obtained from them.

Data analysis

Statistical analyses were done using SPSS Version 15.0 (SPSS Inc. Chicago, Illinois). Preliminary assessments of normality of the distributions of birth weight and gestation length, were done using normal probability plots. Such assessments showed that these outcome variables were normally distributed. Appropriate measures of centrality and of dispersion, as well as frequencies were then computed. Proportions of various outcomes were compared using the the Chi square test. For comparisons of mean birth weight, and gestation length by maternal infection status, the ANOVA technique was used. During the various stages of this analytic procedure, assessments were done to ascertain whether the data met the independence and homogeneity of variance assumptions. The Welch F statistic was used as the robust test for equality of means, where homogeneity of variance assumption was violated. Two different Post hoc tests were done; the Hochberg's GT2 test was used where samples sizes were different and the Gaems-Hoewell procedure where homogeneity of variance assumption was violated. The associations between low birth weight, preterm delivery and maternal infection status, as well as other factors were investigated using simple logistic and multiple logistic regression techniques. Assessment of the strength of these associations was done using odds ratios (ORs) and their 95% confidence intervals (CI). The covariates used in the multiple regression models were included, if they were significantly associated with any of the outcome measures in the bivariate analyses, or if they were known to be associated with these outcomes based on previous studies. Overall, the level of significance was set at $p < 0.05$. For the Post Hoc tests, however, this was set at $p < 0.0167$ (0.05 divided by the number of comparisons/tests).

RESULTS

Background, socio-demographic and other characteristics of study participants

The total number of 1,154 pregnant women recruited into the study comprised 443 HIV-positive women and 711 HIV-negative women. A little over 30% (361) of the 1,154 enrolled were lost to follow up before delivery (128 HIV-positive and 233 HIV-negative). Excluded from the analyses were 32 cases (7 pair of babies who were products of multiple deliveries, 11 stillbirths and 13 with missing/inadequate data). This left 761 (295 HIV+, and 466 HIV-) mother-infant dyads with complete data for the analysis. Details of the study profile including the rates of the various adverse perinatal outcomes by maternal HIV infection status are presented in Figure 1.

In Table 1, comparisons were made of the baseline background and socio-demographic characteristics of the women on whom data at delivery were available with that of those who were lost to follow-up. These two groups of women did not differ significantly in terms of their age distribution, gestation length at first antenatal visit, occupation and socio-economic stratification (Table 1). However, they differed significantly with respect to marital status, level of education and place of residence.

Prevalence of LBW, PTD by some selected maternal characteristics

The overall prevalence of LBW was 15.4% and PTD, 18.4%. By maternal HIV infection status, prevalence of LBW was 22.5% among neonates born to HIV-positive women, and 14.1% among those born to HIV-negative women. Comparatively, PTD was higher among HIV-positive mothers (24.4% versus 13.6%). Further comparison of these rates by maternal infection status are presented in Figure 2. Relating these events to the various maternal infections status period showed a clear trend. Overall, HIV-infected women were more likely to have a LBW or PTD than uninfected women. To place these results in a broader context of maternal infections or dual infections at the two time points, the incidence of the above adverse perinatal outcomes were compared against seven different maternal infection statuses (Figure 2). The incidence of these events was pronounced in the neonates whose mothers were co-infected with malaria and HIV either at first antenatal visit or at delivery. Dramatically, higher were the incidences of these events in mothers who were co-infected with HIV and malaria both at their first antenatal visit and at delivery (Figure 2).

Maternal infection status and infant outcomes (birth weight and gestation length)

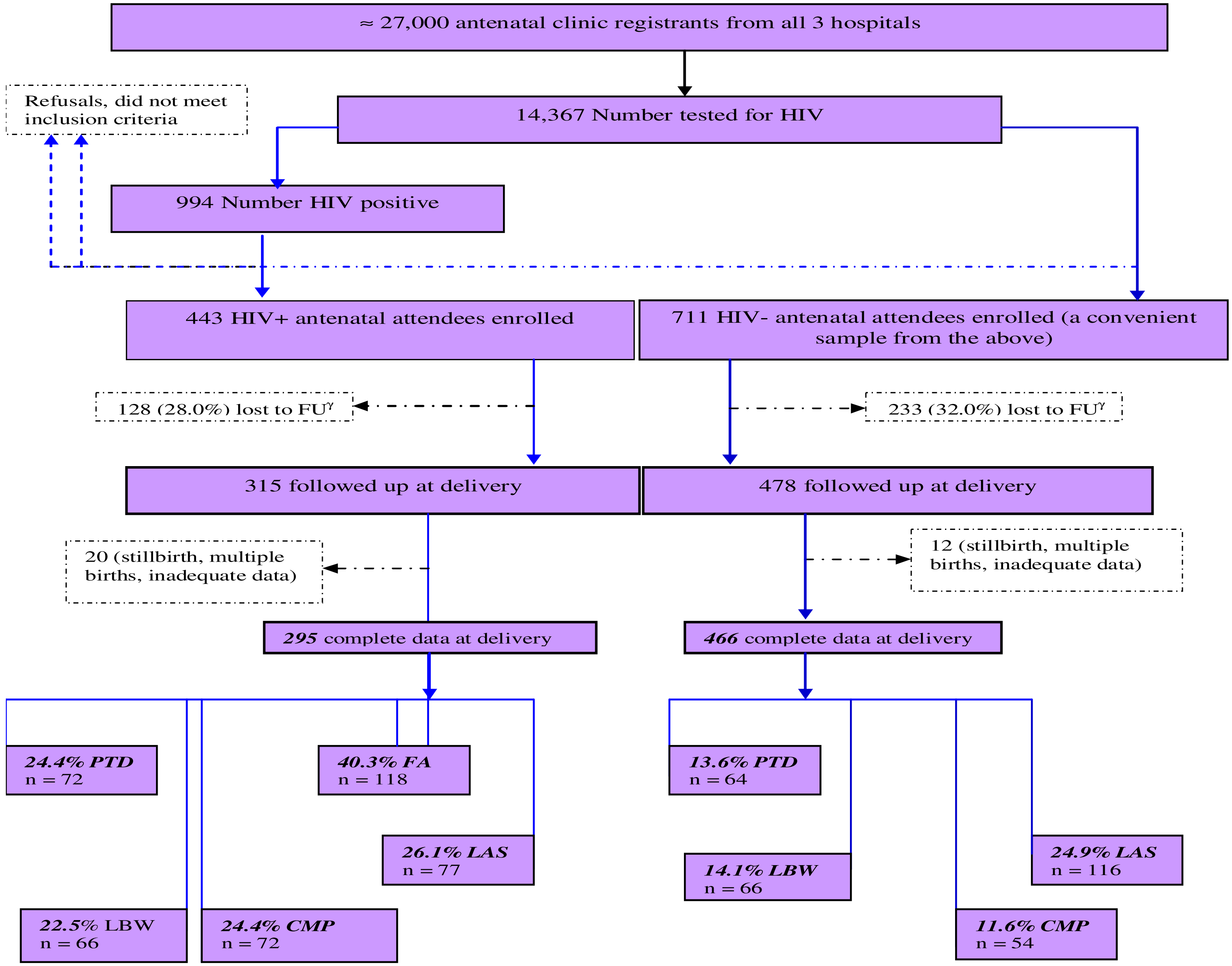
We explored the association between maternal infection status and birth weight, gestation length. The following variants of maternal infection categories were used in these comparisons:

