HIV INFECTION, MALARIA, AND PREGNANCY: A PROSPECTIVE COHORT STUDY IN KIGALI, RWANDA

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Abstract. In order to study the relation between human immunodeficiency virus (HIV) infection and malaria in women, during and after pregnancy, a prospective cohort study was initiated at the Centre Hospitalier de Kigali in Rwanda through routine voluntary and confidential HIV screening in antenatal clinics. At inclusion in the cohort of all HIV-positive and an equivalent number of HIV-negative pregnant women, between 21 and 28 weeks of gestation, sociodemographic characteristics and medical history during the current pregnancy were collected; screening for malaria (tick blood smear) and anemia and a CD4 lymphocyte count were systematically performed. Each woman enrolled had a monthly follow-up until 6 months after delivery. A clinic was implemented that was accessible and free of charge to every woman during the study period between scheduled visits. Malaria infection was systematically screened in case of fever or other compatible symptoms. The cohort included 228 HIV-positive and 229 HIV-negative women. At inclusion, malaria prevalence was 8.0% in HIV-positive women and 3.5% in HIV-negative women (P < 0.04). Over the study period, the incidence of malaria was 6.2 per 100 women-months in the HIV-positive group and 3.5 in the HIV-negative group (relative risk [RR] = 1.7, 95% confidence interval [CI] = 1.4–2.3). The bulk of the difference occurred postpartum. The Kaplan-Meier 9-month probability of remaining free of malaria infection was 51.8% in HIV-positive women and 65.2% in HIV-negative women (P = 0.013). When taking account in the same multivariate model (including HIV infection, primiparity, CD4 lymphocytes, anemia, and education level), positive HIV serostatus remained the only factor significantly associated with malaria infection (RR = 1.4, CI = 1.1–1.6; P = 0.016). Our study prospectively documents the association between malaria and maternal HIV infection and highlights the increased risk of malaria occurrence in all HIV-infected women. Strategies to reduce the malaria morbidity during pregnancy should be reinforced in areas of high HIV seroprevalence.

INTRODUCTION

In sub-Saharan Africa, human immunodeficiency virus (HIV) infection is one of the most recent endemic infectious diseases. On the other hand, Plasmodium falciparum malaria is one of the most ancient and prevalent infectious diseases. The World Health Organization (WHO) estimates that by 1994, cumulatively over 14 million people in Africa had been infected by HIV.1 Heterosexual transmission is predominant on this continent, and most of the HIV-infected women are in their reproductive years, leading to serious adverse outcomes for pregnancy.2 Issues such as the interaction between HIV disease, pregnancy, and malaria deserve special attention. It has been estimated that between 270 and 480 million clinical malaria cases may occur every year.3 P. falciparum is estimated to cause between 1.4 and 2.6 million deaths per year in Africa, and more than 50% of women have malaria infection during pregnancy.4,5 During pregnancy, risks of maternal anemia, abortion, stillbirth, prematurity, and low birthweight are increased by malaria infection.4,5

In areas of high malarial transmission, humoral and cell-mediated immunity to the parasite is impaired in pregnant women, especially in primigravidas.6 HIV reduces the host’s immune response to infectious agents. Most studies, however, suggest that there is no significant biological relation between HIV infection and P. falciparum malaria in adults and children.2,40 Recently, studies have found an interaction between malaria and HIV infection during pregnancy and a possible role of placental malaria infection in increasing the risk of vertical transmission of HIV.11–15

The aim of the present report is to estimate the incidence of malaria infection during pregnancy and the postpartum period and to examine the relationship between HIV and malaria infections in Kigali, the capital city of Rwanda.

SUBJECTS AND METHODS

Setting. A prospective cohort of pregnant women was enrolled at the Maternal and Child Health Clinic of the Centre Hospitalier de Kigali in Rwanda between July 1992 and August 1993. The general aim of this study was to evaluate the effect of HIV-1 infection on pregnancy. The main objectives were, first, to document pregnancy outcomes after screening and treating common genital infections, and second, to study the maternal postpartum complications in HIV-positive in comparison to HIV-negative women. Approval for conducting the study was obtained from the Rwandan Ministry of Health. Details about the counseling and HIV-testing procedures and the main findings of the study have been given elsewhere.16,17

