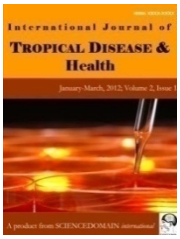

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# Malarial Morbidity and Postnatal HIV Infection in Breastfeeding HIV-exposed Infants

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***Authors' contributions***

*All co-authors have seen and approved the content of this article. Each has participated in the concept and design, analysis, data interpretation, writing or revision of this manuscript. We did not receive any external writing assistance in the development of this manuscript.*

***Research Article***

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## ABSTRACT

**Background:** For at-risk HIV-negative individuals, whether malarial morbidity increases the likelihood of HIV infection when exposed is unknown. Hence, we investigate the

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malaria-associated risk of postnatal HIV infection in 1804 breastfeeding infants of HIV-positive women from Dar es Salaam, Tanzania.
**Methods:** Six-week-old HIV-negative infants were followed until breastfeeding cessation or postnatal HIV infection. HIV-1 status was determined by a DNA PCR test. Malarial morbidity was diagnosed by physicians using a combination of clinical symptoms and laboratory tests. For analytic purposes, malaria was distinguished by diagnostic specificity as: (1) clinical; (2) probable, where laboratory testing is requested for parasitemia; and (3) blood smear-confirmed. Hazard ratios (HR) and 95% confidence intervals (CI) for the risk of HIV infection were estimated from multivariate Cox regression models.
**Results:** Mean follow-up duration was 6.2 months (standard deviation=2.4 months), during which 91 new HIV infections developed and clinical malaria was diagnosed in 594(32.3%) children, including 283 (15.5%) probable and 80(4.4%) confirmed malaria episodes. Infants ever diagnosed with clinical and probable malaria were at 73% (95%CI:1.11 - 2.69) and 100% (95%CI:1.17-3.42) higher risk of postnatal HIV infection, respectively. This risk increased by 39% (95%CI: 1.08-1.80) and 59% (95%CI: 1.00-2.32), respectively, per episode increment in clinical and probable malarial; however, confirmed malaria was not significantly associated with HIV incidence (HR=2.09; 95%CI: 0.74 - 5.91).
**Conclusion:** We found positive associations between child malarial infection and postnatal HIV infection among breastfeeding HIV-negative children of HIV-positive women. These findings suggest that malaria prevention in such infants may decrease the risk of HIV mother-to-child-transmission. However, specific future studies using laboratory-confirmed malaria in HIV-negative but HIV at risk populations are needed to substantiate these findings.

*Keywords: HIV/AIDS; malaria; co-infection; children; hiv incidence; breastfeeding; Sub-Saharan Africa.*

## 1. INTRODUCTION

Malaria and human immunodeficiency virus (HIV) are leading causes of death in Sub-Saharan Africa (SSA) [1], where millions are at risk of co-infection [2]. Up to a 30% co-infection rate has been reported in HIV-infected adults [3], among whom frequent and severe malaria episodes, CD4 T-cell count declines, and higher viral loads were observed [4]. For at-risk HIV-uninfected individuals, whether malaria promotes HIV acquisition is unknown [5], with no epidemiologic evidence that the malaria-associated decline in CD4 T-cell count [6,7] and increase in viral load [4]correlate with HIV incidence. However, results from CD4 T-cell infectability studies within *Plasmodium falciparum* antigen-stimulated pro-inflammatory cytokine milieu [8]suggest that immune responses to malaria in at-risk HIV-negative persons promotes HIV infection through parasite-induced immune activation, [9] up-regulation of viral expression in macrophages [10], and enhanced expression of CCR5 and CXCR4 co-receptors that facilitate HIV cell entry [11].

Women of reproductive age constitute the majority of HIV-infected persons in SSA[12]. Many bear children and breastfeed post-delivery despite the breastfeeding-associated risk of HIV mother-to-child-transmission (MTCT) [13]. Breastfeeding of HIV-exposed but uninfected infants by their HIV-positive mothers has serious health implications for the newborn's health. In the malaria-endemic regions of SSA, in particular, such a newborn is at simultaneous high risk of malaria and HIV infections. On the one hand, the passive anti-