i. HIV- Mal[r-d-]:- women with no HIV and also no malaria both at recruitment and at delivery.
ii. HIV- Mal[r+d-]:- women with no HIV but have malaria at recruitment and not at delivery.
iii. HIV+ Mal[r-d+]:- women with HIV, no malaria at recruitment but malaria positive at delivery.
iv. HIV+ Mal[r+d+]:- HIV-positive women with malaria both at recruitment and at delivery.

Two analytical approaches were employed. In the first approach, analysis of variance (ANOVA) was used to test the differences in the means of birth weight and gestation length across the above maternal infection categories. The second approach made use of logistic regression technique in assessing the strength of the associations between maternal infection characteristics and the two infant adverse outcomes of interest.

Birth weight/LBW and maternal infection status

The effect of no infection (HIV- Mal[r-d-]), single maternal malaria infection (HIV- Mal[r+ d-]/ HIV- Mal[r-d+]), dual infection at recruitment (HIV+ Mal[r+ d-]), at delivery (HIV+ Mal[r- d+]), and at both time points (HIV+ Mal[r+d+]) on mean birth weight are presented in Figure 3. This clearly shows that the mean birth weight of a neonate differed significantly by maternal infection status; $F(3, 722) = 11.23$; $p < 0.001$. Giving the disparity in the sample sizes across the test groups, the Hochberg GT2 Post Hoc test was the appropriate choice for the multiple comparisons. These comparisons while affirming the overall ANOVA test, specifically showed that, HIV-negative women with malaria infection only at recruitment on average delivered babies who weighed 123 g less; mean difference = 0.123 kg; 95% CI (0.001 to 0.244) compared to HIV-negative women without malaria at recruitment. Those dually infected at recruitment delivered babies weighing 195 g less; mean difference = 0.195 kg; 95% CI (0.035 to 0.356). These differences in



FU (follow up); γHigh antenatal attendance and very low institutional delivery is a very common phenomenon in these settings; PTD:- Preterm delivery; FA:- Fetal anemia; LAS:- Low Apgar score; LBW:- Low birth weight; CMP:- Cord malaria parasitemia

Figure 1. The study profile: June 2005 to August 2008 and the main adverse perinatal outcomes.

Table 1. Baseline background, and socio-demographic characteristics of women on whom complete data at delivery were available compared with those who were lost to follow-up.

Characteristic	Participants at delivery	Participants lost to follow-up	P value
Age			
19 years or younger	79 (10)	31 (8.4)	
20-24 years	181 (23.8)	88 (24.0%)	0.587
20-24 years	501 (65.8))	248 (67.6)	
Trimester at recruitment			
First	69 (9.1)	39 (10.6)	
Second	332 (43.9)	167 (45.5)	0.540
Third	355 (47.0)	161 (43.9)	
Marital status			
Married	349 (45.9)	160 (43.4)	
Single	239 (31.4)	65 (17.6)	<0.001
Divorced/Separated	2 (0.3)	1 (0.3)	
Cohabiting	171 (22.5)	143 (38.8)	
Religion			
Christian	468 (61.7)	331 (89.7)	
Islam	277 (36.5)	31 (8.4)	<0.001
Traditionalist	13 (1.7)	4 (1.1)	
None	1 (1.0)	3 (8.0)	
Place of residence			
Rural	471 (62.1)	280 (75.9)	<0.001
Urban	288 (37.9)	89 (24.1)	
Subject's level of education			
Nil	79 (10.4)	31 (8.4)	
Primary	172 (22.7)	101 (27.4)	
Middle/JSS	272 (35.9)	171 (46.3)	<0.001
Secondary/Post secondary	180 (23.7)	55 (14.9)	
Tertiary	55 (7.3)	11 (3.0)	
Partner's level of education			
Nil	45 (5.3)	12 (3.3)	
Primary	68 (9.6)	39 (10.8)	
Middle/JSS	260 36.6)	159 (44.2)	0.040
Secondary/Post secondary	228 (32.1)	105 (29.2)	
Tertiary	110 (15.5)	45 (12.5)	
Occupation of subject			
Not working	143 (18.8)	53 (14.4)	
Peasant farming	22 (2.9)	10 (2.7)	
Services	576 (75.7)	297 (80.5)	0.431
Clerical work	5 (0.7)	3 (0.8)	
Professional/technical/administrative work	15 (2.0)	6 (1.6)	
Occupation of partner			
Not working	39 (5.1)	17 (4.6)	0.773
Peasant farming	74 (9.7)	41 (11.1)	

Table 1. Continued.

