Am. J. Trop. Med. Hyg., 85(2), 2011, pp. 202–206
doi:10.4269/ajtmh.2011.10-0589

Placental Malaria and Mother-to-Child Transmission of Human Immunodeficiency Virus-1 in Rural Rwanda

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Abstract. We conducted a nested case-control study of placental malaria (PM) and mother-to-child transmission (MTCT) of human immunodeficiency virus-1 (HIV-1) within a prospective cohort of 627 mother-infant pairs followed from October 1989 until April 1994 in rural Rwanda. Sixty stored placentas were examined for PM and other placental pathology, comparing 20 HIV-infected mother-infant (perinatal transmitter) pairs, 20 HIV-uninfected pairs, and 20 HIV-infected mothers who did not transmit to their infant perinatally. Of 60 placentas examined, 45% showed evidence of PM. Placental malaria was associated with increased risk of MTCT of HIV-1 (adjusted odds ratio [aOR] = 6.3; 95% confidence interval [CI] = 1.4–29.1), especially among primigravidae (aOR = 12.0; 95% CI = 1.0–150; $P < 0.05$). Before antiretroviral therapy or prophylaxis, PM was associated with early infant HIV infection among rural Rwandan women living in a hyper-endemic malaria region. Primigravidae, among whom malaria tends to be most severe, may be at higher risk.

INTRODUCTION

Malaria and human immunodeficiency virus (HIV) are among the most prevalent infectious diseases worldwide, with ~2.7 million new HIV infections, two million acquired immunodeficiency syndrome (AIDS)-related deaths, and nearly one million malaria deaths occurring globally in 2008.[1,2] In the context of co-infection with both HIV and malaria, mounting evidence suggests that interaction between the two occurs synergistically, with each disease contributing to substantial excess infections and dissemination of the other.[3] Co-infection is of special concern for pregnant women, of whom there are an estimated 25 million at risk for malaria infection each year in hyper-endemic regions of sub-Saharan Africa.[4,5] The HIV-infected women may have reduced immunity to malaria infection and increased risk of placental malaria and clinical disease.[6,7] Conversely, co-infection with malaria is associated with increased maternal HIV viral load[8] and adverse health outcomes.[3,6,9]

Among HIV-1-infected pregnant women, severe and recurrent malaria infection with placental malaria (PM) may increase the risk of mother-to-child transmission (MTCT) of HIV, yet studies assessing the relationship between PM infection and MTCT of HIV-1 have reported varying results.[10–15] A study conducted in rural Rakai District in Southern Uganda reported a significant association between PM and MTCT after controlling for HIV viral load.[14] In contrast, a study conducted in Mombasa, Kenya did not observe an association between PM and MTCT,[13] and data from the urban HIVNET 024 trial did not show this association overall, although an association was observed in a subset of women with low viral load at baseline.[10] A study in rural Nyanza Province, Kenya reported an increased risk of MTCT of HIV-1 in women with high density PM compared with low density PM, but no overall increased risk of MTCT with PM.[15] Discrepancies in findings among these studies may be attributed to variations in study population, prevalence of HIV or malaria, disease duration, seasonal timing, or host response in different settings.[14] We examined the relationship between PM and MTCT of HIV-1 among antiretroviral treatment-naive women and infants enrolled in a prospective cohort study in Butare, Rwanda from 1989 to 1994.

