Clinical Malaria Diagnosis in Pregnancy in Relation to Early Perinatal Mother-to-Child-Transmission of HIV: A Prospective Cohort Study

Published in final edited form as:
HIV Med. 2014 May ; 15(5): 276–285. doi:10.1111/hiv.12111.

Clinical Malaria Diagnosis in Pregnancy in Relation to Early Perinatal Mother-to-Child-Transmission of HIV: A Prospective Cohort Study

AE Ezeamama, PhD[1], **C Duggan, MD, MPH**[2,3], **KP Manji, MBBS,MMED, MPH**[4], **D Spiegelman, ScD**[5,6], **E Hertzmark, MS**[5], **RJ Bosch, PhD**[6,7], **R Kupka, ScD**[2,8], **JO Okuma, MS**[2], **R Kisenge**[9], **S Aboud, MD, MMED**[9,10], and **WW Fawzi, MD, DrPH**[2,5,11]

[1]Department of Epidemiology & Biostatistics, The University of Georgia, B.S. Miller Hall, Room 125, 101 Buck Rd, Athens, GA 30602, USA

[2]Department of Nutrition, Harvard School of Public Health (HSPH), Boston, MA

[3]Division of Gastroenterology and Nutrition, Children's Hospital, Boston, MA

[4]Department of Pediatrics and Child Health, School of Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[5]Department of Epidemiology, HSPH, Boston, MA

[6]Department of Biostatistics, HSPH, Boston, MA

[7]Center for Biostatistics in AIDS Research, HSPH, Boston, MA

[8]United Nations Children's Fund, Regional Office for West and Central Africa, Dakar, Senegal

[9]Department of Pediatrics and Child Health, School of Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[10]Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[11]Department of Global Health and Population, HSPH, Boston, MA

Abstract

Objectives—We prospectively investigated fever symptoms and maternal diagnosis of malaria in pregnancy (MIP) in relation to child HIV infection among 2,368 pregnant HIV-positive women and their infants, followed-up from pregnancy until birth and 6 weeks post-delivery in Tanzania.

Methods—Doctors clinically diagnosed and treated MIP and fever symptoms during prenatal healthcare. Child HIV status was determined via DNA-PCR. Multivariable logistic regression

Corresponding Author: Amara E. Ezeamama, PhD. Department of Epidemiology & Biostatistics, The University of Georgia, B.S. Miller Hall, Room 125, 101 Buck Rd, Athens, GA 30602, USA; aezeamam@uga.edu.

Conflict of Interest Statement

The authors do not have commercial (e.g., pharmaceutical stock ownership, consultancy) or other associations that pose a conflict of interest in the collection, analysis, presentation and interpretation of this data.

models estimated relative risks (RR) and 95% confidence intervals (CI) for HIV mother-to-child-transmission (MTCT) by 6th week of life.

Results—Mean gestational age at enrollment was 22.2 weeks. During follow-up, 16.6% had ≥1 MIP diagnosis, 15.9% reported fever symptoms and 8.7% had both fever and MIP diagnosis. Eleven percent of HIV-exposed infants were HIV-positive by 6 weeks. The RR of HIV MTCT was statistically similar for infants whose mothers were ever vs. never clinical MIP diagnosed (RR=1.24, 95%CI:0.94–1.64), were diagnosed with 1 vs. 0 clinical MIP episode (RR=1.07;95%CI:0.77–1.48) and had ever vs. never reported fever symptoms (RR=1.04, 95%CI: 0.78,1.38) in pregnancy. However, HIV MTCT risk increased by 29% (95%CI:4–58%) per MIP episode. Infants of women with ≥2 vs. 0 MIP diagnoses were 2.1 times more likely to be HIV infected by 6weeks old (95%CI:1.31–3.45).

Conclusions—Clinical MIP diagnosis, but not fevers, in HIV-positive pregnant women was associated with elevated risk of early HIV MTCT suggesting that malaria prevention and treatment in pregnant HIV-positive women may enhance the effectiveness of HIV prevention in MTCT programs in this setting. Future studies using laboratory confirmed malaria is needed to confirm this association.

Keywords

Co-infection; Malaria; AIDS; HIV mother-to-child-transmission; HIV-exposed Infant

Introduction

The vast majority of human immunodeficiency virus (HIV)-infected children acquire the infection through mother-to-child transmission (MTCT) *in utero*, around the time of labor and delivery, or postnatally through breastfeeding.(1, 2) Approximately 70% of all perinatally-acquired HIV transmission occurs early (i.e., *in utero*, intra partum or within 6 weeks) of an exposed infant's life.(2) Prior investigations have identified several factors associated with early HIV transmission, including maternal (low CD4 cell count, high viral load, and lack of antiretroviral therapy during pregnancy, mastitis, and breast abscess), delivery (non-cesarean section birth and absence of prophylactic nevirapine use), and child (low birth weight and prematurity) factors.(2–4)

HIV and malaria infections represent the most important health problems in Sub-Saharan Africa (SSA), where these infections overlap and co-infection is common.(5) An estimated 24.3% to 37% of HIV-positive pregnant women in the malaria-endemic regions of SSA are co-infected with malaria.(6–8) Malaria infection up-regulates the expression of CCR5 receptors in placental macrophages(9), increases peripheral and placental HIV-1 viral load(8), and thereby raises the possibility of HIV MTCT for infants of pregnant HIV-positive women.

