Malaria Parasitemia and CD4 T Cell Count, Viral Load, and Adverse HIV Outcomes Among HIV-Infected Pregnant Women in Tanzania

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Abstract. We examined the cross-sectional relationships between malaria parasitemia and CD4 T cell count and viral load among human immunodeficiency virus (HIV)-infected pregnant women. We then followed women to investigate whether or not baseline parasitemia predicted CD4 T cell counts or viral loads >90 days post-baseline or predicted time to HIV disease stage 3 or 4 or acquired immune deficiency syndrome (AIDS)-related death (ARD). Parasitemia level was nonlinearly associated with viral load at baseline and among measurements taken >90 days post-baseline; women with low baseline parasitemia, versus none, had higher viral loads at both time points. Any baseline parasitemia predicted an increased rate of ARD among women with baseline CD4 T cell counts ≥500 cells/μL (ratio rate [RR] = 2.6; 95% confidence interval [CI] = 1.1–6.0; P test for heterogeneity = 0.05). Further study is warranted to determine whether or not parasitemia is especially detrimental to individuals with lower levels of immunosuppression or chronic low parasitemia.

INTRODUCTION

Since 2003, great strides have been made in the provision of life-saving antiretroviral therapy (ART) to individuals who are infected with human immunodeficiency virus (HIV) in sub-Saharan Africa. In spite of these efforts, recent estimates suggest that approximately two-thirds of HIV-infected individuals in need of ART do not receive it. Furthermore, the majority of adults living with HIV has not reached advanced stages of disease and therefore, may not be eligible for ART according to current treatment guidelines. The identification of modifiable risk factors for HIV disease progression and death will inform interventions to reduce the incidence of these events and prolong the time before ART is needed.

Worldwide, millions of individuals living with HIV reside in areas where malaria is endemic, and malaria is a leading cause of morbidity among individuals infected with HIV in sub-Saharan Africa. Growing evidence suggests a detrimental synergy between these two infectious diseases. Among adults living in malaria-endemic areas, HIV-related immune suppression seems to increase vulnerability to malaria parasitemia (henceforth, parasitemia) or clinical malaria. Malaria may also facilitate HIV replication through incitement of cytokine production and immune-cell activation. An increased rate of HIV replication that leads to a lasting elevation in viral load could, in turn, hasten immune system decline and HIV disease progression. Previous studies among HIV-infected individuals have reported an association between elevated viral load and malaria infection or clinical malaria. At present, there is insufficient evidence to determine whether or not malaria-induced changes in CD4 T cell counts or viral loads translate to an increased rate of HIV disease progression or death in areas of stable malaria transmission.

In this paper, we examined the relationship between baseline parasitemia and HIV-related outcomes in the context of a randomized micronutrient supplementation trial conducted among HIV-infected pregnant women in Dar es Salaam, Tanzania. First, we studied whether or not malaria parasitemia was associated with CD4 T cell count or viral load in cross-sectional analysis. We then followed women to investigate whether or not baseline parasitemia predicted CD4 T cell counts or viral loads >90 days post-baseline or predicted time to HIV disease stage 3 or 4 or acquired immune deficiency syndrome (AIDS)-related death (ARD). We also examined if the relationship between malaria parasitemia and these outcomes varied according to the baseline level of host immunosuppression.

METHODS

Study population and location. The study population consisted of 1,078 HIV-infected pregnant women who enrolled in a double-blind, placebo-controlled randomized trial at a participating antenatal clinic in Dar es Salaam, Tanzania, to examine the effects of daily micronutrient supplements on HIV disease progression and mortality. Trial results and a detailed description of the trial design have been published elsewhere. Ethical approvals for the trial were obtained from Research and Publications Committee of Muhimbili University College of Health Sciences, the Ethical Committee of the Tanzania National AIDS Control Program, and the Institutional Review Board of the Harvard School of Public Health. Informed consent was obtained from all women. The study enrollment took place from April 1995 through July 1997, a time during which women did not have access to ART in Tanzania. Women were followed until August 2003. Malaria is endemic in Dar es Salaam, and stable transmission occurs all year. The national annual incidence of malaria disease was estimated to be 400–500 per 1,000 in the general population in 2003. Bed nets were reported to have been used by all or some children in an estimated 52% of urban Tanzanian households in 1999; however, only a small percentage of these bed nets (10%) had been previously treated with insecticides.