Services	434 (57.0)	219 (59.3)	
Clerical work	128 (16.8)	56 (15.2)	
Professional/technical/administrative work	86 (11.3)	36 (9.8)	
Subject's SE index score			
Low	186 (27.0)	76 (22.4)	
Middle	471 (68.3)	252 (74.3)	0.117
High	33 (4.8)	11 (3.2)	

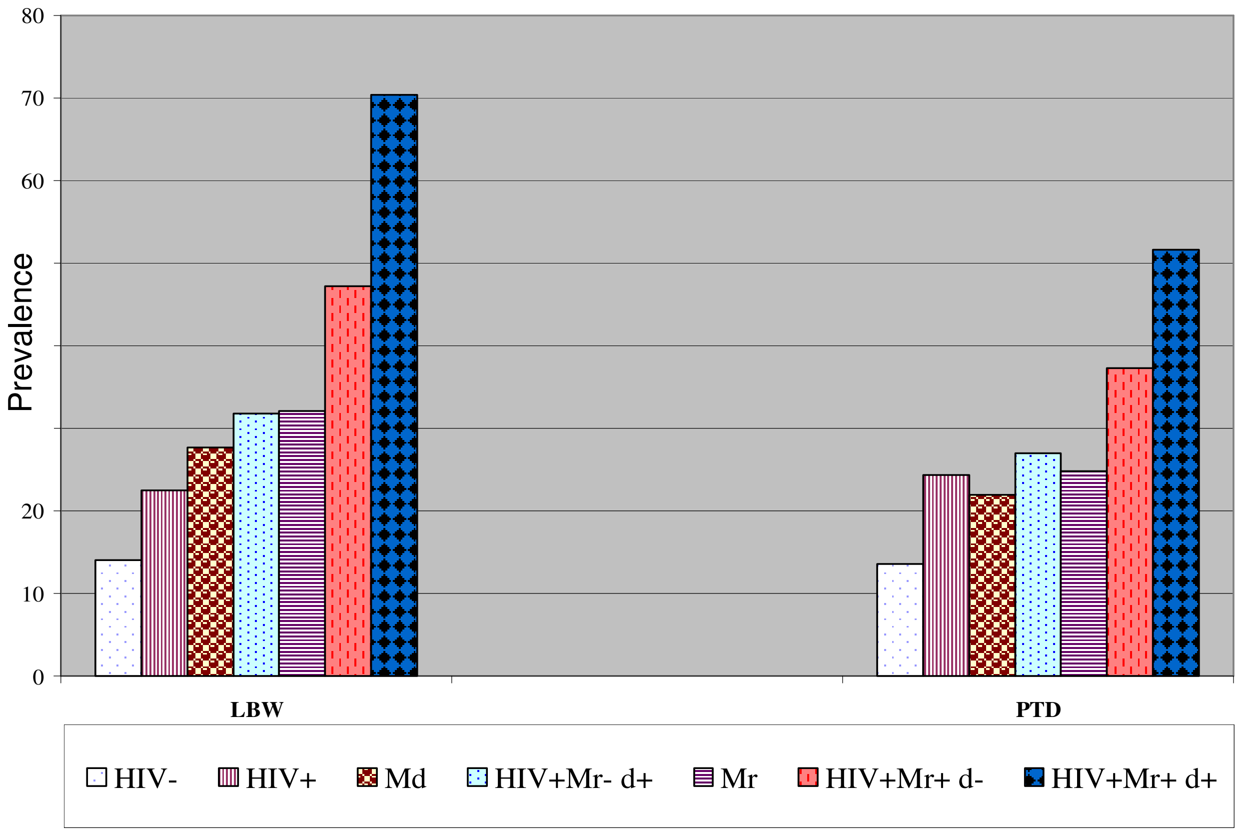
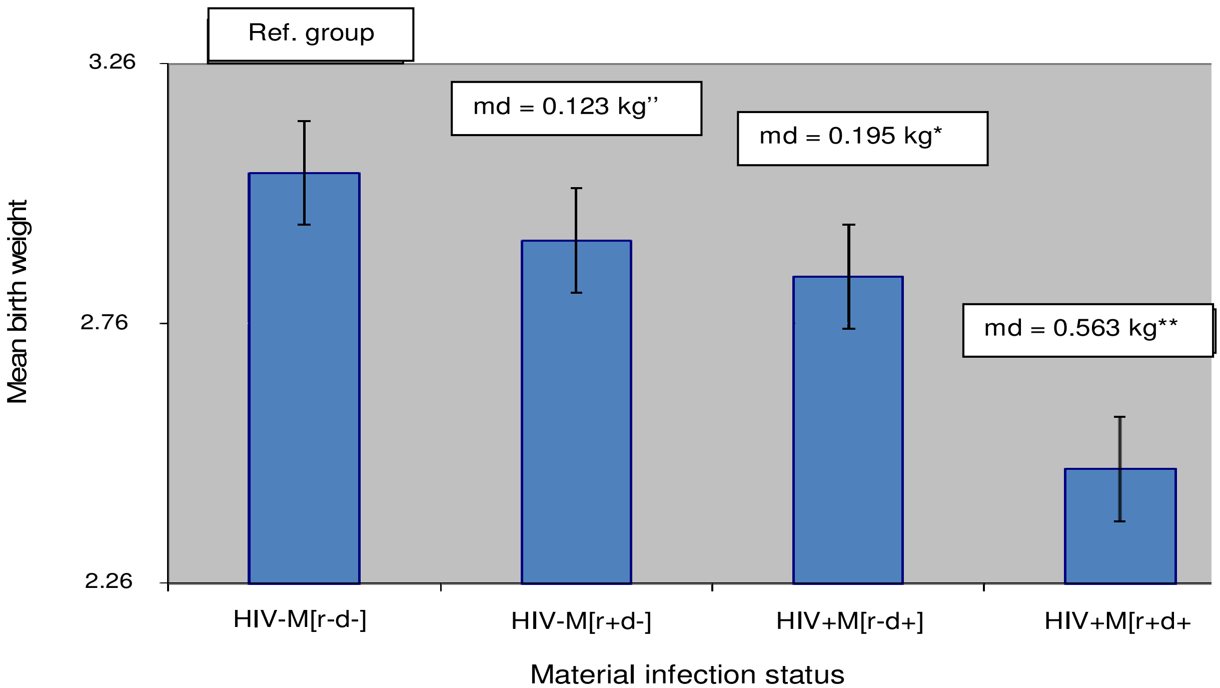