METHODS

Study population and design. This case-control study was nested within a prospective cohort study conducted between October 1989 and April 1994 of 318 HIV-1 seropositive and 309 HIV-1 seronegative women enrolled during pregnancy at one of five collaborating health centers in Butare Province, a rural but densely-populated region of southern Rwanda with an altitude between 1450 and 1850 meters above sea level. All women were offered HIV-1 antibody testing during their first prenatal visit; HIV prevalence was 9.3% overall.[16] Cohort enrollment procedures have been described elsewhere.[17,18] Women were enrolled in the cohort at the second prenatal visit (median, 32 completed weeks of gestation) and encouraged to deliver at one of two maternity wards serving the region. Lymphocyte measurements were taken at the time of cohort enrollment. In case of home delivery, the placenta was collected by a study team member within 6 hours of birth and neonatal examinations were performed by a physician within 48 hours of birth. Mother-infant follow-up was carried out at 6-week intervals during the first year of life and at 4-month intervals thereafter until the child reached 3 years of age or when the study ended in April 1994.[18] The follow-up rate among mother-infant pairs in the study was 96% in the first year postpartum and the MTCT rate by 12 months of age was estimated at 25%.[19] Psychosocial support and individual counseling regarding prevention of HIV-1 transmission were available to women and their partners on a weekly basis at study

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clinics; however, antiretroviral treatment was not available at the time of the study. Approval for this study was granted by the Rwandan Ministry of Health and the Johns Hopkins University School of Hygiene and Public Health Institutional Review Board (IRB). An exempt approval was granted by the Stanford University human subjects' protection panel for analysis of the data.

This study design is a case-control study nested within the original cohort, comparing 20 HIV-infected mother-infant pairs (perinatal transmitters), 20 HIV-infected mothers who did not transmit to their infant (non-transmitters), and 20 HIV-uninfected mother-infant pairs. Non-transmitters were selected at random from all such women in the cohort with placenta specimens available. Infant HIV infection was determined by DNA polymerase chain reaction (PCR) performed on peripheral blood mononuclear cells collected at 4–6 weeks of age.[20] In surviving infants, antibody tests were performed at 9, 12, 16, and 20 months of age to confirm HIV status. Twenty mother-infant pairs fulfilled the study inclusion criteria as "transmitters": 1) an adequate placenta specimen was available for pathological examination, and 2) the infant was found positive by HIV DNA PCR at 4–6 weeks of age. Information on maternal CD4 count, CD4%, ultrasensitive p24 antigen level, delivery outcome, and sexual history were also available. Twenty non-transmitter pairs and 20 HIV-negative mother-infant pairs were randomly selected among all other mother-infant pairs in the study and compared with regard to PM and other clinical and demographic variables.

Histological examination. At delivery, the placenta, fetal membranes (chorion and amnion), and an umbilical cord sample were collected for microscopic examination at the National University of Rwanda project laboratory.[17] Of 627 mother-infant pairs enrolled in the cohort study, 543 (87%) placentas were collected and microscopically examined at the project laboratory. Each placenta was also weighed and any physical abnormality (such as infarction) was noted. Detailed PM evaluations were conducted at the Johns Hopkins University School of Medicine within 3 years of placenta collection. A large section of fetal placenta comprising an area of at least 2 cm^2 was examined histologically. In addition, a section of chorioamniotic roll consisting of at least 2 cm^2 of chorioamniotic tissue and a cross section of cord vessels were examined. Formalin-fixed, paraffin-embedded placental tissues were sectioned for hematoxylin and eosin (H&E) staining. Giemsa staining and immunohistochemistry were performed on the placental disk with a primary monoclonal antibody (3A4) to *Plasmodium falciparum* and a secondary peroxidase antibody.[21,22]

Focal villitis and infiltration of the intervillous space with macrophages containing birefringent hemazoin pigment and/or the presence of malarial organisms were considered evidence of active malarial infection[23] and were categorized as malaria positive. Malaria-negative placentas did not have any of the typical changes seen in active malarial infection, including focal villitis, cellular infiltration of the intervillous space, chorioamnionitis, or vasculitis. Occasional placentas had only minimal hemazoin pigment without the presence of parasites. These were deemed indicative of past infection and were categorized as malaria negative. Immunohistochemical analysis was performed as a confirmatory diagnostic method (with no discordant results) using the avidin-biotin complex technique with peroxidase as the substrate for color reaction with 3,3′-disminobenzidine tetrahydrochloride. Matched isotype controls at the same concentration were used. The pathologist reading the slides was blinded to all clinical data and laboratory test results.