malaria immunity transferred through breastfeeding is highly desirable because it will protect the newborn from malaria [14]; on the other hand, for infants of HIV-positive women, the shedding of HIV virus in breast milk is a serious risk factor for postnatal HIV infection [15]. The World Health Organization's (WHO) prevention of MTCT guidelines attempts to balance this inherent tension, while optimizing the likelihood of HIV-free survival for infants of HIV-positive women. WHO recommends exclusive breastfeeding in tandem with antiretroviral therapy (ART) prophylaxis for the mother and child during breastfeeding for 6 months [15]. Despite the near-universal breastfeedingand the dual threat of HIV and malaria infections for infants of HIV-positive women in SSA, how malaria affects postnatal HIV infection has not been specifically investigated. Therefore, we conduct this prospective cohort study to test the hypothesis that malarial morbidity in HIV-uninfected breastfeeding babies of HIV-positive mothers increases their risk of postnatal HIV infection.

## 2. MATERIALS AND METHODS

### 2.1 Design

This is a nested prospective cohort study designed to investigate the association between child malarial morbidity and postnatal HIV infection in breastfeeding HIV-negative children of HIV-positive women. The parent study is a randomized, placebo-controlled trial of the efficacy of daily supplementation with vitamins B, C, and E in decreasing the incidence of select childhood morbidities among infants of HIV-positive women in Dar es Salaam, Tanzania. The parent study included follow-up of the mother-child pair until the child's 24th month of life. For this nested study, follow-up duration is restricted to the period of breastfeeding, when infants of HIV-positive women are at risk of postnatal HIV MTCT.

### 2.2 Setting

Malaria is endemic in Dar es Salaam, with stable transmission year round. The annual prevalence of malaria in Dar es Salaam is 12%, with an estimated 1.28 infectious mosquito bites per person per year [16] Among children less than 5 years old, malaria is identified as the cause of one in three deaths and is the primary reason for at least 40% of medical consultations [17]. With respect to HIV prevalence in the study area at study initiation, HIV prevalence in antenatal clinics was 8.4% [18] and pediatric HIV constituted 9% of prevalent HIV infections.

### 2.3 Inclusion/Exclusion Criteria for Study Participation

Pregnant women who intended to maintain residence in Dar es Salaam for two or more years post-delivery and who received medical care during pregnancy at either the Makuti or Tama Study Clinics as part of the Muhimbili, Dar es Salaam and Harvard research collaboration project were enrolled in the parent study between February 2004 and July 2007. Children whose mothers were unable to return for follow-up, those with serious congenital anomalies that would interfere with compliance with study procedures, and twins andmultiple birth infants were excluded.

All study participants received stipends for transportation to and from the study clinic for monthly scheduled visits, during which medical and detailed nutritional assessments were made. HIV testing, complete blood count, and CD4 measurements were repeated every 6 months. Study participants were encouraged to return to the clinic for management of illnesses that occurred outside of scheduled visits.

## 2.4 Standard of Care

During pregnancy, all mothers received multivitamins, folate, iron, and intermittent preventive malaria therapy that used sulfadoxine-pyrimethamine at the 20th and 30th weeks of gestation. At study initiation, maternal ART was limited to nevirapine administered prophylatically during labor to prevent intra-partum HIV infection. Beginning in July 2005, mothers were routinely evaluated for ART eligibility for their own health as access to antiretroviral drugs widened through the President's Emergency Plan for AIDS Relief (PEPFAR) and other programs. All newborns in the study were given nevirapine within 72 hours of birth or as close to birth as possible for infants not born in hospitals. In addition, all infants were prescribed cotrimoxazole until 6 months of age, after which only breastfeeding and HIV-infected children remained on cotrimoxazole.

## 2.5 Primary Determinant: Malarial Morbidity

Malaria was assessed during monthly and unscheduled visits based on physician diagnosis of patients' clinical symptoms as malaria, with or without laboratory testing to confirm parasitemia. All diagnoses were accompanied by anti-malarial prescription, most commonly amodiaquine, sulfadoxine-pyrimethamine, or fansidar. Lab tests were typically ordered in this setting to confirm the presence/absence of treatment-resistant malaria and complicated or cerebral malaria and, thus, reflected heightened clinical suspicion [19]. When diagnosis is accompanied with a requisition for a parasitemia test, children were classified as having clinical malaria if the result of such a test (when available) were either positive or unknown. This approach, which is typical clinical practice in many resource-constrained settings, is consistent with recommendations of the integrated management of childhood illnesses (IMCI). However, such diagnoses are limited by low specificity, which can result in possible over-diagnosis of malarial morbidity [20]. To accommodate this common clinical practice and allow for explicit examination of any differences in our results by malaria diagnostic specificity, we adopted a sensitivity analysis-type approach in defining malarial morbidity, as was done in a previous study [19]. In brief, we created two additional classes of malaria diagnoses, probable and confirmed malaria, based on the requisition of a blood test for the malaria parasite and the presence/absence of laboratory results, given that a test was ordered. Probable malaria included all diagnoses accompanied by a malaria parasite test requisition for which the result of testing was either positive or unknown. Confirmed malaria included the subset of diagnoses accompanied with lab tests, with test results available and positive for parasitemia.