Figure 2. Prevalence of low birth weight and preterm delivery by maternal infection status. HIV- ⇒ HIV-negative women; HIV+ ⇒ HIV-positive women; Mr⇒ malaria positive at recruitment; Md ⇒ malaria positive at delivery; Mr+d- ⇒ malaria positive at recruitment and negative at delivery; Mr+d+⇒ malaria positive both at recruitment and at delivery; HIV+Mr-d+ ⇒HIV-positive women without malaria at recruitment but have malaria parasitemia at delivery.

mean birth weight were particularly marked in women dually-infected both at recruitment and at delivery. These women delivered babies weighing on average 563 g less than those of uninfected counterparts; mean difference = -0.563.5 kg, 95% CI (-0.855 -0.272). Collectively these findings demonstrate that maternal infection with HIV and malaria has a profound negative effect on birth weight. This bivariate level analysis shows a significantly increased risk of LBW among HIV+ women who had malaria parasitemia at recruitment OR = 4.4; 95% CI (2.3 to 8.4), and at delivery OR = 2.5; 95% CI (1.1 to 3.7). This risk was statistically more pronounced in neonates whose mothers were dually infected with HIV and malaria at both time points – at recruitment and at delivery OR = 11.3; 95% CI (4.6 to 27.4; Figure 4). In accord with its generally negative effects on birth weight, preterm delivery in this instance was a strong risk factor for LBW. In particular, newborns delivered before the 37th week of gestation were over 30-fold as likely, as those who were delivered on or after the 37th week to have weighed less



Overall test: F (3 722) = 11.24; p < 0.001; Error bars represent 95% Confidence interval for the mean.
Ref. group = Group compared with in the Post Hoc analysis (Hochberg GT2 test); "p < 0.05; *p < 0.016; **p < 0.001

Figure 3. Association between maternal infection status and birth weight.

than 2500 g OR = 31.5; 95% CI (15.0 to 66.1).

These risks persisted in the multiple logistic regression model, where a number of covariates were controlled for. PTD, malaria infection at recruitment and at delivery remained significant predictors of LBW. One other factor significantly associated with LBW was maternal HIV status (Table 2). Taken together, these data indicate that neonates born to women exposed to malaria and HIV infections during pregnancy as well as those born before term are at particular risk of LBW.

Gestation length/PTD and maternal infection status

Presented in Figure 5 are results showing the existence of statistically significant differences in gestation length by maternal infection categories. Of note here, the homogeneity of variance assumption was violated (Levene Statistic [4.321; df1:df2 (3: 739); p = 0.005). To ensure validity of the test outputs therefore, a more robust test of equality of means (the Welch F statistic) was used. This again affirmed the assumption that, significant differences exist in mean gestation length across the maternal infections categories; F (3, 123) = 7.40; p < 0.001. In this case, the Gaemes-Howell Post Hoc test was chosen. This showed that on average, maternal infections (single or dual) were associated with shorter gestation length. However, this was more pronounced and statistically significant in the group of women who were dually infected both at recruitment and at delivery. Compared to their HIV-uninfected and malaria negative counterparts, this group of women delivered two (2) weeks earlier; mean difference = -2.08 weeks 95% CI (-3.28 to -0.88), p < 0.001. These differences persisted, but nevertheless attenuated when the HIV+ M[r+d+] group was compared with the HIV-M[r+d-] group and also with the HIV+M[r-d+].

In Figure 6, the associations between PTD and some clinical and nonclinical maternal factors are presented. Among HIV-positive women, maternal CD4 count beyond a certain threshold (more than 350 cells per ml) were significantly protective against PTD. On the contrary, pregnant women with malaria had an increased risk of delivering preterm. Malaria infection (both at recruitment and at delivery) was associated with almost 4-fold risk of delivering before the 37th week of gestation OR = 3.96; 95% CI (1.8 to 8.5). Women with one-time malaria infection – recruitment, but not at delivery also had higher relative odds of delivery preterm (Figure 6). Maternal MUAC < 23.5 cm, and maternal anaemia (defined as hemoglobin concentration < 11.0 g/dl) were both not significantly associated with PTD. These associations were further evaluated in a multiple logistic regression model including maternal malaria infection at recruitment,

