Placental Malaria and Perinatal Transmission of Human Immunodeficiency Virus Type 1

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Prevalence of placental malaria in human immunodeficiency virus (HIV) type 1–infected and –uninfected women and the effect of placental malaria on genital shedding and perinatal transmission of HIV-1 were examined. Genital samples for HIV-1 DNA RNA were collected during labor. Infants were tested for HIV-1 at 1 day and 6 weeks postpartum. Placental malaria was diagnosed by histopathological examination: 372 placentas of HIV-1–infected women and 277 of HIV-1–uninfected women were processed. A higher prevalence of placental malaria was seen in HIV-1–infected women. No association was found between placental malaria and either maternal virus load, genital HIV-1 DNA, or HIV-1 RNA. Placental malaria did not correlate with in utero or peripartal transmission of HIV-1.

A high prevalence of both human immunodeficiency virus (HIV) type 1 and malaria infections is found among pregnant women in sub-Saharan Africa. Although coinfection with HIV-1 and malaria must be common, few studies have investigated their potential interaction.

Hoffman et al. [1] have shown that, in nonpregnant adults, malaria infection increases HIV-1 load. Also, during pregnancy, the presence of malaria parasites was associated with a higher HIV-1 load [2, 3]. Studies of pregnant women in Malawi and Kenya suggest that HIV-1 infection reduces the ability to control malaria infections [4, 5]. Impaired cytokine responses, especially that of interferon-γ, to malaria antigens were found in placentas of HIV-1–infected women and may contribute to increased susceptibility to malaria [6]. The coexistence of maternal HIV-1 infection and placental malaria was also strongly associated with an increased risk of infant morbidity and mortality [7]. The authors hypothesized that this increased risk resulted from an increase in mother-to-child transmission (MTCT) of HIV-1, due to the presence of placental malaria. In placental tissue in vitro, malarial antigens have been shown to up-regulate cytokines (tumor necrosis factor–α) by placental blood mononuclear cells, resulting in increased replication of HIV-1 [8–10]. Additionally, placental malaria causes inflammatory changes by mononuclear cell infiltration in the intervillous space [11], causing a higher presence of HIV-1–presenting cells and leading to placental-tissue damage. A high maternal virus load and cervicovaginal shedding of HIV-1 have been associated with an increased risk of MTCT [12–14].

We hypothesized that an increased placental HIV-1 load, due to the presence of malaria parasites, might also be associated with increased excretion of HIV-1 in the genital tract, thus increasing the risk of MTCT. We conducted a study that aimed to (1) evaluate and compare the prevalence and correlates of placental malaria in HIV-1–infected and HIV-1–uninfected women, (2) analyze the effect of placental malaria on shedding of HIV-1 in genital-tract secretions during labor, and (3) evaluate the effect of placental malaria on MTCT of HIV-1.

Subjects, materials, and methods. A chlorhexidine vaginal-lavage study was conducted from March 1996 to April 1999 in the Maternity Unit of the Coast Provincial General Hospital (CPGH), Mombasa, Kenya [15]. Only women with uncomplicated labor ending in a spontaneous vaginal delivery of a singleton were included. Severe illness, such as malaria, was an exclusion criterion. The prevalence of HIV-1 in the selected population was 14%. Plasmodium falciparum is endemic in the region. Malaria prophylaxis during pregnancy is part of the Kenyan health policy. Ninety-nine percent of mothers breastfeed [15]. The present study was performed by use of materials and data from patients recruited in the nonintervention arm of the chlorhexidine study. No lavage was performed on the women analyzed in this report. No antiviral prophylaxis was given, because, at the time of the study, it was not available. The ethical committees of the CPGH and the University of Ghent approved the study, and study participants provided consent for their participation.

Details on sample collection and laboratory procedures have been published elsewhere [14, 15]. A sample of cervicovaginal secretions, for detection of HIV-1 DNA and RNA by polymerase...
chain reaction (PCR), was obtained during labor. A thick peripheral biopsy sample was obtained from the placenta and was fixed in 10% formalin. Venous blood samples for HIV-1 testing of mother and child were obtained at 1 day postpartum, after participants had received pretest counseling and provided informed consent to participate in the study. Maternal blood was also tested for syphilis (rapid plasma reagin [RPR] test) and hemoglobin, and maternal virus load was determined in a sub-sample of HIV-1–infected women. Infants were again tested for HIV-1 at a follow-up visit at 6 weeks postpartum. An infant was classified as having been infected in utero when PCR results were positive for HIV-1 at 1 day postpartum; an infant was classified as having been infected during delivery and/or early postpartum (peri-partum transmission) when PCR results were negative at 1 day postpartum but positive at 6 weeks postpartum [15]. Data on demographic and socioeconomic status of the mother and weight and physical examination of the infant were collected by use of a standard questionnaire.

