INFECTION AND IMMUNITY, Jan. 2011, p. 298–304 Vol. 79, No. 1
0019-9567/11/$12.00 doi:10.1128/IAI.00814-10

Persistence of *Plasmodium falciparum* Parasites in Infected Pregnant Mozambican Women after Delivery▽

Elisa Serra-Casas,[1,2] Clara Menéndez,[1,2] Carlota Dobaño,[1,2] Azucena Bardají,[1,2] Llorençc Quintó,[1] Jaume Ordi,[1,3] Betuel Sigauque,[2,4] Pau Cisteró,[1] Inacio Mandomando,[2,4] Pedro L. Alonso,[1,2] and Alfredo Mayor[1,2]*

Barcelona Centre for International Health Research (CRESIB), Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Universitat de Barcelona, Barcelona, Spain[1]; Manhiça Health Research Centre (CISM), Maputo, Mozambique[2]; Department of Pathology, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain[3]; and National Institute of Health, Ministry of Health, Maputo, Mozambique[4]

Received 27 July 2010/Returned for modification 18 October 2010/Accepted 26 October 2010

Pregnant women are susceptible to *Plasmodium falciparum* parasites that sequester in the placenta. The massive accumulation of infected erythrocytes in the placenta has been suggested to trigger the deleterious effects of malaria in pregnant women and their offspring. The risk of malaria is also high during the postpartum period, although mechanisms underlying this susceptibility are not known. Here, we aimed to identify host factors contributing to the risk of postpartum infections and to determine the origin of postpartum parasites by comparing their genotypes with those present at the time of delivery. To address this, blood samples were collected at delivery ($n = 402$) and postpartum ($n = 354$) from Mozambican women enrolled in a trial of intermittent preventive treatment in pregnancy (IPTp). *P. falciparum* was detected by real-time quantitative PCR (qPCR), and the parasite merozoite surface protein 1 (*msp-1*) and *msp-2* genes were genotyped. Fifty-seven out of 354 (16%) women were infected postpartum as assessed by qPCR, whereas prevalence by optical microscopy was only 4%. Risk of postpartum infection was lower in older women (odds ratio [OR] = 0.34, 95% confidence interval [CI] = 0.15 to 0.81) and higher in women with a placental infection at delivery (OR = 4.20, 95% CI = 2.19 to 8.08). Among 24 women with matched infections, 12 (50%) were infected postpartum with at least one parasite strain that was also present in their placentas. These results suggest that parasites infecting pregnant women persist after delivery and increase the risk of malaria during the postpartum period. Interventions that reduce malaria during pregnancy may translate into a lower risk of postpartum infection.

Sequestration of erythrocytes infected by mature forms of *Plasmodium falciparum* is a characteristic feature of malaria infections (31). This phenomenon is thought to play a crucial role in the pathogenesis of severe malaria disease (22). Organ-specific sequestration of *P. falciparum* is particularly remarkable during pregnancy when massive numbers of infected erythrocytes accumulate in the intervillous spaces of the placenta (46). This situation, referred to as placental malaria, has been suggested to contribute to poor delivery outcomes (5). Placental tropism of the parasite is mediated through a specific *P. falciparum* erythrocyte membrane protein (PfEMP1) codified by *var2csa*, which mediates adherence to the placental receptor chondroitin sulfate A (CSA) (13, 36). Malaria susceptibility in pregnant women has been explained as a consequence of the lack of antibodies blocking adhesion of infected erythrocytes to placental CSA (14). However, immunity to CSA-binding parasites and reduced risk of poor delivery outcomes have been associated in some studies (9, 14, 41) but not in others (4, 6, 11, 39). Furthermore, the high incidence of malaria episodes observed a few weeks after delivery (8, 15, 34) suggests that other mechanisms may be also involved in the susceptibility of pregnant women to malaria.