Exposure and covariate assessment. After randomization, women completed baseline interviews regarding socio-demographic characteristics and medical history, and they were asked to provide blood, stool, and vaginal-fluid specimens for...
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detection of malaria parasites, intestinal parasites, and sexually transmitted infections. Follow-up consisted of monthly clinic visits throughout pregnancy and thereafter for a minimum of 2 years. To diagnose malaria, thick and thin blood films were air-dried and stained with 5% Giemsa at pH 7.2 for 20 minutes. Presence of asexual *Plasmodium falciparum* malaria parasites was determined by microscopic examination of stained slides. A slide was considered negative when no parasites were detected in the process of counting 200 leukocytes on a blood film. Quality control for smear microscopy was ensured through multiple mechanisms. First, known negative and positive control slides were included in every microscopic examination of stained slides. Second, all results from microscopic examination of stained slides were verified by a second testing laboratory technologist, and any discrepant results were resolved by a third senior laboratory technologist. The study laboratory also participated in the World Health Organization (WHO)/National Institute for Communicable Diseases (NICD) proficiency testing program. Women with malaria parasites and other infections received treatment, free-of-charge, in accordance with the Tanzania Ministry of Health treatment guidelines. Chloroquine was the first-line drug for treatment of uncomplicated malaria until August 2001, when it was changed to sulfadoxine/pyrimethamine because of high levels of treatment failure with chloroquine.23

**Outcomes assessments.** A blood specimen was requested at baseline and every 6 months thereafter for the enumeration of CD4 T cell counts using the FACSCount system (Becton Dickinson, San Jose, CA). For a random sample of 415 women, plasma viral load was quantified at a minimum of one time point using the Roche Amplicor HIV-1 monitor version 1.5 assay (Roche Diagnostics Corp., Indianapolis, IN), which has a detection limit of 400 copies/mL. For these analyses, results below this limit (1.6% of all viral load results) were assigned a value of 0.20: maternal age, gestational age, body mass index (BMI; kg/m²), mid-upper arm circumference, year of recruitment, WHO HIV disease stage, primiparity, medical antecedents, presence of coprevalent parasitic and sexually transmitted infections, and socio-demographic characteristics (education level, marital status, per person daily food expenditure, and reliance on others for financial support). We did not consider multivitamin use as a potential confounder in this study, because we expected that randomization would have yielded comparable exposure to the multivitamin interventions for women with and without baseline parasitemia. Final multivariable models included all potential confounders that changed the effect estimate by ≥ 10% in either direction as well as other established risk factors for the outcomes. Viral loads were transformed to the log₁₀ scale. The missing-indicator method was used to account for missing covariate data. We excluded extreme outlying values for log₁₀ viral load and CD4 T cell counts as well as values for individuals with outlying within-person standard deviations for these outcomes. Because we hypothesized that the effect of parasitemia on all outcomes could differ according to host immunological status, we stratified each final model by baseline CD4 T cell count group (<200 cells/μL, 200–499 cells/μL, ≥500 cells/μL) and tested for heterogeneity across strata using Cochran’s Q test.

**RESULTS**

Because they lacked a baseline malaria slide result, 12 of 1,078 women who enrolled in the study (1.1%) were excluded from these analyses. Of the remaining 1,066 women, 199 (18.7%) had slide-confirmed parasitemia at the time of study enrollment. Table 1 displays baseline characteristics of the study participants. Women with and without parasitemia were similar; however, women with parasitemia tended to be primiparous (prevalence ratio [PR] = 1.3; 95% confidence interval [CI] = 1.1–1.6; *P* value = 0.01), depend entirely on others for financial support (PR = 1.1; CI = 1.02–1.2; *P* = 0.02), and have hookworm infection (PR = 1.6; CI = 1.1–2.3; *P* = 0.01).