The HIV-1 status of the mother was determined by ELISA (Detect-HIV; Biochem ImmunoSystems). A second HIV ELISA (Recobigen; Cambridge Biotech) confirmed the results for samples found to be positive by the screening ELISA performed in the local research laboratory.

Maternal HIV-1 load was determined in the reference laboratory in Ghent. Quantification was performed by use of the Cobas Amplicor HIV-1 Monitor assay (version 1.5; Roche Diagnostics).

The HIV-1 status of infants born to HIV-1–positive women was established by DNA PCR analysis in the AIDS Reference Laboratory, University of Ghent. HIV Pol primers described by Fransen et al. [16] and HIV Env primers described by Simmonds et al. [17] were used for amplification of the HIV-1–specific DNA found in Kenyan HIV-1 strains. Two positive HIV-1 PCR tests were necessary before a sample was considered to be positive.

HIV-1 DNA-PCR analysis of cervicovaginal samples was performed by use of the same procedure as described for the blood samples. Purified RNA was DNAs treated and amplified in a reverse-transcriptase (RT) PCR using the Pol primers and the Titan One Tube RT-PCR System (Roche Diagnostics).

The fixed placental biopsy samples were embedded in paraffin wax, were prepared, and were stained (Giemsa, hematoxylin-eosin, and periodic acid–Schiff staining) in the Histopathology Laboratory of the University of Antwerp. A total of 375 placenta from HIV-1–infected women, as well as 277 from HIV-1–uninfected women, were processed. The HIV-1–uninfected control subject was a woman who gave birth next to the HIV-1–infected woman. A pathologist, experienced in tropical parasitology, examined all the slides for the presence of malaria parasites, malarial pigment, placental morphology, and signs of infections other than malaria. Standardized questionnaires were used to report results. Quality control, performed by a second pathologist, confirmed our findings. We defined placental malaria as the presence of parasites and/or pigments in red blood cells or the presence of pigments in monocytes in the intervillous space of the placenta, which is, according to Bulmer et al. [18], classified as “active placental malaria.”

Epi-Info (CDC) was used for data entry, and SPSS (version 10.0; SPSS) was used for analysis. For the univariate analysis of categorical variables, Pearson’s χ² test or Fisher’s exact test was applied where appropriate. For continuous variables, we used the independent sample t test after checking normality and equality of variance, on the basis of Levine’s test, at 5% significance level.

Results. A total of 372 placentas (3 were discarded) from HIV-1–infected women were analyzed, as were 277 placentas from uninfected control subjects. The overall prevalence of (active) placental malaria was 7.1% (46/649). Data on placental malaria are presented in table 1. Women with placental malaria were more often primigravidas and were less frequently secundigravidas. Placental malaria was significantly associated with HIV-1 infection. Age, anemia, low birth weight, and RPR seroreactivity did not differ between the 2 groups.

The role of placental malaria in the transmission of HIV-1 to the infant was evaluated in the 372 mother-infant pairs. The prevalence of (active) placental malaria was 9.1% (34/372) in these HIV-1–infected women. Being primigravida was again significantly associated with the presence of placental malaria. The prevalence of placental malaria was significantly lower in women who were secundigravidas. No association was found between placental malaria and the maternal virus load. Also, no significant difference in age, anemia, low birth weight, or RPR seroreactivity could be observed in the presence or absence of placental malaria (table 2).

The relationship between placental malaria and genital-tract

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placental malaria positive (n = 603)</th>
<th>Placental malaria negative (n = 46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), years</td>
<td>4.3 (23.7)</td>
<td>22.6 (4.5)</td>
<td>.082</td>
</tr>
<tr>
<td>Anemia</td>
<td>374 (62.0)</td>
<td>31 (67.4)</td>
<td>.469</td>
</tr>
<tr>
<td>Severe anemia</td>
<td>79 (13.1)</td>
<td>9 (19.6)</td>
<td>.217</td>
</tr>
<tr>
<td>Syphilis RPR</td>
<td>22 (3.5)</td>
<td>5 (11.1)</td>
<td>.333</td>
</tr>
<tr>
<td>Primigravida</td>
<td>185 (30.8)</td>
<td>22 (47.6)</td>
<td>.017</td>
</tr>
<tr>
<td>Secundigravida</td>
<td>203 (33.8)</td>
<td>8 (17.4)</td>
<td>.022</td>
</tr>
<tr>
<td>Multigravida</td>
<td>213 (35.4)</td>
<td>16 (34.8)</td>
<td>.928</td>
</tr>
<tr>
<td>HIV-1 infected</td>
<td>338 (56.1)</td>
<td>34 (73.9)</td>
<td>.018</td>
</tr>
<tr>
<td>LBW</td>
<td>89 (14.9)</td>
<td>9 (19.6)</td>
<td>.400</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of subjects, unless otherwise noted. Anemia, hemoglobin <10 ng/dL; HIV-1, human immunodeficiency virus type 1; LBW, low birth weight (<2500 g); multigravida, ≥2 pregnancies; RPR, rapid plasma reagin; severe anemia, hemoglobin <8 ng/dL.
The prevalence of placental malaria was higher in HIV-1–infected women than in HIV-1–uninfected women (9.1% vs. 4.3%), a finding that is in agreement with results from other studies in Africa [5, 19]. It has been shown that this difference remains significant even when malaria prophylaxis is provided [19].