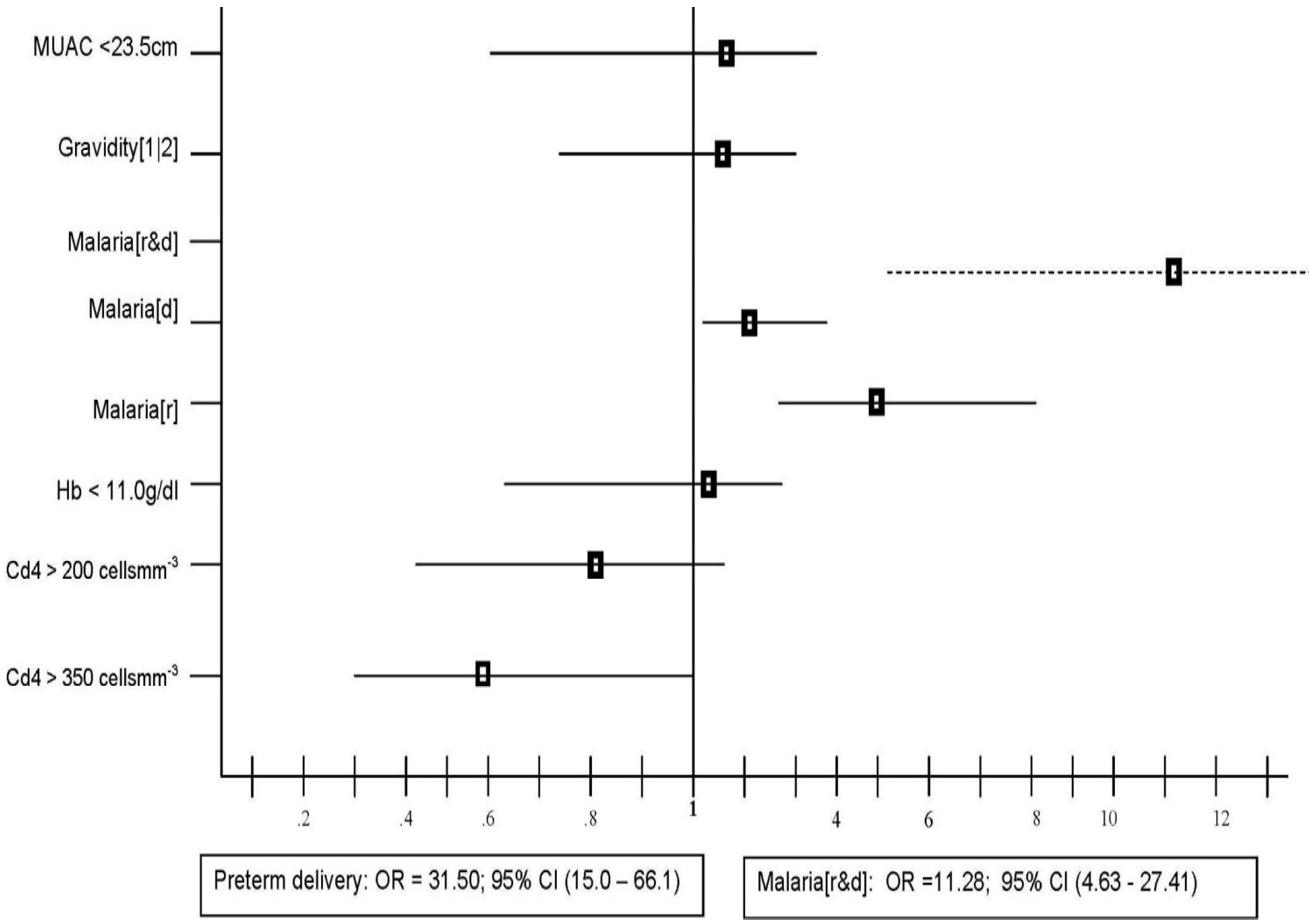


Figure 4. Risk factors associated with LBW (bivariate analysis; ORs with their 95% CI).

Table 2. Predictors of low birth weight (LBW): Multiple logistic regression analysis[1].

Predictors		95% CI for the AOR		
	B (SE)	Lower bound	AOR	Upper bound
β0	-5.15 (1.01)			
Malaria at recruitment	2.57 (0.72)	3.18	13.03*	53.33
Malaria at delivery	2.17 (0.83)	1.72	8.77*	44.76
CD4 count	0.21 (0.42)	0.54	1.24	2.82
HIV status	1.89 (0.99)	1.95	6.65*	46.47
PTD	3.66 (0.44)	16.22	38.74*	92.53

Model for LBW included the following variables: Malaria at recruitment, Malaria at delivery, CD4 count, HIV status, and PTD.Model Summary: -2 Log likelihood 146.09, Cox and Snell R Square 39; Nagelkerke R Square 60; *p value significant at 0.05 level. AOR = Adjusted Odds Ratio. [1]It did not escape our speculation that the inclusion in the model simultaneously, the variants of maternal infection categories such as malaria infection at recruitment, malaria infection at delivery could make the models prone to the biasing effect of collinearity. Of note here, the version of the SPSS used in the analysis did not have the option for producing collinearity diagnostics in logistic regression. Nevertheless, statistics such as tolerance and VIF values were obtained by running a linear regression analysis using the same outcome and predictors. The tolerance and VIF values from the outputs showed that multicollinearity was not a cause for concern.