Nevertheless, there is little research on the potential contribution of coincident infections, such as malaria in pregnancy (MIP) to early HIV MTCT among HIV-exposed infants of HIV-infected women in Sub-Saharan Africa (SSA); when available, results have been inconclusive.(6–8, 10–16) In a short-term prospective study of infants born to 277 HIV-

positive Kenyan women and 372 HIV-negative hospital-based controls, Inion *et al.* found no association between placental malaria and *in-utero* or peripartal transmission of HIV-1 by 6 weeks.(13) Maternal placental malaria was associated with lower risk of child HIV-positive status at 1 month of life among 207 pregnant HIV-positive women, each provided with a long-lasting insecticide-treated bed net with randomization to either intermittent preventive malaria therapy with sulfadoxyl-pyrimethamine or a placebo.(17) Another study of 512 HIV-positive mothers from Kenya found complex associations between maternal placental malaria and perinatal HIV MTCT.(6) On the one hand, low-density placental malaria was an independent protective risk factor for HIV MTCT. This association varied by maternal viral load such that, among mothers with low HIV viral load, low-density placental malaria protected against HIV MTCT, whereas, among mothers with high HIV viral loads, high density placental parasitemia was associated with elevated risk of HIV MTCT.(6) Further, a large, multi-site, randomized, placebo-controlled trial of antibiotics for reduction of chorioamnionitis that included pregnant HIV-positive women from three SSA countries, Malawi, Zambia, and Tanzania, found no association between placental malaria and MTCT at birth.(14) However, among women with low baseline viral load, placental malaria was positively associated with HIV MTCT at birth.(15) On the other hand, a study of HIV-positive mothers from the Rakai District in Uganda found a twofold higher risk of HIV MTCT for HIV-positive mothers with placental malaria relative to those without placental malaria.(7, 10) Most recently, a small case control study of placentas of 40 HIV-infected mother-child pairs, which included 20 whose infants were perinatally HIV infected and 20 whose infants were HIV-exposed but uninfected, as well as 20 HIV-uninfected mother-child pairs, reported six fold higher odds of child perinatal HIV-infection for mothers with placental malaria.(11) Both studies that found significant positive associations between in-pregnancy malaria and HIV MTCT were implemented prior to availability of antiretroviral therapy for pregnant HIV-infected African women.(10, 11)

In light of these mixed findings and the persisting need to understand the potential role of maternal malarial morbidity during pregnancy in early HIV MTCT in the post-highly active antiretroviral therapy (HAART) era(18), we undertook this prospective cohort study to re-examine the hypothesis that maternal malarial morbidity during pregnancy is positively associated with MTCT. To this end, we used data collected between 2004 and 2008 among HIV-positive mothers whose children were enrolled in a trial of micronutrient supplementation in Dar es Salaam, Tanzania.

Methods

Study population

This is a prospective cohort study of 2,368 singleton, live-born infants whose mothers are HIV-positive, long-term residents of Dar es Salaam, Tanzania. The women were recruited during pregnancy and followed through delivery and child's 24th month of life as part of a micronutrient trial. The study was conducted between June 2004 and May 2008. Per standard of care, all mothers received daily prenatal folate and iron supplements and malaria prophylaxis using sulfadoxine-pyrimethamine (SP) at 20 and 30 weeks of pregnancy. At the study beginning, maternal antiretroviral (ARV) medication was limited to nevirapine

administered prophylactically during labor to prevent intra-partum HIV transmission. Beginning in July 2005, mothers were routinely evaluated for eligibility for HAART due to wider drug availability through the President's Emergency Plan for AIDS Relief (PEPFAR) and other programs. Per standard of care, all newborns received nevirapine within 72 hours of birth and were prescribed cotrimoxazole for prevention of bacterial pneumonia until age 6 months. Beyond 6 months of life, only breastfeeding and HIV-positive children remained on cotrimoxazole. Mothers who were unable to return for follow-up, who had multiple births, or whose children had serious congenital anomalies that would interfere with study procedure compliance were excluded from participation in the trial

Ethical clearance for the conduct of the parent study was provided by the institutional review boards of the Harvard School of Public Health and Muhimbili University of Health and Allied Sciences. The mothers of all children provided written informed consent for their own and the child's participation in the trial. The funder of this study had no role in the design, data collection, analysis, interpretation, or writing of this report. The corresponding author had full access to all the data in the study and the final responsibility for the decision to submit for publication.

Outcome Definition

Child HIV sero-status at 6 weeks is the primary outcome for this study and was accessed by DNA PCR test using Amplicor HIV-1 DNA prototype version 1.5 assay.

Primary Exposure/Determinant

Malarial in pregnancy.

We classified mothers as having clinical MIP when treatment with antimalarial drugs was provided after doctor diagnosis of malaria. Specifically, clinical malaria was diagnosed during prenatal health care via a combination of doctor clinical assessment of patient presenting symptoms as consistent with malaria and laboratory confirmation of parasitemia (where possible). Where lab tests were done, trained laboratory technicians prepared and read thin blood smears stained with Giemsa. Each slide was read in three different fields, and the parasite density per cubic millimeter was estimated from the number of parasites per 200 leukocytes. Of note, in this resource-limited setting, doctor diagnosis of malaria based on patient symptoms is common practice. Therefore, this malaria case definition, although sensitive for identification of malaria, is necessarily limited by lower specificity(19).

For analytic purposes, clinical MIP diagnosis was operationally defined in one of three ways: (a) dichotomously as ever vs. never MIP during index pregnancy, (b) as an ordinal determinant with 0, 1, 2 and ≥3 malarial episodes to estimate the relative risk of HIV MTCT per unit increment in maternal MIP diagnosis, and (c) as a determinant that compares the HIV MTCT risk for infants of mothers with 1 or ≥2 MIP diagnosis to infants whose mothers had 0 MIP diagnosis.

Confounders

Several classes of potential confounders of the malaria HIV-MTCT relationship were defined and adjusted for in multivariable analyses.

I. *Maternal health status:* Five indicators of maternal health during pregnancy at study enrollment were defined. These included (a) immune deficient status (CD4 cell count <350 vs. ≥350 cells/uL); (b) anemia (hemoglobin <8.5g/dL, 8.5 to <11 g/dL vs. ≥ 11 g/dL); (c) HIV World Health Organization (WHO) disease stage (1 vs. ≥1); (d) use vs. non-use of highly active antiretroviral therapy and (e) number of fever symptoms reported by patients and documented by a doctor or nurse during index pregnancy.

II. *Maternal socio-demographic factors:* Marital status, maternal age (in years), and socioeconomic factors were evaluated as potential confounders. Socioeconomic indicators included educational level (≥ 7 vs. <7 years) and presence vs. absence of own income. Marital status was dichotomized as married/cohabiting versus single/ divorced/separated/widowed status.

III. *Maternal obstetric history:* These included parity (number of prior pregnancies to date) and history of adverse pregnancy outcomes in past pregnancies. For the latter, ever vs. never history of early neonatal mortality and ever vs. never history of spontaneous abortion by 7th month of gestation were defined.