Malaria parasite tests were performed using Giemsa-stained thin blood smears made from finger or heel pricks, air dried for 30 minutes. Trained technicians read each blood slide in three different fields, and the parasite density per cubic millimeter was estimated from the number of trophozoites per 200 leukocytes. Malarial morbidity was operationally defined as a time-varying exposure in the following formats: ever vs. never malaria, total malaria episodes to date, and categories of total malaria episodes to date including one, two, or three or more vs. zero episodes. In keeping with previous work, we considered malaria episodes distinct if they occurred 14 days or more apart [21].

## 2.6 Outcome: Postnatal HIV Infection

HIV status of children was assessed at 6 weeks by HIV-1 DNA PCR using Amplicor HIV-1 DNA version 1.5 assays (Roche Molecular Systems, Branchburg, NJ, USA) until 18 months

of age. Thereafter, HIV status was determined by the Murex HIV antigen/antibody (Abbott Murex, UK), followed by the Enzygnost anti-HIV-1/2 Plus (Dade Behring, Marburg, Germany) ELISAs. Discordant results were resolved through Western blot assay[22]. Per protocol of the parent study, real-time HIV tests were done at 6 weeks and 24 months; however, blood samples for future HIV testing were stored every 6 months. Blood samples for children HIV-positive at 24 months were back-tested to estimate the approximate age at HIV infection. For the purpose of this study, children HIV-negative at 6 weeks were at risk of breastfeeding-related postnatal HIV infection until the end of breastfeedingonly[15]. Hence, we limited child malarial morbidity to those diagnoses that occurred during breastfeeding and up to the date of breastfeeding cessation plus an 8-week sero-conversion window. This restriction was necessary to avoid the confusion of temporal sequence between child malarial morbidity and postnatal HIV infection. Per the literature, it may take up to 56 days for HIV antibodies to appear[23]. Because breastfeeding is the primary risk factor for HIV infection for infants HIV-negative at birth, observations were censored for HIV-positive children at either the first HIV-positive blood sample date or the last breastfeeding date plus 56 days, whichever occurred first.

## 2.7 Potential Confounders/Mediators

We considered an extensive array of maternal, child, seasonal, and household factors as potential confounders and/or mediators of the relationship between child malarial morbidity and postnatal HIV infection. Non-time-varying potential confounders measured at baseline included child baseline CD4 T-cell% (<25 vs. $\geq$25), child sex, micronutrient supplementation vs. placebo, low birth weight (<2500 vs. $\geq$2500g), per-capita household daily food expenditure ($\leq$500 vs. >500 Tanzanian Shillings), maternal age (<25, 25-29, 30-34 vs. $\geq$35 years), education (<7, = 7, vs. >7 years), and history of neonatal mortality in prior pregnancies (yes vs. no). The following time-varying covariates were updated monthly: ever vs. never maternal clinical malaria season of patient visit (long rains (November-February) and short rains (March-May) vs. dry season (June-October), child underweight (weight-for-age z-scores <-2 vs. $\geq$-2) and cotrimoxazole use (yes vs. no). In addition, child anemia (hemoglobin<10 g/dL), maternal anemia (hemoglobin<11 g/dL), and maternal CD4 T-cell count (<350vs.>= 350 cells/$\mu$L) values were time varying and updated bi-annually.