**Cross-sectional analyses.** Among 408 women with baseline viral load and parasitemia measurements, the presence of any parasitemia was associated with a 0.34 greater log₁₀ viral...
load (CI = 0.17–0.52; P < 0.0001; Table 2). Baseline CD4 T cell count results were available for 1,008 women; however, two measurements with outlying values were excluded. The association between the presence of any parasitemia and CD4 T cell count was statistically significant only for the subset of women with a baseline value ≥ 500 cells/μL (Table 2). Among this group, parasitemia was associated with a lower CD4 T cell count by 51 cells/μL (CI = −86.6–14.3; P = 0.006; P test for heterogeneity = 0.03). Parasitemia category was not associated with baseline CD4 T cell count (P = 0.24) or baseline CD4 T cell count group (P = 0.95).

CD4 T cell count and viral-load measurements taken > 90 days after baseline. A total of 674 viral-load measurements taken > 90 days after baseline (median = 386 days; interquartile range [IQR] = 182–828) were available for 289 women (median number of measurements per person = 3; range = 1–5). The association between any baseline parasitemia and viral loads after 90 days was not statistically significant (0.19; CI = −0.03–0.40; P = 0.24) or baseline parasitemia, whereas women with higher levels of baseline parasitemia tended to have lower CD4 T cell counts compared with those without parasitemia, whereas women without parasitemia showed CD4 T cell counts that were comparable or slightly higher compared with women without parasitemia (P likelihood ratio test < 0.0001).

Cross-sectional analyses of baseline malaria parasitemia and baseline viral loads and CD4 T cell counts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Log_{10} viral load*</th>
<th>CD4 T cell count*</th>
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<td>No parasitemia</td>
<td>330/408</td>
<td>Referent</td>
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<tr>
<td>Any malaria parasitemia</td>
<td>78/408</td>
<td>0.34 (0.17–0.52)</td>
</tr>
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<td>Low parasitemia</td>
<td>330/408</td>
<td>Referent</td>
</tr>
<tr>
<td>Medium parasitemia</td>
<td>330/408</td>
<td>Referent</td>
</tr>
<tr>
<td>High parasitemia</td>
<td>330/408</td>
<td>Referent</td>
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Table 2

Cross-sectional analyses of baseline malaria parasitemia and baseline viral loads and CD4 T cell counts

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Difference (95% CI)</th>
<th>P value</th>
<th>n/N</th>
<th>Difference (95% CI)</th>
<th>P value</th>
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<td></td>
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</table>

*Models include the following baseline variables: age in years (continuous), previous history of tuberculosis (yes/no), completely dependent on others for financial support (yes/no), gestational age in weeks at study entry (continuous), primiparous (yes/no), low daily per capita food expenditure (yes/no), WHO HIV disease stage (continuous categories: stage 1, 2, or ≥ 3), mid-upper arm circumference (continuous), and missing indicator variables when needed.

† Number of women with corresponding parasitemia status/total number of women.

§ CI = confidence interval.

¶ Low parasitemia < 1,000 parasites/μL; medium: 1,000–10,000 parasites/μL; high: > 10,000 parasites/μL.

‖ P value for likelihood ratio test.

** P value for test of heterogeneity across strata of CD4 T cell count.
parasitemia and progression to HIV disease stage 3 or 4 (Table 4). Overall, parasitemia category did not predict the rate of ARD (P likelihood ratio test = 0.87); however, we observed an elevated mortality rate of borderline statistical significance among individuals with low baseline parasitemia compared with those with no parasitemia (rate ratio [RR] = 1.6; CI = 0.99–2.6; P = 0.05). A statistically significant association between parasitemia and ARD was also observed among women with a baseline CD4 T cell count ≥ 500 cells/μL (RR = 2.6; CI = 1.1–6.0; P = 0.03; P test for heterogeneity = 0.05). Baseline parasitemia did not predict non-ARD among this group (RR = 0.74; CI = 0.08–6.5; P = 0.78).

**DISCUSSION**

Consistent with previous reports, we observed a positive cross-sectional association between parasitemia and viral load among HIV-infected pregnant women. Although we did not find strong evidence in support of an overall association between parasitemia and progression to HIV disease stage 3 or 4 or ARD, the rate of ARD was elevated among two subgroups: HIV-infected individuals with lower levels of immunosuppression and those with low parasitemia. The association between parasitemia and ARD in women with baseline CD4 T cell counts ≥ 500 cells/μL was statistically significant, whereas the relationship between low parasitemia, versus none, and ARD was of borderline statistical significance.