IV. *Delivery factors:* These included cesarean section vs. spontaneous vaginal, assisted breach or vacuum extraction delivery, intra-partum nevirapine administration (yes vs. no), vaginal tears (yes vs. no), episiotomy (yes vs. no), full vs. pre-term birth, and hospital vs. home/other non-hospital delivery.

V. *Child birth characteristics:* These included male vs. female, low (<2500g) vs. normal (≥2500g) birth weight, and full (≥37 weeks) vs. pre-term (<37 weeks) birth.

VI. *Post-delivery factors:* These included any vs. none infant early breastfeeding exposure and maternal breast health. In line with the literature(20), a binary variable was defined to distinguish mothers who reported having cracked nipples, bleeding nipples, an abscess, or other signs of inflammation or infection on physical examination from those without any of these symptoms, between delivery and their child's 6th week of life.

VII. *Seasonality and relevant temporal trends:* Because the intensity of exposure to malaria parasite varies by season, we adjusted for maternal enrollment in the rainy vs. dry seasons in multivariate models.

Statistical Analyses

We built a generalized estimating equations model that assumed a binomial distribution with a log to estimate relative risk and 95% confidence intervals associated with HIV MTCT at 6 weeks, as the outcome, and malaria during pregnancy, as the primary predictor. We adjusted for a wide range of potential confounders in multivariable analyses using a manual

backward selection approach. Based on univariate analyses, all covariates whose association with HIV MTCT had a *p*-value ≤0.20 were included in the final multivariable models.

Separate multivariable models were built to investigate the independent associations between early HIV MTCT and: i) clinical MIP diagnoses and ii) non-specific fever symptoms during pregnancy. A joint multivariable model with mutual adjustment for MIP and fever symptoms was implemented to evaluate the extent to which any association between MIP and early HIV MTCT was mediated by non-specific fevers in HIV-positive pregnant women and vice versa. Finally, we evaluated heterogeneity of the association of maternal MIP diagnosis and early HIV MTCT by levels of the following factors: baseline maternal educational status, baseline maternal immune-deficiency, baseline maternal WHO HIV disease stage, child sex, and prematurity. If the *p*-value associated with the interaction was <0.10, models were re-run (and results provided) within strata of the effect modifier.

Results

Enrolled in the parent study were 2,387 HIV-positive mothers and their HIV-exposed infants. The average gestational age at enrollment was 22 weeks. Of the 2,387, detailed maternal pregnancy health history and HIV status data at 6 weeks was available for the 2,367 mother-child pairs who formed the study base for this nested longitudinal study. 84% of mothers breastfed their infants post-delivery. By the infants 6^{th} week of life, 262 (11.1%) were HIV-positive (Table 1). The prevalence of fever symptoms and ever doctor diagnosed clinical malaria during pregnancy were 16.6% (*n* = 394) and 15.9% (*n* = 376) respectively. Clinical malaria was also diagnosed in 54% of women with fever symptoms in pregnancy. Among women with clinical MIP, 1.6%, 27.5%, and 47.2% of all diagnoses occurred in the 1st, 2nd, and 3rd trimesters, respectively. As many as 30.7% (*n* = 122) of women were clinically diagnosed with ≥≥2 episodes of MIP within the same or across multiple trimesters. The vast majority (96%) of clinical MIP diagnoses was classified as uncomplicated malaria but a laboratory test to confirm parasitemia was ordered in 38% of women only. Test results however were available for only 19 women of whom 18 were positive for malaria parasite (data not shown).

Socio-demographic, obstetric, and at-birth information of mother-child pairs is presented in Table 1. Mothers of children who were HIV positive at 6 weeks did not differ from mothers of children who were HIV negative at 6 weeks with respect to age, educational status, gestational age at study enrollment, gravidity, or marital status. A greater proportion of mothers whose children were HIV negative at 6 weeks, however, delivered in hospitals and received antiretroviral therapy during pregnancy. Similarly, the proportion of mothers who took nevirapine prophylaxis at onset of labor and that of infants who received nevirapine prophylaxis within 72 hours of birth was higher among infants who were HIV negative at six weeks. In addition, a lower percentage of mothers with HIV-negative children at 6 weeks had low birth weight infants, a prior history of early neonatal deaths, and CD4 cell counts < 350 cells/uL at enrollment. However, infants whose mothers had multiple episodes of malaria during pregnancy were over-represented among infants HIV infected at 6 weeks (Table 1).

Based on multivariable models (Table 2), the relative risk of HIV infection at 6 weeks was 24% elevated, but not statistically different, for infants whose mothers were ever vs. never (95% CI:0.94–1.64) diagnosed with clinical malaria and for mothers diagnosed with a single vs. no malaria episodes (RR=1.07, 95%CI:0.77–1.48) during pregnancy. The relative risk of HIV MTCT, however, rose significantly per malaria episode increment during pregnancy (RR=1.29; 95%CI:1.04–1.58), and infants of HIV-positive women with ≥2 malaria diagnoses in pregnancy were more than twice as likely as infants of HIV-positive not diagnosed with MIP women to be HIV infected at 6 weeks (RR=2.12, 95%CI:1.31–3.45). These malaria-associated estimated risks of HIV MTCT were robust to adjustment for variations in infant-related factors, maternal socio-demographic factors, delivery factors, maternal health status in pregnancy, and seasonality. Additional adjustment for potential mediators, including maternal immunologic status at enrollment, use vs. non-use of HAART in pregnancy, and maternal WHO HIV disease stage, slightly attenuated but did not materially change the malaria-associated high risk of HIV MTCT (Table 2). Similarly, the adjustment of multivariable models for number of non-specific fever symptoms reported in pregnancy slightly attenuated but did not ablate the positive association between early HIV MTCT and: i) number of clinical MIP diagnoses in pregnancy (RR = 1.25, 95% CI: 1.00, 1.57) and 2) multiple vs. no MIP diagnosis (RR = 1.92, 95% CI: 1.13, 3.25).