## 2.8 Data Analysis

We used Cox proportional hazards models to estimate univariate and multivariable hazard ratios for time-updated malaria infection in relation to the risk of HIV infection. An Anderson-Gill data structure was used to split each child's follow-up period into pieces of person-time, which reflects the interval between two consecutive visits. Within each interval, beginning and end time were defined as child age at the beginning and end of the interval, respectively. Time-varying factors were updated to reflect their values at the beginning of the visit interval. All potential confounders and established risk factors for HIV infection were adjusted for in multivariable models. Missing confounder values were handled using the missing indicator method. Differences in likelihood ratio tests between nested models were used to test for heterogeneity in the malaria-HIV association by child and maternal immunologic and anemia status, micronutrient supplementation, child underweight, maternal WHO HIV disease stage, and pre- vs. post-PEPFAR enrollment. We specified the proportionality test for all time-dependent covariates to examine the Cox proportional hazards assumption and rejected the null hypothesis of equal hazards for strata of specified covariates if associated *p*-values $\leq$0.05. All analyses were conducted in SAS version 9.1.

## 2.9 Ethical Review & Informed Consent

Ethical clearance for the conduct of this 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 consents for their own and their child's participation in the trial.

## 2.10 Role of Funding Source

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 had the final responsibility for the decision to submit for publication.

## 3. RESULTS

Of the 2,387 randomized children, exclusions were made for lack of HIV information at any time (*n*=27), baseline HIV-positivity (*n*=264), or lack of post-baseline HIV tests (*n*=299). Hence, 1,804 children were included in this analysis, of which 91 became HIV-infected during follow-up (Fig. 1). At 6 weeks postnatal examination (baseline), clinical malaria was diagnosed in 172 HIV-negative infants, of whom only 49 and 15 had probable and confirmed malaria episodes, respectively. Other sociodemographic characteristics of the cohort at baseline (6 weeks) are given in Table 1.

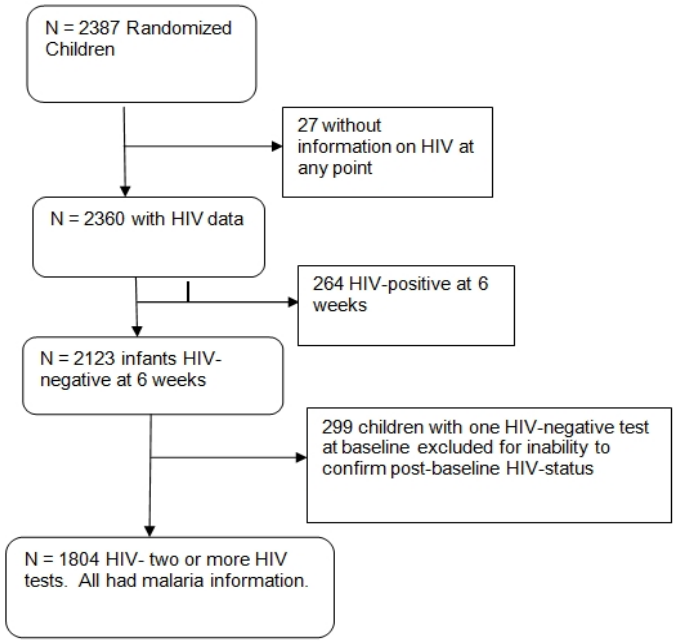


**Fig. 1. Derivation of Analytic Sample**

**Table 1. Description of HIV-positive mothers and HIV-exposed but uninfected infants at mother's enrollment in the study enrollment, delivery or infants' sixth week of life (N = 1804 mother-child pairs)**

| | **Mean (SD)** |
|---|---|
| Child weight (kg) at 6 weeks | 4·5 (0·7) |
| Child CD4 percent at 6 weeks | 38·3(10·4) |
| Child absolute CD4 T-cell Count (cells/uL) at 6 weeks | 2033 (4252) |
| | N (%) |
| Child Sex (male) | 984(54·6) |
| Low Birth weight (< 2500g) | 103 (5·9) |
| Underweight ( WAZ <= -2) at 6 weeks | 129 (7·3) |
| **CD4 T-cell percent distribution at 6 weeks** | |
| <15% | 27 (1·8) |
| 15 – 25% | 110 (7·4) |
| >25% | 1352 (90·8) |
| **Malaria Diagnosis by 6 weeks** | |
| Clinical Malaria | 172 (9·5) |
| Probable Malaria | 49 (2·7) |
| Confirmed Malaria | 15 (0·8) |
| **Maternal Characteristics at Enrollment** | Mean (SD) |
| Maternal Age (years ) | 28·3 (5·0) |
| Maternal CD4 T-cell count (cell/uL) | 550·2 (360·1) |
| Gravidity -including current child | 2·6 (1·3) |
| Maternal Education (years) | 7·1 (2·8) |
| | N (%) |
| Maternal History of >= 1 Neonatal Mortality* | 319 (17·9) |
| Married/living with partner | 1563 (87·6) |
| Household Daily Food expenditure <500 T Sh | 1200 (66.5) |