Statistical analysis. Chi-square (χ^2) tests or Fisher's exact tests were used to test for overall association between HIV-1 transmission and categorical covariates. Double-sided P values ≤ 0.05 were considered statistically significant. The Student's t test was used to test for differences in the mean of continuous variables according to HIV-1 transmission status. The association between PM and MTCT of HIV-1 was evaluated using multivariate logistic regression modeling to estimate crude and adjusted odds ratios (OR and aOR) and 95% confidence intervals (CI). The final multivariate model included potential confounders such as CD4% and ultrasensitive p24 antigen; other factors that were significantly associated with PM in univariate analyses were examined but excluded. CD4% was used as a marker of immunosuppression because absolute maternal CD4 counts have been shown to vary substantially during the antenatal through postpartum period,[24] whereas CD4% has been shown to remain stable from late pregnancy to 6 weeks postpartum.[25] All statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Characteristics of the study population. Characteristics of the study population according to HIV infection and mother-to-child transmission status (transmitter or non-transmitter) are presented in Table 1. Compared with HIV-positive women, HIV-negative women were generally older ($P < 0.005$), reported fewer years of education, and lower household income. Babies born to HIV-negative women were more likely ($P < 0.05$) to weigh 2,500 grams or more at birth than babies of HIV-positive women. HIV-negative women were significantly less likely than HIV-positive women to report any sex partner other than the father of the baby during pregnancy ($P < 0.005$) or any history of a sexually transmitted disease in the past three years ($P < 0.05$).

Compared with non-transmitting women (Table 1), transmitters were somewhat more likely than non-transmitters to be primigravid, to report a sex partner other than the father of the baby during pregnancy, and to report laboratory-confirmed malaria in the past 3 years. Transmitters had significantly lower average CD4+ cell counts than non-transmitters (357 versus 680, respectively). Maternal age, monthly household income, education, number of sexually transmitted diseases in the past 3 years (self-reported), and number of AIDS symptoms were not significantly different between transmitter and non-transmitter mothers.

Placental malaria and MTCT of HIV-1. Of the 60 placentas selected from the mother-infant pairs, 45% showed evidence of PM upon examination, with prevalence of PM differing significantly between transmitting (75%) and non-transmitting (35%) HIV-positive women (Table 2). Placental malaria was significantly associated with increased risk of MTCT of HIV-1 univariately (OR = 5.6; 95% CI = 1.4–21.9). Placental membrane roll inflammation (chorioamnionitis) was also associated with an increased risk of MTCT, although the association was not statistically significant (OR = 4.8; 95% CI = 0.9–27.2). Low maternal CD4/CD8 ratio (< 0.5), low CD4% (< 30%), and low placental weight (< 450 grams) also appeared to be associated with increased risk of MTCT (OR = 2.8; 95%

TABLE 1

Characteristics of mothers at enrollment by HIV-1 mother-to-child transmission status, Butare, Rwanda, 1989–1994*