Non-malaria predictors of HIV MTCT at 6 weeks are presented in Table 3.. The ever vs. never maternal report of fever symptoms was strongly and significantly associated with clinical MIP diagnosis (RR =5.80, 95% CI: 4.91, 6.93 but not with early HIV MTCT in multivariate models mutually adjusted for clinical MIP diagnosis (RR = 1.04., 95% CI: 0.87, 1.29). However, maternal baseline immune-deficiency status, presence of nipple lesion/ inflammation on or before 6 weeks of birth, and infant low birth weight each predicted elevated risks of HIV MTCT at 6 weeks of life. Conversely, the receipt of HAART at any time during pregnancy, maternal possession of own income, and cohabiting or married status were significant protective risk factors for early HIV MTCT (Table 3). The relationship between clinical MIP and the factors identified above was generally invariant within levels of maternal CD4 at enrollment, maternal WHO HIV disease stage at enrollment, child sex, and prematurity. However, the relationship between MIP (per episode increment) and HIV-MTCT was heterogeneous within strata of maternal education (*p*-value for interaction term = 0.05). Specifically, each unit increment in maternal malaria episode was associated with 3.2-fold (95% CI: 1.49–6.71) relative risk of HIV MTCT among mothers with fewer than 7 years of education; whereas the risk of HIV MTCT by 6 weeks was non-significantly elevated among mothers with 7 or more years of education (RR = 1.2, 95% CI:0.94–1.53).

Discussion

In this cohort of HIV-positive mothers from Dar es Salaam, Tanzania, enrolled during pregnancy and followed through delivery, 11% of HIV-exposed infants contracted HIV either *in-utero*, intra-partum, or within the first 6 weeks of life. The 11% HIV MTCT rate observed in this study, is 45% to 62% lower than the typically reported 20% to 29% rate of new HIV infections among HIV-positive treatment naïve pregnant women (21–23) but comparable to the MTCT rates observed in other malaria endemic African settings where

nevirapine prophylaxis was used for PMTCT in the absence of HAART.(24, 25) We found no difference in risk of HIV MTCT for infants of women with 1 versus 0 malaria episodes in pregnancy and women with and without fever symptoms during pregnancy. However, there was a significant elevation of HIV MTCT risk per malaria episode increment. MIP diagnosis was an independent predictor of HIV MTCT at 6 weeks for infants of mothers with two or more malaria episodes, compared to infants whose mothers were not diagnosed with malaria during the pregnancy. These associations were strongest for infants born to women with fewer than 7 years of education. We found no evidence that the positive relationship between MIP diagnosis and HIV MTCT was mediated by fever symptoms.

We confirm the overall trend of lower HIV MTCT with greater access to HAART in pregnancy(26) and the positive associations between HIV MTCT risk and maternal immune deficiency,(20, 27) child low birth weight,(28, 29) and the presence of breast inflammation and nipple lesions in breast feeding HIV-positive women.(20, 30, 31) Similar to prior reports,(32, 33) we find protective associations between indicators of superior socioeconomic position and early HIV MTCT.(32)

Our finding of a statistically significant positive association between MIP and MTCT is corroborated by findings of a twofold higher risk of HIV MTCT among HIV-positive mothers in the Rakai District of Uganda (7, 10) and the recently reported six fold elevated odds of early HIV MTCT for infants of Rwandan HIV-positive women.(11) Our results are further supported by the finding of a higher risk of MTCT among HIV-positive pregnant women with high density placental malaria in Western Kenya(6) and that among pregnant HIV-positive women with low viral load, as recently reported by Msamanga et al.(14)

The above notwithstanding, findings from at least three other epidemiologic studies either do not support our findings, with respect to the relationship of maternal MIP to HIV MTCT. (13, 14) or have results in direct conflict with ours.(17) Specifically, our results are not supported by the overall finding of no association reported by Msamanga et al.(14) In addition, our finding of higher risk of HIV MTCT among pregnant HIV-positive women with malarial morbidity stands in contrast to the findings from a Mozambican cohort of 207 HIV-positive mothers enrolled in a trial of intermittent malaria preventive treatment with two doses of SP versus a placebo.(17)

We note that salient differences between our study and some of the above studies may be partly responsible for these disparate findings: (1) Naniche et al.'s(17) study included fewer subjects than did ours (N = 207 vs. 2,367), involved very few cases of HIV MTCT (N = 19 vs. 262), and had complete data on malaria and HIV MTCT on fewer mother-child pairs (N = 153 vs. 2367), all of which contribute to lower statistical power relative to the present study. These limitations make it difficult to determine whether their findings were true effects or the result of random variation with respect to placental malaria rates and their relationship to HIV MTCT. The findings also may be different when the same question is investigated in the context of a larger, adequately powered sample that includes more mother-child pairs and MTCT events. (2) Despite these potential explanations for the differences in results, rigorously designed large studies using gold-standard malaria diagnostic criteria will be necessary to confirm or refute our findings.

According to the most recent UNAIDS report, there has been an overall 24% reduction in MTCT between 2001 and 2009.(34) This encouraging trend has contributed to the belief that the virtual elimination of MTCT is achievable with the implementation of proven strategies for prevention of HIV transmission.(34) Yet, the 22% prevalence of clinical malarial morbidity in this cohort is comparable to estimates from other investigations among HIV-positive women from the region prior to widespread access to antiretroviral drugs in pregnancy.(6, 7, 10, 13) Of note, the rates of malarial morbidity has been assessed using varied approaches including clinical diagnoses, rapid diagnostic and malaria parasitemia tests. All methods suggest that malaria incidence is on the decline in certain regions of Tanzania particularly in the island of Zanzibar, since 2000.(35) Nevertheless, a significant malaria burden remains, particularly for non-coastal regions in the Republic of Tanzania. Hence, the results of this study provide impetus to provide more comprehensive malaria prevention measures.