** Includes # of children born alive but died within 7 days or after 7 days*
*Estimates based on 1703 to 1804 children, except for child Cd4 percent (n = 1489), absolute CD4 cell count (child = 1690, mother = 1618).*SD = standard deviation.

Median breastfeeding duration for children who became postnatally HIV-positive and those persistently HIV-negative through the end of breastfeeding were 4.7 (variance =6.3) and 4.1 months (variance=5.9), respectively. During follow-up, clinical malaria was diagnosed by a physician in 594(33%) children (mean number of episodes=1.53, SD=0.89). Among these children, laboratory tests were requested for 283 children. However, the results were available for only 81 children, of whom 80 were positive for parasitemia. Median follow-up duration did not differ by postnatal HIV infection status at 5.9 (variance =3.4) months for children who became HIV-positive postnatally versus 5.9 (variance=5.7) months for HIV-uninfected children.

Ever vs. never child clinical, probable, and confirmed malarial morbidity were each positively associated with the risk of postnatal HIV infection; however, statistical significance was attained for clinical and probable malaria only (Table 2). Per episode increment, HIV postnatal infection risk increased by 39% (95%CI:8%-180%) for clinical malaria, 52% (95%CI:0%-232%) for probable malaria, and perhaps as high as 110% (95%CI:-9%-487%) for confirmed malaria episodes. Relative to children without clinical malaria, the risk of postnatal HIV infection increased in a dose-dependent manner for children with one, two, or three or more clinical malaria episodes. Likewise, a non-statistically significant trend of

higher infection risk was evident for categorical comparisons of children with one or two or more versus zero confirmed malaria episodes. These associations were robust to adjustment for several indicators of maternal health status, socio-demographic characteristics, and child cotrimoxazole compliance and child baseline immunologic status (Table 2).

**Table 2. Child Malarial morbidity in relation to postnatal HIV infection among HIV-exposed infants of HIV-positive women from Dar es Salaam, Tanzania**

| | Univariate Model Hazard Ratio (95% CI) | Multivariable Model Hazard Ratio (95% CI) |
|---|---|---|
| **Child Malarial Morbidity** | | |
| ***Ever vs. Never Infection*** | | |
| Clinical Malaria | 1·56 (1·02, 2.39) | 1·73 (1·11, 2·69) |
| Probable Malaria | 1·73 (1·05, 2·85) | 2·00 (1·17, 3·42) |
| Confirmed Malaria | 1·40 (0·51, 3·84) | 2·09 (0·74, 5·91) |
| ***Per episode increment*** | | |
| Clinical Malaria | 1·31 (1·03, 1·67) | 1.39 (1·08, 1·80) |
| Probable Malaria | 1·35 (0·91, 1·99) | 1·52 (1·00, 2·32) |
| Confirmed Malaria | 1·43 (0·65, 3·17) | 2·10 (0·91, 4·87) |
| ***Number of Episodes*** | | |
| Clinical Malaria | | |
| 1 vs. 0 | 1·46 (0·90, 2·38) | 1·66 (1·01, 2·72) |
| 2 vs. 0 | 1·62 (0·79, 3·32) | 1·64 (0·77, 3·45) |
| 3+ vs. 0 | 2·25 (0·88, 5·73) | 2·86 (1·07, 7·65) |
| Probable Malaria | | |
| 1 vs. 0 | 1·97 (1·17, 3·31) | 2·20 (1·27, 3·82 ) |
| 2+ vs. 0* | 0·81 (0·20, 3·32) | 1·07 (0·25, 4·52) |
| Confirmed Malaria | | |
| 1 vs. 0 | 1.20 (0·38, 3·81) | 1·71(0·53, 5·59) |
| 2+ vs. 0* | 2·84 (0·39, 20·84) | 6·87(0·89, 59·86) |