This paper is not the first to report that malaria may be especially detrimental to individuals with higher CD4 T cell counts. In a cohort study in Malawi, Kublin and others 17 found that the harmful association between malaria episodes and viral load was exacerbated for individuals with CD4 T cell counts > 300 cells/μL. The authors proposed that this was a function of relatively more T cells available for HIV viral replication after cytokine simulation by malaria parasites among individuals with higher CD4 T cell counts. 17 Consistent with these findings, in Uganda, Whalen and others 29, 30 observed that the influence of tuberculosis on viral load and mortality was greatest among HIV-infected adults with higher CD4 T cell counts. 29, 30 In this study, we observed statistically significant heterogeneity in the associations between parasitemia and baseline CD4 T cell count and ARD according to CD4 T cell group. For both outcomes, parasitemia seemed most detrimental to women with T cell counts ≥ 500 cells/μL. Similar trends were observed for the associations between baseline parasitemia and viral load and CD4 T cell counts > 90 days post-baseline; however, the heterogeneity was not statistically significant.
We also noted an elevated rate of death of borderline statistical significance among individuals with low baseline parasitemia. It is possible that this is a chance finding; however, it may be that a low baseline parasitemia level, one consequence of acquired malaria immunity that results from repeated malaria infections in endemic settings, is a proxy for a high level of exposure to malaria parasites. Women with frequent exposure to malaria parasites may be at greater risk for maintaining chronic parasitemia and consequently, an elevated viral load. This would be especially true if malaria infection in this group tended to be asymptomatic and left untreated. Asymptomatic infection has been shown to be associated with lower parasitemia levels. This hypothesis is consistent with the harmful cross-sectional relationship that we observed between low parasitemia and viral load at baseline but values similar to those with no baseline parasitemia had elevated viral load. Any parasitemia, rather than symptomatic malaria illness, was a weaker association between malaria and viral load when infection in the absence of clinical malaria. One study reported that parasitemia may be important. In their study, individuals who were cured of malaria 45 days after a clinical episode. If parasitemia alone influences viral load or CD4 T cell count, interventions to prevent and interrupt chronic parasitemia, which may not be clinically evident, may improve HIV-related health outcomes among HIV-infected individuals. Such interventions would include those already recommended by the WHO, including the habitual use of insecticide-treated bed nets; however, additional interventions, such as intermittent malaria prophylaxis for HIV-infected individuals, should be evaluated.

Longitudinal analyses were limited by the lack of routinely conducted post-baseline malaria blood smears. Lack of measurement of the duration of parasitemia during the follow-up period may have attenuated effect estimates for the relationship between parasitemia and the study outcomes. We, therefore, cannot discount an association between parasitemia and the prospective outcomes for which we observed null effect estimates or effect estimates that lacked statistical significance. A second potential limitation relates to the stratification of analyses by the baseline CD4 T cell count group. A transient influence of parasitemia on baseline CD4 T cell counts among individuals with higher baseline CD4 T cell counts may have led to some misclassification of baseline immunological status for stratified analyses. The result of this misclassification would be the addition of relatively healthier people (i.e., less immunosuppressed in the absence of malaria) to the strata of individuals with CD4 T cell counts ≥ 200 and < 500 cells/μL. This study was conducted in an era when HIV-infected women did not have access to ART in Tanzania; therefore, these results are not able to be generalized to other populations.
individuals receiving ART. Although women were pregnant during baseline measurements, follow-up extended beyond pregnancy and therefore, results of prospective analyses should be generalized to parous HIV-infected, ART naïve women living in areas with endemic malaria.

In summary, these findings are consistent with a detrimental relationship between malaria and CD4 T cell count and viral load. Further study is warranted to confirm these findings and elucidate the relationship between chronic asymptomatic parasitemia and long-term adverse HIV-related outcomes, such as disease progression and ARD, particularly among HIV-infected individuals with lower levels of immunosuppression.

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