A recent review article suggested that co-infections, including malaria, tuberculosis, and herpes simplex virus type 2, in high HIV-prevalence areas could be important, persistent drivers of HIV transmission(18) in the HAART era. The results reported herein, and the recently reported elevated risk postnatal HIV-infection for breastfeeding infants of HIV-positive women with malaria compared to those without malaria provide empirical evidence in support of this position(36). That the association between MIP diagnosis and HIV infection at birth was slightly attenuated but still statistically significant after controlling for non-specific fever symptoms suggests that observed relationship may reflect malaria-parasite induced immune suppression and HIV viral load amplification. Malarial morbidity is associated with lower immune fitness in HIV-positive persons.(8, 9, 37) The ubiquity of malarial morbidity among pregnant women in SSA may directly counteract the health benefits of increased HAART access for pregnant HIV-positive women and thus has real potential to limit the effectiveness of PMTCT programs in this population. Our results suggest that maternal malarial morbidity in pregnancy is likely to remain an important independent risk factor for HIV MTCT in malaria endemic regions of SSA, even when multiple confounders, including maternal immunologic status, non-specific fever symptoms, and the expected beneficial impact of maternal access to HAART during pregnancy, are accounted for.

Limitations and Strengths

The most important limitation of this study is the use of a clinically defined malaria diagnosis as a surrogate for malarial morbidity. This may have resulted in malaria over-diagnosis in this study. The extent of malaria over-diagnosis is unclear, as our cumulative 16.6% prevalence of clinical MIP is lower than the 20.4% clinical malaria prevalence reported among pregnant women from Moshi Tanzania,(38) the 23% prevalence found among pregnant HIV-positive women from Uganda,(39) and the 21% malaria prevalence among pregnant HIV-positive women from Rwanda, where more rigorous diagnostic criteria were employed.(40) We acknowledge this limitation of our data and stress the utility of rapid diagnostic assays that are increasingly available at reasonable cost to facilitate accurate diagnosis of malaria, whenever possible, even in the most resource-limited settings. This limitation notwithstanding, our study represents the largest prospective investigation of

whether newborns of HIV-infected pregnant women with diagnosed MIP are at elevated risk of HIV-infection within 6-weeks of life in an area of high malaria endemicity, with control for an extensive array of relevant confounders - including number of fever symptoms in pregnancy, maternal HAART use in pregnancy and baseline immunologic status, maternal and child nevirapine prophylaxis, and differences in socio-demographics and child-related characteristics.

Our results suggest that malarial morbidity in pregnant HIV-positive women might be an independent risk factor for HIV MTCT in SSA. Our finding that the association between MIP diagnosis and HIV MTCT was not explained by fever symptoms suggests that MIP in HIV-positive women may elevate HIV incidence co-endemic regions independent of non-specific fever symptoms. Hence, the risk of HIV transmission may remain elevated in HIV-positive women in spite of the increasing HAART availability and improved coverage of PMTCT services. Attainment of the expressed goal of zero MTCT by 2015, as noted in the most recent UNAIDS report in malaria and HIV co-endemic settings, will be enhanced by understanding the contribution of malaria and other co-infections to HIV transmission and ramping up control efforts to mitigate such risks. Given the limitations described above, further studies on this subject, with more rigorous malaria definitions, are warranted to further elucidate the relationship of laboratory-confirmed malarial morbidity in pregnancy and MTCT.

In light of the grave health risks associated with MIP, the special vulnerability of HIV-positive women to malaria, and its potential contribution to elevated risk of HIV MTCT, we believe that large scale adoption and consistent implementation of malaria preventive measures – including universal access to insecticide-treated bed-nets, among HIV-positive pregnant women is warranted. An additional prevention strategy that might improve patient compliance with malaria prevention measures is large scale dissemination of information regarding the possible malaria-associated higher risk of HIV MTCT. Further, all HIV-positive adults may benefit from active, rather than passive, clinical assessment for malaria and its treatment when indicated as part of routine health care for as asymptomatic malaria parasitemia is likely to be common among HIV-positive adults.

Acknowledgements

We thank the study participants from Dar es Salaam, Tanzania for making this study possible. We are grateful to the field staff for their diligence and energy: Rehema Mtonga, Illuminata Ballonzi, Godwin Njiro, Frank Killa, Emily Dantzer, Elizabeth Long and Jenna Golan. Dr. Ezeamama acknowledges, with thanks, salary support during this investigation from the Harvard School of Public Health Yerby Post-doctoral Fellowship program.

The opinions and statements in this article are those of the authors and may not reflect official UNICEF policies.

Funding

This work was supported by the National Institute of Child Health and Human Development Grant#: R01HD043688. CD was supported in part by K24 HD058795.