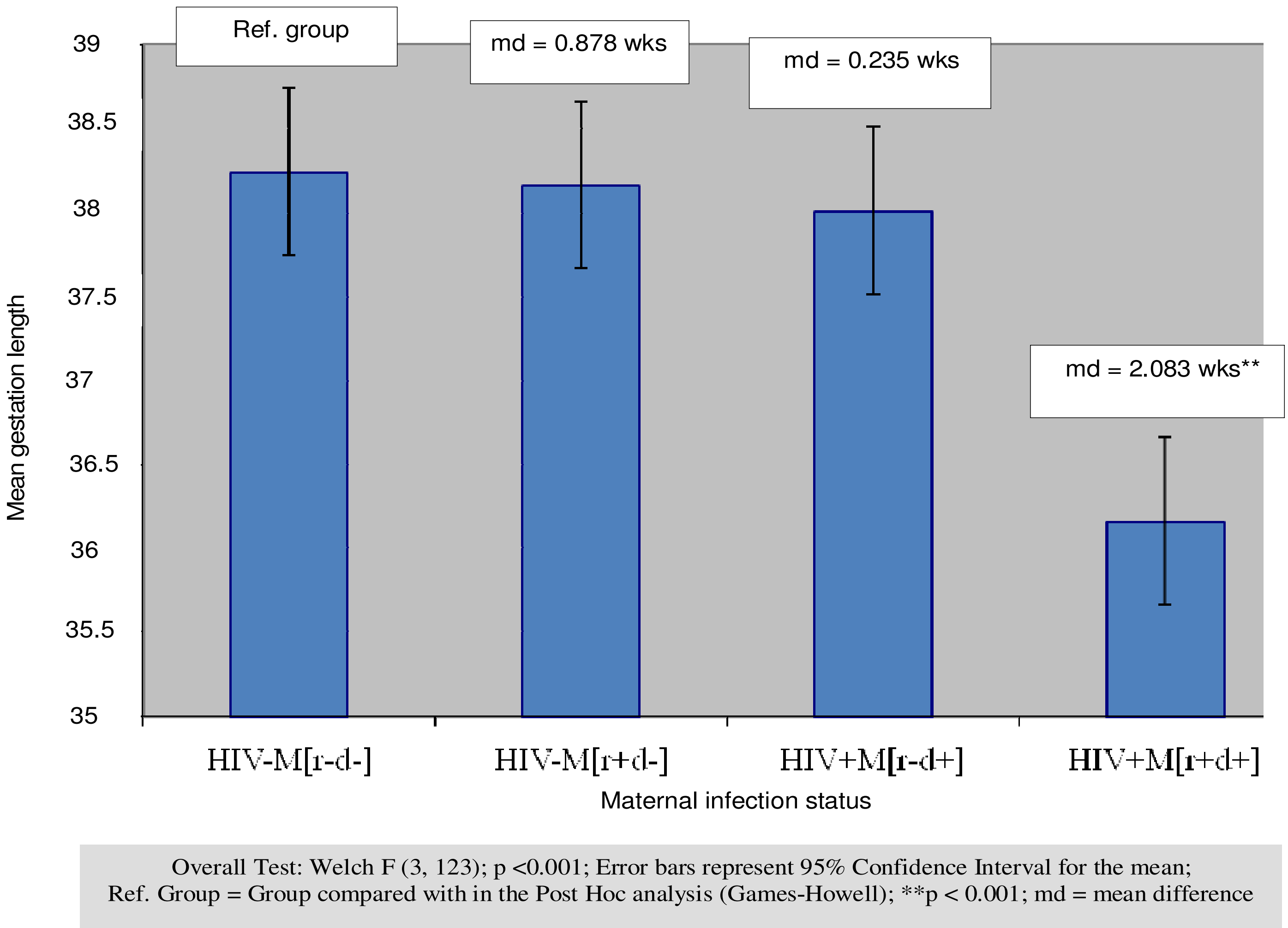


Figure 5. Association between maternal infection status gestation length.

at delivery, malaria infection at these two time points, HIV infection, and CD4 count as predictors. At this stage, malaria at recruitment, malaria at delivery, HIV status, were still significantly associated with PTD (Table 3).

DISCUSSION

Malaria and HIV infections during pregnancy and adverse perinatal outcomes

This study was conducted at three public hospitals; two in the Manya Krobo District (Eastern Region) and one in the Tema Municipality (Greater Accra Region) of Ghana. The study was implemented in a dynamic policy environment with regard to perinatal HIV prevention, and malaria treatment/prevention. Within the period that this study was implemented, there was the shift from the “opt in” to the “opt out” antenatal HIV testing strategies, the shift from the short-course antiretroviral prophylaxis to Highly Active Antiretroviral Therapy (HAART) for pregnant women depending on their CD4 count and other factors, and also the change from Chloroquine monotherapy to Artesunate-Amodiaquine (AS-AQ) combination therapy for malaria treatment. All these took place in the setting where the intermittent preventive antimalarial treatment in pregnancy with SP was being implemented.

Presently at all the three hospitals, programs have been established to deliver both short-course antenatal antiretroviral therapy and full HAART to HIV-positive pregnant women (GHS, 2007) and also to provide IPTp during pregnancy in accordance with recent international policy (WHO/AFRO, 2003). The successful adoption and implementation of these programs can be attributed to the systemic structures that have since the year 2001, been put in place in these areas. It is worthy of note that, the first National PMTCT Pilot Program and Antiretroviral Therapy Program were introduced in the Manya Krobo district in 2001. Presently, this district, the TMA and most