Characteristic	Transmitters *N* (%)	Non-transmitters *N* (%)	HIV-negative *N* (%)
Total†	20	20	20
Maternal age (years)‡			
< 20	10 (50%)	9 (45%)	0 (0%)
20–24	8 (40%)	9 (45%)	12 (60%)
25+	2 (10%)	2 (10%)	8 (40%)
Maternal attained education			
None	3 (15%)	1 (5%)	5 (25%)
1–4 years	5 (25%)	6 (30%)	7 (35%)
5 + years	12 (60%)	13 (65%)	8 (40%)
Household income (Rwandan francs)			
< 1,000	6 (30%)	9 (45%)	7 (35%)
1,000–4,999	9 (45%)	6 (30%)	10 (50%)
5,000+	4 (20%)	4 (20%)	3 (15%)
Number of previous pregnancies			
None	10 (50%)	7 (35%)	6 (30%)
1–2	7 (35%)	11 (55%)	8 (40%)
3+	3 (15%)	2 (10%)	6 (30%)
Baby birth weight§			
< 2,500 grams	9 (45%)	6 (30%)	2 (10%)
2,500 + grams	11 (55%)	14 (70%)	18 (90%)
Any sex partner other than father of baby‡			
None	7 (35%)	11 (55%)	19 (95%)
At least one	12 (60%)	9 (45%)	1 (5%)
History of malaria in the past 3 years			
No malaria	5 (25%)	7 (35%)	7 (35%)
Malaria but not laboratory confirmed	2 (10%)	4 (20%)	6 (30%)
Laboratory confirmed malaria	13 (65%)	8 (40%)	7 (35%)
History of sexually transmitted diseases in the past 3 years§			
None	9 (45%)	11 (55%)	17 (85%)
1	4 (20%)	2 (10%)	2 (10%)
2+	7 (35%)	7 (35%)	1 (5%)
Among HIV-infected women only			
Major or minor symptoms of AIDS			
None	11 (55%)	12 (60%)	
1	8 (40%)	6 (30%)	
2+	1 (5%)	2 (10%)	
CD4%			
< 30%	10 (50%)	4 (20%)	
≥ 30%	10 (50%)	16 (80%)	
CD4/CD8 ratio			
< 0.50	6 (30%)	4 (20%)	
≥ 0.50	10 (50%)	12 (60%)	

* HIV = human immunodeficiency virus; AIDS = acquired immunodeficiency syndrome.
† Some cells do not total 20 because of missing values.
‡ χ^2 test statistic for overall association $P < 0.005$.
§ χ^2 test statistic for overall association $P < 0.05$.

TABLE 2

Number of women, odds ratio (OR), and 95% confidence intervals (CI) for mother-to-child transmission of human immunodeficiency virus-1 (HIV-1) by maternal and placenta characteristics

	Total	Transmitter	Non-transmitter	OR (95% CI)
Placental malaria				
Yes	22	15	7	5.6 (1.4–21.9)
No	18	5	13	1.0 (ref)
Placental membrane inflammation				
Yes	9	7	2	4.8 (0.9–27.2)
No	31	13	18	1.0 (ref)
Placental weight				
< 450 grams	9	6	3	2.4 (0.5–11.5)
≥ 450 grams	31	14	17	1.0 (ref)
Birth weight				
< 2,500 grams	14	8	6	1.7 (0.5–6.4)
≥ 2,500 grams	25	11	14	1.0 (ref)
Number of sex partners during pregnancy				
1	18	7	11	1.0 (ref)
≥ 2	20	11	9	1.9 (0.5–7.0)
Syphilis infection (by VDRL)				
Positive	7	3	4	0.75 (0.1–3.9)
Negative	32	16	16	1.0 (ref)
Maternal CD4/CD8 ratio				
< 0.5	12	8	4	2.8 (0.7–11.9)
≥ 0.5	24	10	14	1.0 (ref)
CD4 percent				
< 30	14	10	4	4.0 (1.0–16.3)
≥ 30	26	10	16	1.0 (ref)
Ultrasensitive p24 antigen				
< 1,000	12	6	6	1.0 (ref)
≥ 1,000	27	14	13	1.1 (0.3–4.2)
Primigravidae				
Yes	16	9	7	1.7 (0.5–6.1)
No	23	10	13	1.0 (ref)
Duration of membrane rupture				
< 4 hours	28	13	15	1.0 (ref)
≥ 4 hours	10	6	4	1.7 (0.4–7.5)

CI = 0.7–11.9, OR = 4.0; 95% CI = 1.0–16.3, OR = 2.4; 95% CI = 0.5–11.5, respectively). Primigravidae, birth weight, ultrasensitive p24 antigen, duration of membrane rupture, syphilis infection, and number of other sex partners during pregnancy were not significantly associated with MTCT in this analysis. Placental malaria was strongly associated with birth weight lower than 2,500 grams ($P = 0.02$, χ^2) but not with other variables.