Placental sequestration of *P. falciparum* complicates detection and quantification of infections during pregnancy, as parasites may be present in the placenta yet not detectable in peripheral blood (44). Moreover, infections at densities below the limit of microscopic detection (submicroscopic infections) contribute to the underestimation of the actual burden of *P. falciparum* infection among pregnant women in areas where malaria is endemic (25, 28, 37). The PCR method has the potential to overcome these limitations by providing a more sensitive estimate of maternal infections (44). Some studies have shown the clinical importance of submicroscopic infections (24, 28, 32) and the utility of quantitative PCR to predict poor delivery outcomes (1, 23). However, no study has assessed the prevalence of submicroscopic infections in women postpartum.

PCR-based genotyping of *P. falciparum* polymorphic markers allows the comparison of peripheral and placental parasite populations infecting women at delivery (17–19, 25, 38). Similar genotyping approaches can be used to determine if malaria infections during the postpartum period are caused by new infections or rather by the same parasites that persist after delivery. However, to date only one study, based on a small number of isolates, has addressed this issue (34).

The aim of this study was, therefore, to assess potential risk factors contributing to *P. falciparum* infection postpartum. To

* Corresponding author. Mailing address: Barcelona Centre for International Health Research, Hospital Clínic, Universitat de Barcelona, Rosselló 132, 08036 Barcelona, Spain. Phone: 34.932275706. Fax: 34.932279853. E-mail: agmayor@clinic.ub.es.

▽ Published ahead of print on 1 November 2010.

achieve this, we first estimated the burden of *P. falciparum* infection among Mozambican women at delivery and during the postpartum period using a PCR-based detection technique. For those women that were infected at both time points, we determined the origin of postpartum parasites by comparing their genotypes with those of parasites present at the time of delivery.

MATERIALS AND METHODS

Study area and population. The study was conducted at the Centro de Investigação em Saúde da Manhiça (CISM) in Manhiça District of southern Mozambique between 2003 and 2005 before intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) was recommended by the Ministry of Health. The characteristics of the area have been described in detail elsewhere (2). Perennial malaria transmission with some seasonality is attributable mostly to *Plasmodium falciparum*, and the estimated entomological inoculation rate for 2002 was 38 infective bites per person per year. IPTp with SP had a protective efficacy against clinical malaria episodes of 74.1% (95% confidence interval [CI], 30.8 to 90.3) after dose 1 and 71.4% (95% CI, 13.1 to 90.6) after dose 2 (30). More than 90% of the pregnant women attend the antenatal care clinic at least once, and 80% of the pregnant women in this area have an institutional delivery (30).

Study design. This study was done in the context of a randomized, double-blind, placebo-controlled trial of IPTp with SP (trial registration number NCT00209781) (30). In brief, women were enrolled in the study, received a long-lasting insecticide-treated net, and were randomized to receive placebo or SP if their gestational age was >12 and ≤28 weeks. SP doses were given twice in the second trimester, at least 1 month apart. Maternal HIV-1 status was determined with the Determine HIV-1/2 rapid test (Abbott Laboratories, North Chicago, IL) and confirmed with the Unigold rapid test (Trinity Biotech, Bray, Ireland). At the time of delivery, thin and thick smears of maternal peripheral blood were collected for examination of malarial parasites according to quality control procedures (3), birth weight was measured, and the maternal hematocrit was quantified in a microcapillary tube after centrifugation. Placental biopsy specimens were collected from the maternal side of the placenta and processed for histological examination as described elsewhere (16). Postpartum capillary blood samples were also collected 4 to 8 weeks after delivery for parasitological and hematocrit determinations. Blood samples from the placenta and periphery of pregnant women were collected at delivery, as well as postpartum, onto filter papers (Schleicher & Schuell number 903TM; Dassel, Germany). Women with a *P. falciparum* infection detected by microscopy in their peripheral blood were referred to receive SP and chloroquine at the pharmacy of the Manhiça Disctrict Hospital. Out of the 1,030 women participating in the IPTp trial, 402 (40%) were included in this study by random selection stratified by HIV status. Written informed consent was obtained from all study participants. The study was approved by the national Mozambican ethics review committee and the Hospital Clínic of Barcelona ethics review committee.