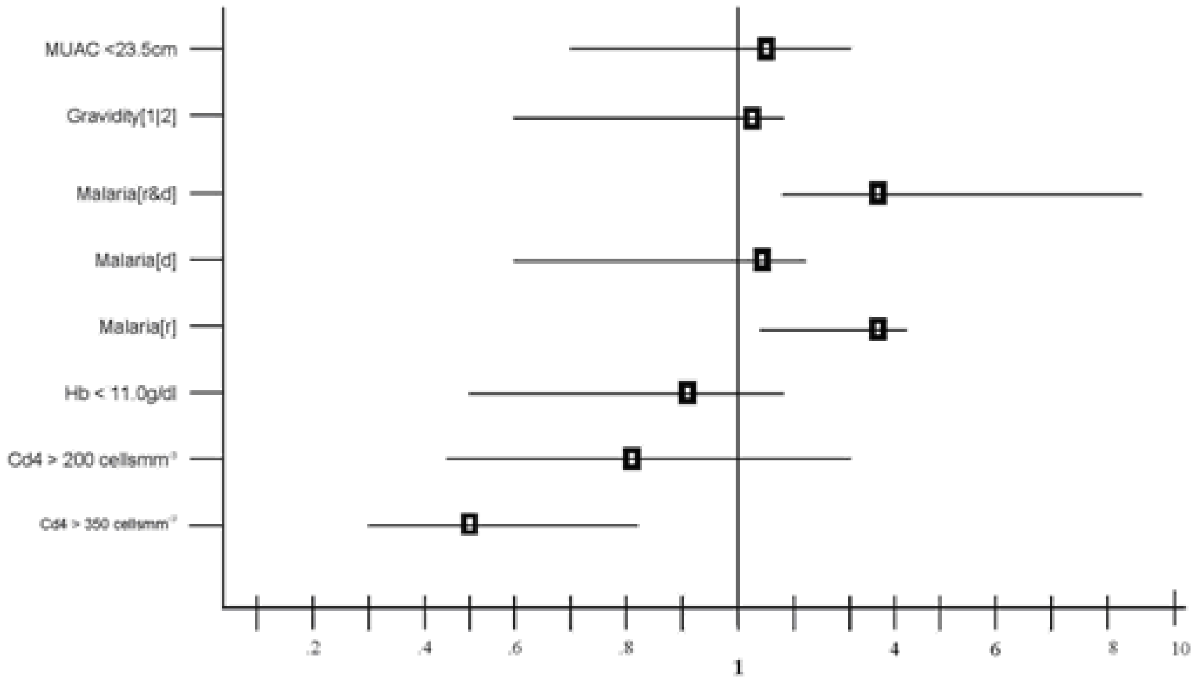


Figure 6. Risk factors associated with PTD (bivariate analysis; ORs with their 95% CI).

Table 3. Predictors of preterm delivery (PTD): Multiple logistic regression analysis.

Predictors	PTD	95% CI for the AOR		
	B (SE)	Lower bound	AOR	Upper bound
β0	-2.46 (0.54)			
Malaria at recruitment	2.26 (0.62)	2.85	9.58*	32.27
Malaria at delivery	1.43 (0.62)	1.23	4.17*	14.18
CD4 count	-0.56 (0.30)	0.32	0.57	1.04
HIV status	2.41 (0.80)	2.33	11.13*	53.30

Model for PTD included the following variables: Malaria at recruitment, malaria at delivery, CD4 count, HIV status, and malaria infection either at recruitment or at delivery. Model Summary: -2 Log likelihood 285.03; Cox and Snell R Square 0.08; Nagelkerke R Square 11. *p value significant at 0.05 level. AOR = Adjusted Odds Ratio

others in Ghana, have a high level of commitment to addressing the issues of HIV and malaria at both the health facility and at the community levels.Of 1,154 pregnant women who were recruited into the study, about 30% were lost to follow-up (LTFU) before delivery. It is, however, worth noting that in terms of age distribution, gestation length at first antenatal visit, occupation, and socio-economic stratification, those women on whom follow up data were available did not differ significantly from those who were lost to follow-up. Also speculating that the baseline clinical outcomes of this group of women on whom data at delivery were available might differ from those who did not come back to deliver at the study hospitals a similar comparison was made.

This analysis again showed that, with the exception of severe anaemia, the two groups of study participants were comparable (data not shown). On this premise, we proceed to discuss the study findings with confidence that the 761 women on whom the main analysis were based, to a large extent represent the overall sample of 1,154 women enrolled into the study.

We, however, acknowledge though that, if an intention to treat analysis had been done, possibly different conclusions would have been made. Nevertheless, this approach which works on the assumption that none of those lost to follow-up suffered the adverse outcomes of interest can open the door to a misleading presentation of study results. It is also worthy of note that, the other alternative strategies available in dealing with this problem by imputing outcomes to those lost to follow-up, in general, all make unverifiable assumptions that may introduce bias in the estimates of treatment effect (Hollis and Campbell, 1999).

Association between birth weight/LBW and maternal infection status

The evaluation on the effect of maternal infection on mean birth weight support the study's hypothesis that the mean birth weight of a neonate differs significantly by maternal infection status; $F(3, 722) = 11.23$; $p < 0.001$ (Figure 3). These differences in mean birth weight were markedly more pronounced in women dually-infected both at recruitment and at delivery. The findings demonstrate that maternal infection with HIV, and/ or malaria has a significant effect on birth weight. Several studies conducted in non-Ghanaian settings have reported similar findings (Ayisi et al., 2003; Leroy et al., 1998; Steketee et al., 1996; Ticconi et al., 2003; Verhoeff et al., 1999; Villamor et al., 2005). For instance, in the study by Ayisi et al. it was reported that compared with women with no malaria or HIV, maternal HIV infection was associated with a 99 g reduction in mean birth weight (Ayisi et al., 2003). Malaria is thought to reduce birth weight through a combination of systemic and local effects (Menendez et al., 2000). That is through malaria-induced anaemia, and or, the effects of placental infection (Ibhanesebhor and Okolo, 1992; Kassam et al., 2006; Okoko et al., 2002). For instance, a high density parasitemia, chronic infection in the placental blood and the associated cellular immune response has been shown to result in the consumption of glucose and oxygen, that would have gone to the foetus. Histopathologic studies of infected placentas have also found thickening of the cytotrophoblastic membranes which may interfere with nutrient transport to the foetus, subsequently leading to LBW (Guyatt and Snow, 2004; Ismail et al., 2000).

The second approach which employed simple and multiple logistic regression techniques revealed that, the risk of LBW was highest in women dually infected at two time points (at recruitment and at delivery). The data presented suggest a significantly increased risk of LBW among HIV+ women who had malaria parasitaemia at recruitment OR = 4.4; 95% CI (2.3 to 8.4), and at delivery OR = 2.5; 95% CI (1.1 to 3.7). This risk was statistically more pronounced in neonates whose mothers were dually infected with HIV and malaria at both time points – at recruitment and at delivery OR = 11.3; 95% CI (4.6 to 27.4). This observation also concurs with those of Villamor et al., who found HIV infection to be an independent risk factor for LBW (Villamor et al., 2005).

In the study, Ticconi and others, it was specifically reported that infants born to HIV-positive mothers were more likely to have LBW (< 2500 g) or VLBW (< 1500 g) (Ticconi et al., 2003). This partly confirmed the results of a previous study, which found LBW to be associated with HIV infection on univariate analysis but disappeared after controlling for gestational age (Castetbon et al., 1999). In our analysis, however, the risk persisted in the multiple logistic regression model, where a number of covariates including PTD were controlled for. After controlling for PTD, malaria infection at recruitment and at delivery, as well as HIV remained significant predictors of LBW. Taken together, these findings indicate that neonates born to women exposed to malaria and HIV infections during pregnancy are at particular risk of LBW. Neonates of mothers who had malaria at both time points were worse off. This may reflect the effect of chronic malaria infection on foetal growth.