REFERENCES

1. Horvath T, Madi BC, Iuppa IM, Kennedy GE, Rutherford G, Read JS. Interventions for preventing late postnatal mother-to-child transmission of HIV. Cochrane database of systematic reviews (Online). 2009:CD006734.
2. Mmiro FA, Aizire J, Mwatha AK, et al. Predictors of early and late mother-to-child transmission of HIV in a breastfeeding population: HIV Network for Prevention Trials 012 experience, Kampala, Uganda. Journal of acquired immune deficiency syndromes (1999). 2009; 52:32–39. [PubMed: 19617849]
3. Read JS. Preventing mother to child transmission of HIV: the role of caesarean section. Sexually transmitted infections. 2000; 76:231–232. [PubMed: 11026874]
4. Spensley A, Sripipatana T, Turner AN, Hoblitzelle C, Robinson J, Wilfert C. Preventing mother-to-child transmission of HIV in resource-limited settings: the Elizabeth Glaser Pediatric AIDS Foundation experience. American journal of public health. 2009; 99:631–637. [PubMed: 18703458]
5. Abu-Raddad LJ, Patnaik P, Kublin JG. Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. Science (New York, NY. 2006; 314:1603–1606.
6. Ayisi JG, van Eijk AM, Newman RD, et al. Maternal malaria and perinatal HIV transmission, western Kenya. Emerging infectious diseases. 2004; 10:643–652. [PubMed: 15200854]
7. Brahmbhatt H, Sullivan D, Kigozi G, et al. Association of HIV and malaria with mother-to-child transmission, birth outcomes, and child mortality. Journal of acquired immune deficiency syndromes (1999). 2008; 47:472–476. [PubMed: 18332766]
8. Mwapasa V, Rogerson SJ, Molyneux ME, et al. The effect of Plasmodium falciparum malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. AIDS (London, England). 2004; 18:1051–1059.
9. Tkachuk AN, Moormann AM, Poore JA, et al. Malaria enhances expression of CC chemokine receptor 5 on placental macrophages. The Journal of infectious diseases. 2001; 183:967–972. [PubMed: 11237815]
10. Brahmbhatt H, Kigozi G, Wabwire-Mangen F, et al. The effects of placental malaria on mother-to-child HIV transmission in Rakai, Uganda. AIDS (London, England). 2003; 17:2539–2541.
11. Bulterys PL, Chao A, Dalai SC, et al. Placental malaria and mother-to-child transmission of human immunodeficiency virus-1 in rural Rwanda. The American journal of tropical medicine and hygiene. 2011; 85:202–206. [PubMed: 21813835]
12. Gallagher M, Malhotra I, Mungai PL, et al. The effects of maternal helminth and malaria infections on mother-to-child HIV transmission. AIDS (London, England). 2005; 19:1849–1855.
13. Inion I, Mwanyumba F, Gaillard P, et al. Placental malaria and perinatal transmission of human immunodeficiency virus type 1. The Journal of infectious diseases. 2003; 188:1675–1678. [PubMed: 14639538]
14. Kupka R, Msamanga GI, Aboud S, Manji KP, Duggan C, Fawzi WW. Patterns and predictors of CD4 T-cell counts among children born to HIV-infected women in Tanzania. Journal of tropical pediatrics. 2009; 55:290–296. [PubMed: 19158163]
15. Msamanga GI, Taha TE, Young AM, et al. Placental malaria and mother-to-child transmission of human immunodeficiency virus-1. The American journal of tropical medicine and hygiene. 2009; 80:508–515. [PubMed: 19346367]
16. Naniche D, Lahuerta M, Bardaji A, et al. Mother-to-child transmission of HIV-1: association with malaria prevention, anaemia and placental malaria. HIV medicine. 2008; 9:757–764. [PubMed: 18651857]
17. Naniche D, Lahuerta M, Bardaji A, et al. Mother-to-child transmission of HIV-1: association with malaria prevention, anaemia and placental malaria. HIV Med. 2008
18. Barnabas RV, Webb EL, Weiss HA, Wasserheit JN. The role of co-infections in HIV epidemic trajectory and positive prevention: a systematic review and meta-analysis. Aids. 2011
19. Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: a brief review. The Korean journal of parasitology. 2009; 47:93–102. [PubMed: 19488414]

20. Fawzi W, Msamanga G, Spiegelman D, et al. Transmission of HIV-1 through breastfeeding among women in Dar es Salaam, Tanzania. Journal of acquired immune deficiency syndromes (1999). 2002; 31:331–338. [PubMed: 12439210]
21. Bertolli J, St Louis ME, Simonds RJ, et al. Estimating the timing of mother-to-child transmission of human immunodeficiency virus in a breast-feeding population in Kinshasa, Zaire. The Journal of infectious diseases. 1996; 174:722–726. [PubMed: 8843208]
22. Bulterys M, Chao A, Dushimimana A, et al. Multiple sexual partners and mother-to-child transmission of HIV-1. Aids. 1993; 7:1639–1645. [PubMed: 8286074]
23. Wabwire-Mangen F, Gray RH, Mmiro FA, et al. Placental membrane inflammation and risks of maternal-to-child transmission of HIV-1 in Uganda. Journal of acquired immune deficiency syndromes (1999). 1999; 22:379–385. [PubMed: 10634200]
24. Ayouba A, Tene G, Cunin P, et al. Low rate of mother-to-child transmission of HIV-1 after nevirapine intervention in a pilot public health program in Yaounde, Cameroon. Journal of acquired immune deficiency syndromes (1999). 2003; 34:274–280. [PubMed: 14600571]
25. Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet. 1999; 354:795–802. [PubMed: 10485720]
26. Hoffman RM, Black V, Technau K, et al. Effects of highly active antiretroviral therapy duration and regimen on risk for mother-to-child transmission of HIV in Johannesburg, South Africa. Journal of acquired immune deficiency syndromes (1999). 2010; 54:35–41. [PubMed: 20216425]
27. Tournoud M, Ecochard R, Kuhn L, Coutsoudis A. Diversity of risk of mother-to-child HIV-1 transmission according to feeding practices, CD4 cell count, and haemoglobin concentration in a South African cohort. Trop Med Int Health. 2008; 13:310–318. [PubMed: 18397394]
28. Charurat M, Datong P, Matawal B, Ajene A, Blattner W, Abimiku A. Timing and determinants of mother-to-child transmission of HIV in Nigeria. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2009; 106:8–13.
29. Van Dyke RB. Mother-to-child transmission of HIV-1 in the era prior to the availability of combination antiretroviral therapy: the role of drugs of abuse. Life sciences. 2011; 88:922–925. [PubMed: 21439978]
30. Kantarci S, Koulinska IN, Aboud S, Fawzi WW, Villamor E. Subclinical mastitis, cell-associated HIV-1 shedding in breast milk, and breast-feeding transmission of HIV-1. Journal of acquired immune deficiency syndromes (1999). 2007; 46:651–654. [PubMed: 18043320]
31. Semba RD, Kumwenda N, Hoover DR, et al. Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. The Journal of infectious diseases. 1999; 180:93–98. [PubMed: 10353866]
32. Adejuyigbe EA, Fasubaa OB, Onayade AA. Sociodemographic characteristics of HIV-positive mother-child pairs in Ile-Ife, Nigeria. AIDS care. 2004; 16:275–282. [PubMed: 15203421]
33. Vieira AC, Miranda AE, Vargas PR, Maciel EL. HIV prevalence in pregnant women and vertical transmission in according to socioeconomic status, Southeastern Brazil. Revista de saude publica. 2011; 45:644–651. [PubMed: 21739080]
34. UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2010., Joint United Nations Programme on HIV/AIDS. 2010:20.
35. WHO. WHO; 2011. World Malaria Report 2010.
36. Ezeamama AE, Duggan C, Spiegelman D, et al. Malarial Morbidity and Postnatal HIV Infection in Breastfeeding HIV-exposed Infants. International Journal of Tropical Disease & Health. 2013 **In Press**.
37. Barnabas RV, Webb EL, Weiss HA, Wasserheit JN. The role of coinfections in HIV epidemic trajectory and positive prevention: a systematic review and meta-analysis. AIDS (London, England). 2011; 25:1559–1573.
38. Ogundipe O, Hoyo C, Ostbye T, et al. Factors associated with prenatal folic acid and iron supplementation among 21,889 pregnant women in Northern Tanzania: a cross-sectional hospital-based study. BMC public health. 2012; 12:481. [PubMed: 22734580]