In brief, all pregnant women attending the clinic between 21 and 28 weeks of gestation according to ultrasound examination who consented to participate were systematically offered HIV antibody screening by 2 commercial enzyme-linked immunosorbent assays (ELISA; Vironostika HIV Mixt and Vironostika Uniform, Organon Tecknika, Boxtel, The Netherlands). Discordant samples by ELISA were confirmed by a commercial Western blot technique (DuPont de Nemours, Wilmington, DE) using the Centers for Disease Control criteria of interpretation.18 Pre- and posttest counseling was systematically offered in the local language (kinyarwanda).
Thrombocytopenia (%)
Positive thick smear (%)
History of fever during the current pregnancy (%)

Inclusion procedures. Two weeks after the HIV screening test, all HIV-positive women and an equivalent number of HIV-negative women were enrolled in the cohort. Baseline information on age, parity, obstetrical and medical history, and socioeconomic characteristics was collected on standardized questionnaires. A clinical examination was performed by a study physician. Physicians, nurses, and laboratory technicians were blinded to HIV serostatus throughout the study period, except when necessary for medical decisions aimed at improving the management of HIV infection.

A CD4 and CD8 lymphocyte count was performed using a commercial immunomagnetic method (Dynabead T4-T8 Quant., Biosys, France). Hemoglobin (Hb) measurement was performed, and a Hb level of less than 11 g/dL was defined as anemia. Iron supplementation was given when anemia was diagnosed. A platelet measurement of less than 150,000/mm³ was defined as thrombocytopenia.

The diagnosis of malaria infection was based on the recognition of parasites in a thick blood smear film stained with Giemsa. No distinction was made between the different malarial species, *P. falciparum* being by far the most frequent malarial parasite in Kigali. According to national guidelines, standardized treatment with quinine (25 mg/kg) was offered when malaria infection was diagnosed. No antimalarial prophylaxis was recommended.

Follow-up procedures. Until delivery, each woman enrolled had a systematic monthly follow-up. A minimum of 2 antenatal visits were performed. After delivery, mothers were followed every month until 6 months postpartum.

At each antenatal and postpartum visit, information on occurrence of symptoms, illnesses, and treatments taken since the last visit to the project were collected on standardized questionnaires. The project clinic was accessible free of charge to every woman between scheduled visits during the entire study period. Malaria infection was systematically screened in case of fever or other compatible symptoms.

Statistical methods. In each group (HIV-positive and HIV-negative), we estimated the incidence density rate of all episodes of malaria infection and expressed it as the number of events per 100 women-months of follow-up. We then estimated the probability of developing the first episode of malaria infection during the follow-up by the Kaplan-Meier technique. Statistical comparisons between groups were performed with appropriate tests with a significance level of 5%. Relative risks (RR) and odds ratios (OR) were computed and reported with their 95% confidence intervals (CI) to study the association between explanatory variables such as HIV infection and the occurrence of the first episode of malaria. Multivariate analysis of the following factors was performed using the Cox proportional hazard regression method: HIV serostatus, primiparity, education level, CD4 lymphocyte count, and anemia.

RESULTS

Follow-up of the cohort was interrupted by the Rwandese civil war in April 1994. Complete data until 6 months postpartum are available for 457 women (228 HIV-positive and 229 HIV-negative) consecutively enrolled. They represent the study sample for the present report. Table 1 compares baseline data according to HIV serostatus. The 2 groups were comparable in terms of age, employment, history of fever during the current pregnancy, and mean parity. HIV-positive women were less often primiparous (OR = 0.5, CI = 0.3–0.8; *P* = 0.001) and educated (OR = 0.6, CI = 0.4–0.8; *P* = 0.002) than HIV-negative women.

At inclusion, a positive thick smear for *Plasmodium* was found in 8.0% of the 228 HIV-positive women and in 3.5% of the 229 HIV-negative women (OR = 2.4, CI = 1.0–6.1; *P* = 0.04). The prevalence of anemia was significantly higher among HIV-positive than HIV-negative pregnant women (OR = 1.7, CI = 1.1–2.8; *P* = 0.003). The proportion of thrombocytopenia did not differ between the HIV-positive and HIV-negative groups. The mean CD4 lymphocyte count was 553/mm³ (standard deviation [SD] = 300/mm³) in the HIV-positive group and 827/mm³ (SD = 330/mm³) in the HIV-negative group (*P* < 0.0001). The proportion of women with less than 200 CD4 lymphocytes/mm³ was 8.4% in the HIV-positive group and 5% in the HIV-negative group (*P* < 0.0001). The positive relation between HIV serostatus and prevalence of tick smear was not modified when taking into account anemia and CD4 count (data not shown).