Association between gestation length/PTD and maternal infection status

To examine the existence of an association between maternal infection status and gestation length in this study, the ANOVA technique was first used. This also affirmed the hypothesis that significant differences existed in mean gestation length across the maternal infections categories; $F(3, 123) = 7.40$; $p < 0.001$. On average, maternal infections (single or dual) were associated with shorter gestation length. However, this was more pronounced and statistically significant in the group of women who were dually infected both at recruitment and at delivery.

Seven of numerous studies reviewed by Ter Kuile (2004), examined the effect of dual infection with malaria and HIV on birth outcomes including PTD (Ayisi et al., 2003; Leroy et al., 1998; Steketee et al., 1996; Ticconi et al., 2003; Verhoeff et al., 1999; Weng et al., 1998). Although differences in study design limited direct comparisons between these studies, the review reported an increased risk of preterm birth with both HIV and malaria, with the greatest risk in women with dual infection (Ter Kuile et al., 2004). All these seven studies reported on gestational age; suggesting that the effect on birth weight reflects a combined effect of shortened gestational age and IUGR (Ayisi et al., 2003; Leroy et al., 1998; Steketee et al., 1996; Ticconi et al., 2003; Verhoeff et al., 1999; Weng et al., 1998). In a separate but related study in Malawi, Abrams et al. after examining risk factors and mechanisms of PTD in malaria-exposed pregnant women reported that HIV was associated with PTD, while malaria was not (Abrams et al., 2004), contrary to the findings by Noble et al. where both maternal HIV infection and malaria history, among Zimbabwean women were associated with preterm delivery (Noble et al., 2005).

In our analysis, the effect of maternal infection persisted but nevertheless attenuated when the comparisons were made between maternal single infection versus dual infection at two time points or dual infection only at one time point (only at recruitment) versus dual infection at two time points. Put together, the data demonstrates that maternal dual infection with HIV and malaria during the course of pregnancy has a significantly negative effect on gestation length.

In the second approach, associations between PTD and some clinical and non-clinical maternal factors were assessed using simple and multiple logistic regression techniques. PTD is defined as labor resulting in birth before 37 completed weeks/259 days of gestational age. This definition, promulgated by the World Health Organization (WHO) and the International Federation of Gynecology and Obstetrics (FIGO), originated from a statistical analysis of the distribution of gestational age at birth, based on the first day of the last menstrual period (WHO, 1976). This definition was used for this analysis.

Among HIV-positive women, maternal CD4 count greater than 350 cells/mm3 was significantly protective against PTD. On the contrary, maternal malaria infection had an increased risk of delivering preterm. Dual infection with malaria and HIV both at recruitment and at delivery was associated with almost 4-fold risk of delivering before the 37th week of gestation. Although the precise effect of malaria infection on PTD is uncertain, malaria-infected placentas according some authorities have been shown to frequently carry antibodies, cytokines and macrophages which are indicative of active immune-response and this response may stimulate early labor (Guyatt and Snow, 2004; Ismail et al., 2000).

The current PTD rate of 24.4% was comparable to those reported by earlier studies whose study participants were on antiretroviral therapy (ART). At the initiation of our study, ART uptake was low (4.9%) and only 10 of the 62 HIV-positive women who delivered prematurely were on ART. Even though the benefits of ART are known, concerns have been raised that maternal receipt of these drugs during pregnancy may be associated with adverse outcomes including PTD (Marti et al., 2007; Saraceni et al., 2005; Suy et al., 2006; Thorne and Newell, 2003; Thorne and Newell, 2007; Tuomala et al., 2002). In the studies by Marti et al. and Thorne and Newell among HIV+ women receiving antiretroviral therapy, premature delivery rates were 29 and 24.9% respectively (Marti et al., 2007; Thorne and Newell, 2007). Further studies controlling the effect of ART on PTD are needed to shed more light on this.

Conclusions

This study demonstrates that newborns of women infected with HIV and/or malaria are at heightened risk of LBW and PTD. The risks were more pronounced in women dually infected with HIV and malaria at first antenatal visit and also at delivery.

Recommendations

In the settings where both malaria and HIV are co-endemic, routine screening of pregnant women for both malaria and HIV at antenatal visits, successful treatment of women who are found to have malaria and avoidance of re-infection during the course of the pregnancy may reduce the incidence of the various adverse outcomes investigated in this study. Innovative integration of malaria screening and management into PMTCT programs in these areas, may bring some synergy into these interventions, which are currently vertical in nature. While malaria control programs could specifically consider malaria interventions for pregnant women with HIV, PMTCT programs could also consider the possibility of taking advantage of contacts with healthcare systems to deliver malaria prevention interventions. Inclusion of malaria tests in the routine assessment of HIV-related immunosuppression would particularly be useful. Also intensification of the education of women at antenatal clinics on the risks of malaria in pregnancy, the various means of preventing malaria, such as the use of insecticide treated nets and the use of IPT could equip women with self-protection tools.

ACKNOWLEDGMENTS

We thankfully acknowledge all the women who consented and enrolled into the study, the dedication of our research nurses, research assistants and the laboratory technicians at the three study sites (Tema General Hospital, Atua Government Hospital and St Martins de Porres Hospital).

The authors also thank the Manya Krobo Queen Mothers Association, the Family Health International and the Manya Krobo District Health Directorate for their guidance during the community entry stages of the research. The entire cost of the field work was catered for by the TALIF Project # CHSR/001/2005 of the School of Public Health, College of Health Sciences, University of Ghana, Legon.

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