39. De Beaudrap P, Turyakira E, White LJ, et al. Impact of malaria during pregnancy on pregnancy outcomes in a Ugandan prospective cohort with intensive malaria screening and prompt treatment. Malaria journal. 2013; 12:139. [PubMed: 23617626]

40. Ivan E, Crowther NJ, Rucogoza AT, et al. Malaria and helminthic co-infection among HIV-positive pregnant women: prevalence and effects of antiretroviral therapy. Acta tropica. 2012; 124:179–184. [PubMed: 22940013]

Table 1

Socio-demographic, Obstetric History and at-birth Description of HIV-infected Mothers and their newborns from Dar es Salaam, Tanzania

	Overall	Child HIV-positive at 6 weeks	Child HIV-negative at 6 Weeks	P-value
		N = 262 (11.1%)	N = 2105 (88.9%)	
	Mean (SD)	Mean (SD)	Mean (SD)	
Mother's Age (years)	28.5 (4.9)	28.8 (4.94)	28.6 (4.95)	0.5170
Mother's Years of Education	7.2 (2.7)	7.2 (2.33)	7.19 (2.79)	0.6195
GA at enrollment (weeks)	22.2 (5.30)	22.2 (5.39)	22.1 (5.29)	0.7276
	N (%)	N (%)	N (%)	
Married/Co-habiting	1996 (85.0)	213 (80.7)	1783 (85.6)	0.1782
Mother has own Income	809 (34.5)	79 (29.9)	730 (35.0)	0.1001
Post-PEPFAR Enrollee	1449 (61.2)	154 (58.8)	1295 (61.5)	0.3955
Obstetric History	N (%)	N (%)	N (%)	
Neonatal Deaths prior pregnancies	351 (15)	60 (22.7)	291 (14.0)	0.0008
Spontaneous abortions (prior pregnancies)	437 (18.5)	52 (19.9)	385 (18.3)	0.5377
Gravidity (including current pregnancy)				
1	531 (23.6)	56 (21.5)	475 (23.0)	Ref
2	772 (32.9)	83 (31.4)	689 (33.0)	0.9619
3	524 (22.3)	67 (25.4)	457 (21.9)	0.2805
4+	499 (21.3)	55 (20.8)	444 (21.3)	0.8541
Malaria during Pregnancy	N (%)	N (%)	N (%)	
Ever vs. Never	513 (21.6)	64 (24.1)	450 (25.1)	0.3210
# of Malaria Episodes				
0	1855 (78.3)	180 (76.0)	1537 (78.6)	0.0178
1	391 (16.5)	39 (14.9)	352 (16.7)	
2	101 (4.3)	18 (6.9)	83 (3.3)	
3+	21 (0.9)	6 (2.3)	15 (0.7)	
Delivery Details and Infant Characteristics	N (%)	N (%)	N (%)	
Mothers that took nevirapine at labor onset	711 (30.0)	66 (25.2)	645 (30.6)	0.0703
In Hospital Delivery	2011 (85.7)	214 (81.8)	1797 (86.2)	0.0241
Vaginal Tears	351 (15.0)	49 (18.6)	396 (19.0)	0.8632
Female Sex	1083 (45.7)	131 (50.0)	952 (45.2)	0.1417
C-Section	275 (11.7)	23 (8.7)	252 (12.1)	0.1076
Full term Birth	1774 (75)	1578 (75.7)	186 (70.5)	0.0623
Low Birth Weight	157 (6.7)	42 (15.9)	115 (5.5)	<0.0001
Infant received nevirapine within 72 hours	1691 (71.4)	170 (64.9)	1521 (72.2)	0.0132
Breast fed between delivery and six weeks	1989 (84.0)	230 (87.8)	1759 (83.5)	0.0759
Maternal Health, Immune/Lab Details				
Anemia at enrollment	643(27.2)	89 (34.0)	554 (26.3)	0.0085
Breast Pain/Inflammation, Cracked or Bleeding Nipple by 6-weeks	32 (1.4)	6 (2.4)	26 (1.2)	0.1629

	Overall	Child HIV-positive at 6 weeks	Child HIV-negative at 6 Weeks	P-value
		N = 262 (11.1%)	N = 2105 (88.9%)	
CD4 count < 350 cells/uL at enrollment	774 (34.0)	102 (45.6)	672 (32.9)	<.0001
HIV disease Stage >1 at enrollment	383 (16.2)	43 (16.4)	340 (16.1)	0.9116
On ARV at any time during pregnancy	166 (7.1)	5 (1.9)	161 (7.6)	0.0006

Table 2

Mother-to-child HIV transmission by 6-weeks in relation to clinical malaria diagnosis by a doctor during pregnancy among HIV-positive mothers and their HIV exposed infants from Dar es Salaam, Tanzania

		Univariate Association	Adjusted Model (1)**	Adjusted Model (2)**
Maternal Malaria in Pregnancy	**n/N**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Never Clinical Malaria	209/1974	1.27 (0.96, 1.68)	1.24(0.94, 1.64)	1.25 (0.93, 1.63)
Ever Clinical Malaria	53/394	1.00	1.00	1.00
Per episode increment in clinical malaria	n/a	**1.31 (1.08, 1.61)**	**1.29 (1.04, 1.58)**	**1.29 (1.05, 1.59)**
# of Doctor Diagnosed Clinical Malaria				
0	209/1974	1.00	1.00	1.00
1 vs. 0	38/332	1.08 (0.78,1.50)	1.07 (0.77, 1.48)	1.09 (0.80, 1.48)
2+ vs. 0	15/62	**2.27 (1.39, 3.71)**	**2.12 (1.31, 3.45)**	**1.99 (1.20, 3.27)**

RR = Relative Risk; CI = confidence Intervals

* n = number of sero-conversions by 6 weeks; N = number of mothers within strata of maternal malarial morbidity in pregnancy. Estimates are from a GEE model modeling Sero-conversion at 6 weeks as the dependent variable. Model assumed a binomial distribution with logit link. All adjusted covariates reflect their baseline values.