***P. falciparum* detection and genotyping by PCR.** DNA was extracted from a drop of 50 μl blood onto filter paper with an ABIPrism 6700 automated nucleic acid work station (Applied Biosystems) and finally resuspended in 200 μl of water, according to the manufacturer's instructions. Five microliters of DNA samples were screened for *P. falciparum* DNA by real-time quantitative PCR (qPCR) described elsewhere (28). A negative-control sample with no template DNA was also run in all reactions.

P. falciparum infections were genotyped if parasites were detected by qPCR in more than one of the matched samples (placental, peripheral at delivery and postpartum) collected from study participants. Merozoite surface protein 1 (*msp-1*) and *msp-2* genes were amplified through nested PCR (40) by using 5 μl of template DNA in the primary PCR and 2 μl of the product obtained to initiate the secondary PCR. The glutamate-rich protein gene was not used in this study due to its limited diversity among the parasite population in the Manhiça area (data not shown). Amplification products were electrophoresed in 2.5% agarose (LM Sieve:agarose [3:1]) and visualized by UV transillumination. The products of paired samples were loaded side by side for better comparison of the size and the number of PCR fragments. Each *P. falciparum* genotype was characterized on the basis of the fragment size of the PCR product of each locus. Size alleles were allocated into bins of 20-base pair-size ranges. Genotypic profiles of peripheral-placental matched samples were termed "divergent" if none of the *msp-1* and *msp-2* genotypes in one sample were present in the other, "identical" if the same *msp-1* and *msp-2* genotypes were observed in both samples, and "overlapping" if there was partial sharing of genotypes. Postpartum infections were considered persistent if at least one *msp-1* genotype and one *msp-2* genotype were also present at delivery among women with paired infections. To eliminate the possibility of misclassifying infections with common variants as persistent (20), a postpartum episode was considered indeterminate if the infections at delivery and during the postpartum period shared only high-frequency (>10%) *msp-1* and *msp-2* genotypes in the parasite population infecting pregnant women from the study area (28).

Definitions and statistical analysis. Pregnant women were classified as first-time mothers (primigravidae [PG]) and those with at least one previous pregnancy (multigravidae [MG]). Age was categorized as ≤20, 21 to 25, and >25 years. Microscopic peripheral infection was defined as the presence of asexual parasites assessed by optical examination of blood smears. Active placental infection was defined as the presence of parasites in histological sections. Submicroscopic infections were those undetected by microscopy or placental histology which gave a positive qPCR result. Anemia was considered if the hematocrit was <33%, and low birth weight (LBW) if the birth weight was <2,500 g. The sensitivity and specificity of microscopy were calculated by considering qPCR as the gold standard. Statistical analysis was performed using Stata 10.0 (StataCorp, College Station, TX). Prevalences of matched infections at delivery and during the postpartum period were compared by the use of McNemar's test. Logistic regression models were used to estimate the association of infection at delivery or during the postpartum period with parity, age, HIV, IPTp, parasitemia in other compartments, and maternal anemia. Multiplicity of infection (MOI), which was estimated as the highest number of *msp-1* or *msp-2* alleles detected in the sample, was analyzed by Poisson regression models, taking into account the matching between samples collected from the same woman. Multivariate models were estimated to assess the independent associations of age, parity, HIV, and IPTp with maternal infection. These models were estimated using a forward-stepwise procedure, using *P* values below 0.05 and above 0.10 from the likelihood ratio test as enter and remove criteria, respectively. *P* values less than 0.05 were considered statistically significant.