The mean follow-up was 9.2 months (SD = 9.9) for HIV-positive women and 9.4 months (SD = 1.0) for HIV-negative women (*P* = 0.12), allowing the diagnosis of 205 episodes of malaria infection (129 in HIV-positive women and 76 in HIV-negative women). The incidence was therefore 6.2 per 100 women-months in the HIV-positive group and 3.5 per...
### Table 2

Incidence of malaria per 100 women-months during pregnancy and postpartum periods in 228 HIV-infected (HIV+) and 229 uninfected (HIV−) women in the cohort, Centre Hospitalier de Kigali, Kigali, Rwanda, 1992–1994 (N = 257)

<table>
<thead>
<tr>
<th>Time period</th>
<th>HIV+</th>
<th>HIV−</th>
<th>Relative risk*</th>
<th>95% Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion through delivery</td>
<td>227</td>
<td>229</td>
<td>1.1</td>
<td>0.7–1.8</td>
<td>.67</td>
</tr>
<tr>
<td>Inclusion to 1st prenatal visit</td>
<td>227</td>
<td>229</td>
<td>0.6</td>
<td>0.1–5.6</td>
<td>.44</td>
</tr>
<tr>
<td>1st prenatal to 2nd prenatal visits</td>
<td>221</td>
<td>220</td>
<td>1.0</td>
<td>0.1–7.9</td>
<td>.84</td>
</tr>
<tr>
<td>2nd prenatal to delivery</td>
<td>208</td>
<td>207</td>
<td>1.7</td>
<td>0.7–3.5</td>
<td>.26</td>
</tr>
<tr>
<td>Delivery through 6 months postpartum</td>
<td>191</td>
<td>196</td>
<td>2.2</td>
<td>1.5–3.2</td>
<td>.0001</td>
</tr>
<tr>
<td>Delivery to 3 months postpartum</td>
<td>191</td>
<td>196</td>
<td>1.9</td>
<td>1.1–3.3</td>
<td>.02</td>
</tr>
<tr>
<td>3 months to 6 months postpartum</td>
<td>156</td>
<td>153</td>
<td>2.5</td>
<td>1.5–3.9</td>
<td>.0001</td>
</tr>
<tr>
<td>Inclusion through 6 months postpartum</td>
<td>227</td>
<td>229</td>
<td>1.7</td>
<td>1.4–2.3</td>
<td>.0001</td>
</tr>
</tbody>
</table>

* HIV+ versus HIV−.

100 women-months in the HIV-negative group (RR = 1.7, CI = 1.4–2.3; P < 0.0001) between inclusion and 6 months postpartum (Table 2). The incidence was comparable between the 2 groups during the last trimester of pregnancy, but there was a significantly higher risk of malaria among HIV-positive women than among HIV-negative women in the postpartum period (Table 2).

Kaplan-Meier estimates of the risk of malaria infection during the study period were performed in 210 HIV-positive and 221 HIV-negative women, excluding the 18 HIV-positive and 8 HIV-negative women with a positive thick drop at inclusion. Malaria infection–free survival was 86.9% in HIV-positive and 89.2% in HIV-negative women after 3 months of follow-up; these probabilities dropped to 78.1% in HIV-positive and 81.3% in HIV-negative women at 6 months, and at 9 months, 51.8% of HIV-positive and 65.2% of HIV-negative women remained free of malaria infection (log-rank test, P = 0.013; Figure 1).

Taking into account 5 baseline variables with a P-value below 0.20 in univariate analysis, positive HIV serostatus remained the only variable significantly associated with occurrence of malaria infection during follow-up in the Cox proportional hazard regression model (RR = 1.3, CI = 1.1–1.7; P = 0.016; Table 3).