Table 3 shows the results of multivariate regression modeling of MTCT by PM adjusting simultaneously for maternal CD4% and ultrasensitive p24 antigen. The PM was independently associated with significantly increased risk of MTCT of HIV-1 after adjusting for maternal CD4% and ultrasensitive p24 antigen (aOR = 6.3; 95% CI = 1.4–29.1), with the strongest association seen among primigravidae (aOR = 12.0; 95% CI = 1.0–150; $P < 0.05$). Maternal CD4% below 30 was also marginally associated with increased risk of MTCT in this model (aOR = 4.8; 95% CI = 1.0–24.0). Ultrasensitive p24 antigen was not associated with MTCT of HIV-1 in univariate or multivariate analysis. Inclusion of other measured variables as potential confounders did not change the adjusted odds ratios appreciably.

TABLE 3

Adjusted odds ratio (aOR) and 95% confidence intervals (CI) for mother-to-child transmission of human immunodeficiency virus-1 (HIV-1) by simultaneous examination of placental malaria, maternal CD4%, and maternal p24 antigen

	aOR (95% CI)
Placental malaria*	
Yes	6.3 (1.4–29.1)
No	1.0 (ref)
CD4 percent	
< 30	4.8 (1.0–24.0)
≥ 30	1.0 (ref)
Ultrasensitive p24 antigen	
< 1,000	1.0 (ref)
≥ 1,000	1.1 (0.2–5.2)

DISCUSSION

In this nested case-control study of rural Rwandan mothers followed from 1989 to 1994, we found that PM was independently associated with MTCT of HIV-1, particularly among primigravidae, before the regional availability of antiretroviral therapy or prophylaxis. Our results are consistent with a recently published study conducted in rural Rakai, Uganda,[14] but differ from findings reported in Blantyre, Malawi,[11] Nairobi,[12] Mombasa,[13] and Nyanza Province, Kenya.[15] These differences may be explained in part by the varying dynamics of malaria infection and epidemiology in different settings and altitudes. The PM may have a greater effect on MTCT of HIV-1 in rural as opposed to urban areas, possibly caused by higher malaria disease burden among pregnant women or reduced access to malaria treatment in rural areas. Differences in PM diagnostic techniques may also account for some of the observed differences among studies. In this study, PM was diagnosed by H&E staining, Giemsa staining, and immunohistochemistry, which serve as markers for chronic infection and have been shown to be more sensitive than peripheral or placental blood films.[26] The histopathological findings of our study are characteristic of chronic or acute active malarial infection.[23] Our results suggest that PM may partially explain the observed seasonal variation in rates of MTCT in some settings.[27] Primigravidae, among whom malaria tends to be most severe, may be at higher risk. Our finding that primigravidae with observed PM were at higher risk of MTCT of HIV than multigravidae may reflect a protective effect of pregnancy-specific malaria immunity, which has been shown to be absent in the first pregnancy.[6]

The PM may affect MTCT through several possible mechanisms, including up-regulation of CCR5 chemokine co-receptor expression on placental macrophages as a consequence of malaria infection.[28] In addition, compromised integrity of the placenta caused by inflammation may increase tissue susceptibility to viral infection and promote increased placental virus load.[11,29] We did not observe an association between HIV-1 MTCT and ultrasensitive p24 antigen level, a proxy for viral load, suggesting that local rather than systemic virus replication may be responsible for vertical transmission.