RESULTS

Among the 402 women with a singleton pregnancy included in the study, 104 (26%) were PG, 203 (50%) had received SP as IPTp, and 194 (48%) were HIV positive. Mean maternal age was 24.2 (standard deviation [SD], 6.4). One hundred sixty-eight (42%) mothers had anemia at delivery, and 45 (11%) delivered an LBW baby. No differences in terms of parity, age, prevalence of malaria infection, anemia, and LBW were observed between the subgroup of women selected for this study and the 1,030 total women participating in the randomized trial (all *P* values were >0.422, Fisher's exact test) (30). Filter papers were available for 402 (100%) peripheral samples taken at delivery, 350 (87%) placental samples, and 354 (88%) peripheral samples taken postpartum (median number of days after delivery, 32; interquartile range, 31 to 42). The prevalence of postpartum anemia was 23% (80 out of 353 women [1 missing datum]).

***P. falciparum* infection detected in women at delivery and postpartum.** Prevalences of qPCR-detected *P. falciparum* infections (32% in placental blood, 30% in peripheral blood at delivery, and 16% in postpartum peripheral blood) were higher than those detected by placental histological examination (active infections, 16%) or by light microscopy (12% in peripheral blood at delivery and 4% in postpartum peripheral blood) ($P < 0.001$ for all comparisons) (Table 1). All blood slide-positive samples taken from the peripheral blood of women at delivery ($n = 47$) and postpartum ($n = 14$) were also positive by qPCR, whereas 7 out of the 55 placental active infections determined by histology were negative by qPCR. The specificity of microscopic and histologic diagnoses was high (97% to 100%) (Table 1). Prevalences of submicroscopic infection at delivery were similar in peripheral (73/352, 21%) and placental (62/288, 22%) samples ($P = 0.781$) and higher

TABLE 1. Prevalence of *P. falciparum* maternal infection and sensitivity/specificity of microscopy and placental histology

Parameter	*P. falciparum* infection at:		
	Periphery (delivery)	Placenta (delivery)	Periphery (postpartum)
Prevalence by optical examination, no. of positive samples/total no. of samples (%)[a]	47/399 (12)	55/343 (16)	14/354 (4)
Prevalence by qPCR, no. of positive samples/total no. of samples (%)	120/399 (30)	110/343 (32)	57/354 (16)
Sensitivity, no. of positive samples by optical examination and qPCR/no. of positive samples by qPCR (%)	47/120 (39)	48/110 (44)	14/57 (25)
Specificity, no. of negative samples by optical examination and qPCR/no. of negative samples by qPCR (%)	279/279 (100)	226/233 (97)	297/297 (100)

[a] Active infections by microscopy or histology.

than the prevalence of submicroscopic infection detected postpartum (43/340, 13%; $P = 0.018$).

The multivariate analysis showed that parity was independently associated with peripheral infections at delivery detected by microscopy and qPCR, with active placental infections detected by histology, and with postpartum infections detected by microscopy (Tables 2 and 3). On the other hand, maternal age was found to be independently associated with placental and postpartum infection when parasites were detected by qPCR (Tables 2 and 3). IPTp with SP was associated with a lower risk of microscopic and submicroscopic infections at delivery, both in the periphery and placenta (Tables 2). The univariate analysis showed that the risk of infection detected by qPCR postpartum was higher among women with qPCR-detected peripheral infection (odds ratio [OR] = 3.83, 95% CI = 2.04 to 7.21) and qPCR-detected placental infection (OR = 4.65, 95% CI = 2.45 to 8.82) ($P < 0.001$, in both cases). However, the multivariate analysis showed that this association was found only for placental infection detected by qPCR (OR = 4.20, $P < 0.001$) or placental submicroscopic infection (OR = 5.14, $P < 0.001$) (Table 3). No association was found between HIV and *P. falciparum* infection detected at delivery or postpartum (Tables 2 and 3). Risk of maternal anemia at delivery was found to be independently associated with HIV infection (OR = 2.30, 95% CI = 1.42 to 3.70; $P = 0.001$) and the presence of a placental active infection detected by histology (OR = 1.95, 95% CI = 1.04 to 3.67; $P = 0.038$) or qPCR (OR = 2.13, 95% CI = 1.29 to 3.53; $P = 0.003$). In the postpartum period, anemia tended to be associated with microscopically detected infections (OR = 2.31, 95% CI = 0.73 to 7.32; $P = 0.153$), although this trend was not found for qPCR-detected infections (OR = 1.02, 95% CI = 0.50 to 2.06; $P = 0.961$).