** **Model 1**: covariates include: a) Maternal factors – presence vs. absence of breast/nipple lesions or inflammation, education, age, marital status, malaria prophylaxis at enrollment (self-reported yes vs. no), mother having own income, gravidity (2, 3, 4+ vs. 1), history of neonatal deaths in previous pregnancies, history of still births in previous pregnancies and Hemoglobin <11 vs.≥ 11 at study enrollment; b) Delivery factors: c-section vs. other form of delivery, presence/absence of vaginal tears or episiotomy, full vs. pre-term birth and location of delivery (Muhimbili hospital vs. other); c) Child factors: male vs. female infant, low birth weight, ever vs. never breast fed status, and d) Study relevant secular trends: Pre vs. post pepfar birth (Child born <=July 2005 vs. > July 2005) and season of mother's recruitment into study: long & short rains vs. dry season.

** **Model 2**: Adjusted for all of the above including the following potential mediators: maternal ARV status during pregnancy, maternal WHO stage at enrollment, maternal immunity during pregnancy at enrollment i.e. enrollment CD4 (<350 vs. ≥ 350 cells/uL) at enrollment and intra-partum administration of nevirapine.

Table 3

Non malaria predictors of Mother-to-child HIV transmission by 6-weeks among HIV-positive mothers and their HIV exposed infants from Dar es Salaam, Tanzania

Maternal Health Indicators	With Exposure n/N	Without Exposure n/N	Univariate Model RR (95% CI)	Adjusted Model ** RR (95% CI)
Maternal CD4 < 350 at enrollment	94/607	168/1761	1.83 (1.42, 2.35)	**1.84 (1.44, 2.36)**
Ever vs. Never maternal fever during pregnancy	53/376	209/1992	1.34 (1.01, 1.78)	1.04 (0.78, 1.38)
Maternal anemia at Enrollment				
Moderate Anemia (Hgb 8.5 to <11 vs. ≥ 11 g/dL)	79/587	183/1781	1.38 (1.00, 3.24)	1.25 (0.97, 1.62)
Severe Anemia (Hgb <8.5 vs. ≥ 11 g/dL)	10/56	252/2312	1.77 (1.07, 1.79)	1.61(0.93, 2.81)
On ARV in pregnancy	5/166	257/2202	0.30 (0.13, 0.73)	**0.20 (0.08, 0.50)**
Maternal Obstetric History in Prior Pregnancies/Deliveries				
≥1 vs. 0 Early child death*/spontaneous abortion	96/771	166/1597	1.25 (0.97, 1.61)	1.19 (0.92, 1.54)
Breast Feeding & Maternal Breast/Nipple Health				
Any vs. no breast feeding (birth to 6-weeks of life)	230/1989	32/379	1.40 (0.94, 2.10)	1.27 (0.85, 1.90)
Any vs. no Breast lesion/pain/inflammation, cracked or bleeding nipple (birth to 6-weeks of post-partum)	6/32	256/2336	1.69 (0.81, 3.51)	**1.90 (1.02, 3.55)**
Delivery Factors/Infant Characteristics				
Nevirapine prophylaxis given to:				
Both mother at labor onset & baby within 72 hours of birth	60/644	202/1724	0.66 (0.49, 0.90)	0.75 (0.53, 1.08)
Baby only within 72 hours of birth	110/1047	152/1321	0.74 (0.57, 0.97)	0.84 (0.60, 1.17)
Mother at labor onset only	5/60	257/2308	0.59 (0.25, 1.40)	0.67 (0.29, 1.53)
C-Section vs. other form of birth	23/275	239/2093	0.74 (0.49, 1.12)	0.83 (0.55, 1.25)
Premature Birth (< 37 vs. ≥ 37 weeks)	49/348	213/2020	1.35 (1.02, 1.81)	1.01 (0.75, 1.35)
Female vs. Male sex	131/1083	131/1285	1.19 (0.94, 1.49)	1.13 (0.91, 1.41)
Low (<2500g) vs. normal (≥2500g) Birth Weight	40/156	222/2212	2.65 (1.96, 3.57)	**2.30 (1.71, 3.07)**
In Hospital vs. home/other location of Delivery	212/2026	50/342	0.70 (0.50, 0.98)	0.99 (0.66, 1.48)
Economic/Socio-demographic/Temporal Factors				
Married/cohabiting vs. single/divorced/widowed/separated	222/2060	40/308	0.82 (0.60, 1.13)	**0.73 (0.54, 1.00)**
Has own income vs. no income	78/813	184/1555	0.81 (0.63, 1.04)	**0.76 (0.59, 0.98)**
Maternal Education (≥7 vs. <7 years)	234/2046	28/322	1.32 (0.90, 1.92)	1.31(0.91, 1.92)

* Self-reported maternal history of a live born child dying within 7 days of birth or spontaneous abortion by pregnancy month 7. Current pregnancy is not included.

n = number of sero-conversions by 6 weeks; N = number of mothers-child pairs with or without given exposure. Estimates are from a GEE model modeling Sero-conversion at 6 weeks (randomization) as the dependent variable. Model assumed a binomial distribution with log link. All adjusted covariates reflect their baseline values.

** **Multivariate model**: adjusted covariates include: a) Maternal factors – education, age, marital status, malaria prophylaxis at enrollment (self-reported yes vs. no), mother having own income, gravidity (2, 3, 4+ vs. 1), history of neonatal deaths in previous pregnancies, history of still births in previous pregnancies and Hemoglobin <11 vs.≥ 11 at study enrollment; b) Delivery factors: c-section vs. other form of delivery, presence/ absence of vaginal tears or episiotomy, full vs. pre-term birth and location of delivery (Muhimbili hospital vs. other); c) Child factors: male vs. female infant, low birth weight, d) Infant breastfeeding status (yes vs. no) and maternal breast/nipple health and e) Study relevant secular trends: Pre vs. post pepfar birth (Child born <=July 2005 vs. > July 2005) and season of mother's recruitment into study: long & short rains vs. dry season as well as the following potential mediators: maternal ARV status during pregnancy, maternal WHO stage at enrollment, maternal CD4 at enrollment (<350 vs. ≥ 350 cells/uL) at enrollment and intra-partum administration of nevirapine.