This study has several limitations, including its small sample size. In agreement with requirements from the Rwandan Ministry of Health, most specimens collected from cohort women and infants were kept at the National University of Rwanda project laboratory and these were all destroyed as a result of the civil war in April 1994, severely limiting the number of additional specimens that could be tested. Another limitation is the lack of data on maternal viral load as a measure of HIV disease severity (only ultrasensitive p24 antigen was available). The strengths of the study include the nested case-control design, random selection of control placentas, high follow-up rate of the cohort, high rate of placental retrieval after delivery, and precise exposure and outcome assessment, all of which serve to reduce selection and information bias and hence improve the precision of our findings. In addition, this analysis focused on data and placenta specimens from treatment-naive pregnant women and infants in rural East Africa, yielding results that could not be duplicated in the present-day highly active antiretroviral therapy (HAART) era.

Although antiretroviral prophylaxis and treatment have been shown to reduce rates of MTCT more than 10-fold,[30] many HIV-infected pregnant women, especially in malaria-endemic rural areas of sub-Saharan Africa, do not yet have access to antiretroviral treatment or prophylaxis.[31] Prevention and treatment of malaria among pregnant women through established interventions such as insecticide-treated bed nets and artemisinin combination anti-malarial treatment, as well as control strategies under development to interrupt parasite transmission dynamics, may reduce rates of MTCT of HIV-1, particularly in hyper-endemic areas with high rates of malaria transmission among rural households.[32,33] Although no studies have reported on the association between PM and MTCT of HIV-1 in the context of maternal HAART during pregnancy, we speculate that the impact of placental malaria on the risk of MTCT of HIV-1 in these mothers may be limited, particularly among those with undetectable virus. However, the pathological changes caused by PM including focal villitis, cellular infiltration of the intervillous space, chorioamnionitis, and vasculitis could provide a portal of entry for infected maternal cells into the fetal circulation despite suppression of HIV-1 RNA. Additional studies in malaria-endemic settings among HIV-infected mothers may elucidate the relationship among HAART, PM, and MTCT of HIV-1 and the biological and epidemiological factors that underlie this relationship. In the current context of maternal and infant antiretroviral prophylaxis and treatment, studies of drug interactions and potential toxicities when antiretroviral therapy and antimalarials are co-administered in pregnancy are also needed.

Received October 17, 2010. Accepted for publication March 22, 2011.

Acknowledgments: We gratefully acknowledge the Rwandan women whose participation made this study possible; the faculty and staff of the Centre Universitaire de Santé Publique (CUSP) in Butare, Rwanda; the staff of the health centers of CUSP, Sovu, Save, Rango, and Matyazo; and all the dedicated staff members of the National University of Rwanda–Johns Hopkins University AIDS Research Project in Butare, Rwanda from 1989 through 1994. We thank Homayoon Farzadegan for DNA PCR analysis. Ann Duerr contributed significantly to the initial phase of the cohort study and the malaria sub-study. Rich Respess's laboratory conducted the ultra-sensitive p24 antigen determinations. Paula Nawrocki, Andrea Imredy, and Ellen Taylor provided invaluable assistance to the study. Jules Kajugiro was responsible for collecting placentas soon after birth. We are especially grateful to the late Edmond Mugabo, the head laboratory technician responsible for processing of placenta specimens, and Jean-Baptiste Kurawige who was the primary physician responsible for neonatal and infant clinical examinations in the study. Edmond Mugabo and Jean-Baptiste Kurawige were among the many study staff and health care personnel who perished during the Rwanda genocide of April–June 1994. We thank Mary Glenn Fowler, Chin-Yih Ou, Lawrence Slutsker, and Rick Steketee for providing helpful comments on an earlier draft. We also thank Dmitri Petrov, members of the Petrov Lab at the Stanford University Department of Biology, and Allan Campbell for helpful discussion and comments on the manuscript.

Financial support: This study was supported by grants from the National Institute of Child Health and Human Development (HD22496 and HD25785) and from the World AIDS Foundation (114-96024). PLB is supported by the UCLA/Caltech Medical Scientist Training Program (MSTP) and the Paul and Daisy Soros Fellowships for New Americans.

Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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