*Estimates are derived from Cox Proportional Hazards models where time to HIV infection is the dependent variable. The relative hazard of HIV incidence is estimated. CI = Confidence Intervals. In addition to the covariates shown above, the multivariable model adjusted for: child sex, micro-nutrient supplementation vs. Placebo, birth weight, per-capita household daily food expenditure, maternal age, maternal education, maternal history of neonatal mortality and child anemia status. All covariates are set to their values at the beginning of respective intervals.*

**:Few children were diagnosed with three or more probable or confirmed malaria episodes while breastfeeding; therefore highest exposure category consists of two or more malaria episodes.*

There was no relationship between postnatal HIV infection and child micronutrient supplementation (HR=0.98; 95%CI:0.64-1.49) or child baseline CD4%<25 (HR=1.28; 95%CI:0.77-2.14) level. Likewise, there was no association between child postnatal HIV infection risk and the following indicators of maternal health status: maternal malaria, maternal anemia status, or maternal ARV use (Table 3). However, poor maternal immunologic status, as indicated by CD4 T-cell count <350 vs. ≥350 cells/µl, was associated with a nearly three times higher risk of postnatal HIV infection. In comparison, current maternal ARV use exhibited a non-statistically protective association with child postnatal HIV infection (Table 3). We found no evidence of heterogeneity in the association between child malarial morbidity and postnatal HIV infection by maternal or child immunologic status, anemia, micronutrient supplementation, maternal enrolment in the pre vs. post-PEPFAR era, or child cotrimoxazole compliance.

**Table 3. Maternal health status indicators in relation to postnatal HIV infection among HIV-exposed infants of HIV-positive women from Dar es Salaam, Tanzania**

| Maternal Health Indicators | Univariate Model Hazard Ratio (95% CI) | Multivariate Model Hazard Ratio (95% CI) |
|---|---|---|
| Ever vs. never clinical malaria | 1·23 (0·78, 2·00) | 1·03 (0·59, 1·79) |
| Current vs. never anemia | 1·39 (0·91, 2·13) | 1·14 (0·62, 2·10) |
| CD4 <350 vs. >= 350 cells/uL | 3·45 (2·28, 5·24) | 2·82 (1·75, 4·54) |
| Current vs. never ARV use | 1·06 (0·49, 2·30) | 0·62 (0·28, 1·42) |

*Estimates are derived from Cox Proportional Hazards models where time to child HIV infection is the dependent variable. The relative hazard of HIV incidence is estimated. CI = Confidence Intervals. In addition to above covariates, the multivariable model adjusted for: child malarial morbidity, child anemia, child sex, micro-nutrient supplementation vs. placebo, birth weight, per-capita household daily food expenditure, maternal age, maternal education and maternal history of neonatal mortality. All covariates are set to their values at the beginning of respective follow-up visits.*

## 4. DISCUSSION

We provide the first prospective evidence, to our knowledge, of an association between child clinical and probable malarial morbidity and subsequent risk of postnatal HIV infection among breastfeeding infants of HIV-positive women at high risk of acquiring both infections. The risk of HIV infection increased by between 39% and 52%, and may possibly be as high as 110%, for each additional episode of malaria. We found evidence of a dose-dependent rise in risk of postnatal HIV infection for children with clinical and confirmed malaria relative to children without malaria. Except for low maternal immunologic status, which was associated with higher risk of postnatal HIV infection, other indicators of maternal health, including maternal malaria, did not independently predict child postnatal HIV infection.

Our findings are consistent with the results of two epidemiologic studies conducted in Kenya. The first, a simulation study of the consequence of extensive HIV and malaria overlap in SSA, concluded that malaria and HIV interact non-additively and increase the burden of both diseases in the region [2]. The second, an ecological study, found twice the odds of being HIV-infected for adults who reside in high vs. low *P. falciparum* prevalence areas[24].