In the present study, active placental malaria was not associated with higher maternal virus load. In vitro experiments showed that higher placental virus load was seen when malaria antigen was present [8–10]. It might be that this local increase in virus concentration is not reflected in the systemic maternal virus load. In many studies, blood HIV-1 load remains the most important predictor for transmission [12, 13]. This would make acute maternal malaria infection around the period of delivery a risk factor for transmission. Genital shedding of HIV-1 remains, nevertheless, an independent predictor for transmission [12, 14, 22]. The hypothesis that active placental malaria would increase the rate of perinatal transmission, by higher levels of genital shedding of HIV-1, was, however, not confirmed by our results. The rate of peripartum transmission, measured at 6 weeks postpartum, was not significantly different for women with placental malaria, compared with those for women without placental malaria.

A weakness of the present study is that it was performed on biopsy samples from a previously completed trial that was not designed to focus on malaria infection. Therefore, no additional examinations could be performed while processing. We conclude that active placental malaria was more common in placentas of HIV-1–infected women, but our study could not show any relationship between placental malaria and MTCT of HIV-1. To be able to make final conclusions, prospective studies in which maternal parasitemia and HIV-1 load, placental and umbilical parasitemia, and placental and umbilical HIV-1 load are collected and correlated need to be performed.

Acknowledgments

We thank the Coast Provincial General Hospital Administration and the staff of the maternity unit, for their cooperation.

Table 2. Correlates of placental malaria in human immunodeficiency virus (HIV) type 1–infected women (n = 372).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placental malaria negative (n = 338)</th>
<th>Placental malaria positive (n = 34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), years</td>
<td>24.1 (4.2)</td>
<td>22.8 (4.8)</td>
<td>.096</td>
</tr>
<tr>
<td>Anemia</td>
<td>222 (65.7)</td>
<td>22 (64.7)</td>
<td>.909</td>
</tr>
<tr>
<td>Severe anemia</td>
<td>55 (16.3)</td>
<td>9 (26.5)</td>
<td>.133</td>
</tr>
<tr>
<td>Syphilis RPR</td>
<td>4.4</td>
<td>0</td>
<td>.210</td>
</tr>
<tr>
<td>Maternal HIV-1 load (SD), log10</td>
<td>4.0941 (0.9057)</td>
<td>4.1149 (0.9689)</td>
<td>.921</td>
</tr>
<tr>
<td>Primigravida</td>
<td>94 (28.0)</td>
<td>17 (50.0)</td>
<td>.008</td>
</tr>
<tr>
<td>Secondigravida</td>
<td>116 (34.5)</td>
<td>6 (17.6)</td>
<td>.048</td>
</tr>
<tr>
<td>Multigravida</td>
<td>37.5</td>
<td>11 (32.4)</td>
<td>.554</td>
</tr>
<tr>
<td>LBV</td>
<td>58 (17.4)</td>
<td>7 (20.6)</td>
<td>.645</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of subjects, unless otherwise noted. Anemia, hemoglobin <10 g/dL; LBW, low birth weight (<2500 g); multigravida, ≥2 pregnancies; RPR, rapid plasma reagin; severe anemia, hemoglobin <8 g/dL. *P* < .05 was considered to be significant.

sheding of HIV-1 during labor was examined in a subset of 224 mothers (224/372). DNA and RNA were detected by PCR in 56.6% (3 samples were inconclusive) and 24.5% of the maternal cervicovaginal secretions collected during labor, respectively. Shedding of HIV-1 in maternal genital secretions of HIV-1 DNA (60.0% vs. 56.2%; *P* = .745) and HIV-1 RNA (20.0% vs. 25.0%; *P* = .620) was not significantly different in the presence or absence, respectively, of placental malaria.

At 1 day postpartum, 20 of 372 infants born to HIV-1–infected women tested positive for HIV-1 by PCR, resulting in a rate of in utero transmission of 5.4%. Eighty-eight percent of the children (327/372) were also tested at 6 weeks postpartum; 64 of 324 (3 results were inconclusive) infants were positive for HIV-1 by PCR, which resulted in a total MTCT rate of 19.6%. Hence, 46/324 children were infected intrapartum and early postpartum (peripartum transmission rate, 14.2%).

In the present study, active placental malaria was not associated with higher maternal virus load.
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References