### DISCUSSION

Although women are estimated to represent more than half of the population of HIV-infected people worldwide and although malaria is one of the most prevalent infectious diseases in sub-Saharan Africa, longitudinal studies looking at the impact of malaria on HIV-infected pregnant women have only been reported from rural Malawi.11–14

In our study, the risk of developing malaria infection was 1.7 times higher for HIV-positive women than for HIV-negative women in the last trimester of pregnancy and in the first 6 months postpartum. The bulk of this difference occurred postpartum. Our findings are in agreement with the Malawi studies, which have shown a strong association between malaria and HIV infection in pregnant women.11–14 Steketee and others observed a prevalence and a mean density of parasitemia significantly higher in 152 HIV-positive

![Figure 1](image-url)  
**Figure 1.** Malaria infection-free survival time by Kaplan-Meier method from 21 to 28 weeks of gestation for the cohort, Centre Hospitalier de Kigali, Kigali, Rwanda, 1992–1994 (210 HIV-positive and 221 HIV-negative women).
infected women than in 2,601 HIV-negative women at 6 months of pregnancy and also at delivery.\textsuperscript{11} Bloland and others suggested that placental malaria infection heightens the risk of exposure to maternal HIV infection on postneonatal mortality (n = 2,608).\textsuperscript{12} Steketee and others suggested that HIV infection was a risk factor for parasitemia at antenatal clinics in 4,127 women.\textsuperscript{13,14} Our study allowed a cohort as-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hazard ratio</th>
<th>95% Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV serostatus</td>
<td>1.33</td>
<td>1.10–1.72</td>
<td>.016</td>
</tr>
<tr>
<td>Primiparity</td>
<td>1.08</td>
<td>0.90–1.15</td>
<td>.16</td>
</tr>
<tr>
<td>Education</td>
<td>1.04</td>
<td>0.66–1.73</td>
<td>.88</td>
</tr>
<tr>
<td>Lymphocytes CD4 count (&lt;200/mm(^3))</td>
<td>0.96</td>
<td>0.48–1.64</td>
<td>.89</td>
</tr>
<tr>
<td>Anemia at inclusion</td>
<td>1.06</td>
<td>0.81–1.29</td>
<td>.31</td>
</tr>
</tbody>
</table>

At inclusion in the cohort, the prevalence of positive thick drop was higher in HIV-positive than in HIV-negative pregnant women. Comparable observations have been made in pregnant women or other adults in Malawi,\textsuperscript{11,14} in Zaire,\textsuperscript{8} and in Tanzania.\textsuperscript{28} Recently, a significant association between HIV infection and malaria prevalence early in gestation has been reported independently of maternal age.\textsuperscript{15} In contrast, another study performed in Kigali, where the prevalence of positive thick drop was 9%, did not find a relationship between HIV infection and malaria.\textsuperscript{10} The fact that pregnant women in Allen and colleagues’ study\textsuperscript{10} lived in various areas of Kigali and only within the city limits in our study could explain this difference.

In our prospective design, the possibility that biases could explain the association between HIV and malaria infections was minimized. Diagnosis of malaria was done in a standard fashion for all study subjects and by a laboratory technician blinded to HIV serostatus. The consecutive enrollment of the cohort during a 12-month period limited the potential selection bias in relation to seasonality. The number of participants lost to follow-up was minimal, and follow-up procedures, including free and indiscriminate access to the medical center and reimbursement of transport, diagnosis, and treatment expenses led to unbiased estimates of incidence of malaria infection.

The mechanisms of the association between malaria and maternal HIV infection are potentially numerous. First, impaired cell-mediated immunity in HIV-infected women could influence the frequency and course of malaria infection, as HIV infection interferes with the maintenance of immune recognition of malaria.\textsuperscript{20,21} It has also been reported that CD4 count decreases during the third trimester in both HIV-infected and uninfected pregnant women and that it levels off after delivery in HIV-infected mothers, whereas it increases in uninfected mothers.\textsuperscript{22} This change of the immune status around the time of delivery could explain in our study the sustained risk of malaria in HIV-positive women in the postpartum period. Second, an impairment of the immune response to certain malaria antigens,\textsuperscript{23} a modification of the secretion of cortisol during pregnancy,\textsuperscript{24} and the role of genetic factors\textsuperscript{25} have also been suggested. Very recently, the role of \textit{P. falciparum} infection on modulation of HIV pathogenesis by activation of lymphocytes and stimulation of the viral replication through the production of cytokines has been hypothesized.\textsuperscript{26} The notion of a pyrogenic threshold for the occurrence of symptomatic malaria and the ability of cytokines to induce fever have also been underlined independently of HIV infection.\textsuperscript{27} A down-regulation of this py-

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