*In vitro* studies of immune response to malaria and HIV infections provide biologically plausible mechanisms in support of our finding that HIV-exposed sero-negative infants could be at higher risk of HIV infection following bouts of malaria. First, CD4 T-cells, in which HIV preferentially replicates, have a dual role in adaptive anti-malarial immune response, starting with initial elaboration of pro-inflammatory cytokine expression[25,26] and transitioning to expansion of counter-regulatory anti-inflammatory cytokines through interaction with B-cells to minimize the risk of developing complicated malaria[27]. The up-regulation of TNF-$\alpha$ and other pro-inflammatory cytokines in response to acute malaria infections creates conditions favorable to HIV acquisition[28], as inflammatory cytokines act directly on the HIV long-terminal repeats to up-regulate viral replication[29,30]and to increase the population of cell types (macrophages) susceptible to HIV infection[31]. Second, *in-utero* HIV exposure has been associated with immune-dysregulations characterized by higher pro-inflammatory cytokine expression during infancy relative to HIV unexposed neonates[29]. The extent of immune-dysregulation is more profound for children whose mothers had detectable viral loads during pregnancy [32]. We speculate that our finding of malaria-associated risk of HIV infection in this sample is mediated through malaria-induced up-regulation of pro-inflammatory cytokine responses, a phenomenon that limits the capacity of infants with

malaria to mount an effective counter-regulatory anti-inflammatory response and, thereby, creates conditions favorable for HIV acquisition. Specific future studies are needed to confirm or refute our findings and to elucidate the proximate mechanisms involved.

The key strengths of this study include its large sample size, prospective cohort design, an analytic approach that restricted the relevant etiologic period for all children by breastfeeding duration, and our ability to make within-person comparisons and have a tight control for important baseline and time-varying confounders: breastfeeding duration, maternal and child immunologic and anemia status, child cotrimoxazole compliance, nutritional status, low birth weight, and socio-demographic characteristics. A major limitation of this study is the use of physician-diagnosed clinical malaria. This definition of malarial morbidity, although reflective of clinical practice in the area, overestimates malarial morbidity. We attempted to address this limitation through sensitivity analyses that used progressively stringent malaria diagnostic criteria. However, the number of malaria events declined steadily as diagnostic specificity increased, which resulted in a limited power to detect associations between confirmed child malaria episodes and postnatal HIV infection. This lack of precision is reflected in the widening of confidence intervals for the most rigorous malaria diagnostic category.

Another important limitation pertains to the 6-monthly timing of HIV tests, which made it difficult to determine with precision the exact timing of HIV infection and raised the potential of reverse causality for the malaria-HIV association. We addressed this issue analytically as follows: (1) We restricted observations for malaria assessment in all infants regardless of eventual HIV-infection status to visits that occurred on or before the end of breastfeeding; and (2) We censored observations for HIV-infected children to the earlier of either the blood sample acquisition date or the end of breastfeeding plus literature informed lag time for detection of HIV-specific immune response. For example, for a given postnatally HIV-infected infant, if the first sample that became HIV-positive was drawn at month 12, but the infant was weaned at 5 months, the infant's effective HIV-positive date for analytic purposes was age at weaning plus HIV sero-conversion window. We acknowledge these limitations, which warrant cautious interpretation of our findings and stress the importance of future, specifically-designed epidemiologic studies that use laboratory-confirmed malaria or malaria rapid diagnostic tests to confirm or refute our findings that malarial morbidity may increase the risk of HIV-acquisition among HIV-negative persons at risk of both HIV and malaria in co-endemic settings. In spite of highlighted weaknesses, the internal consistency of our results across diagnostic categories and their robustness to simultaneous control for a range of confounders is suggestive of a possibly important role for malaria in HIV-acquisition. It remains important to re-examine this hypothesis in a malaria endemic region among malaria and HIV at risk populations.

## 5. CONCLUSION

We have shown that child clinical and probable malarial morbidity may be independent risk factors for postnatal HIV infection in this cohort of breastfeeding children HIV-negative at 6 weeks, born to HIV-infected women. Both HIV-exposed and HIV-infected infants should be actively evaluated for malaria infection and treated in a timely manner, when indicated as part of routine medical care. The adoption of increased clinical vigilance, rather than passive reliance on manifestation of malaria-related symptoms, which is currently the norm, will complement malaria prevention strategies already in place. These strategies include consistent use of insecticide-treated bed nets and malaria chemoprophylaxis of at-risk individuals in regions of unstable malaria transmission by the timely removal of important

sources of systemic inflammatory responses that favor HIV acquisition in malaria and HIV co-endemic settings.

## CONSENT AND ETHICAL APPROVAL

Ethical clearance for the conduct of this 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 consents for their own and their child's participation in the trial.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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

## ROLE OF FUNDING SOURCE

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 had the final responsibility for the decision to submit for publication.

## COMPETING INTERESTS

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.

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