Genotypic profiles at delivery. Among the 350 study women with filter papers available for matched peripheral and placental samples, 140 (40%) presented qPCR-detected infection at least in one of the two compartments. Among these, 27 (19%) were infected only at the periphery, 30 (22%) only at the placenta, and 83 (59%) both at the periphery and the placenta. *P. falciparum msp-1* and *msp-2* were successfully genotyped in matched peripheral and placental samples from 65 (78%) out of the 83 double-positive women. The MOIs for placental (3.65; SD, 1.91) and peripheral (3.54; SD, 1.83) blood at delivery were similar ($P = 0.504$) and were not found to be associated with IPTp, parity, age, and HIV status. Peripheral and placental parasites were genotypically identical in 18 out of the 65 (28%) paired infections, whereas profiles were totally divergent in 4 (6%) matched infections. The remaining 43 (66%) pairs presented overlapping genotypes. Overall, 28 out of 65 (43%) matched samples presented at least 1 genotype that was detected in the periphery but not in the placenta, whereas 36 out of 65 (55%) pairs presented at least 1 genotype in the placenta that was not detected in the periphery.

Postpartum genotypic profiles. Among the 57 women with *P. falciparum* infection detected by qPCR postpartum, 39 (68%) were also positive at the time of delivery, either in peripheral (31/57 [54%]) or placental blood (32/53 [60%]; placental sample not available for 4 women). The postpartum MOI (2.81; SD, 1.92) was significantly lower than the MOI at delivery (defined as the highest MOI among placental and peripheral infection in the same women; 4.03; SD, 2.00) ($P = 0.010$) and also lower in postpartum samples from mothers who had received SP (1.80; SD, 0.92) compared to placebo recipients (3.44; SD, 2.13, $P = 0.017$). Twenty-six postpartum samples were successfully genotyped and compared with matching samples collected from women at delivery (24 paired placental postpartum samples and 19 paired periphery postpartum samples). At least 20 (77%) out of these 26 women were not treated at delivery, as they were negative by standard microscopic examination (i.e., had submicroscopic infections). Based on concordance of both the *msp-1* genotype and *msp-2* genotype, it was found that 13 out of the 26 women (50%) were infected postpartum with a *P. falciparum* parasite that was also present at delivery, either in the placenta (13/24 [54%]) or in peripheral blood (8/19 [42%]) (Table 4). Two paired infections (one placental [postpartum] and another peripheral [postpartum] from the same woman) shared only *msp-1* and *msp-2* common genotypes (>10% prevalence) and were considered indeterminate. After discarding these infections, 12 out of the 26 women (46%) were considered to have an infection at delivery persisting from delivery, either in the placenta (12/24 [50%]) or in peripheral blood (7/19 [37%]) (Table 4). Persistence of genotypes was found till 62 days postdelivery (median, 32 days; range, 28 to 62). The hematocrit of the women with persistent postpartum parasites (34.1%; SD, 3.8) tended to be lower than the hematocrit in women with postdelivery infections by new parasites (37.4%; SD, 3.6; $P = 0.064$).

DISCUSSION

Eighty-five million pregnant women worldwide are exposed annually to *P. falciparum* infection (7). Consequently, all these

TABLE 2. Descriptive and multivariate association between host factors and *P. falciparum* infection in peripheral blood and placenta of pregnant women at delivery

Parameter	Infection at delivery[a]					
	Periphery			Placenta		
	No. of positive samples/total no. of samples (%)	OR[b]	95% CI	No. of positive samples/total no. of samples (%)	OR[b]	95% CI
Microscopy/histology						
Age (yr)						
≤20	23/130 (18)			28/111 (25)		
21–25	9/101 (9)			9/89 (10)		
>25	7/128 (5)			13/109 (12)		
Parity						
PG	21/100 (21)	1		25/87 (29)	1	
MG	18//259 (7)	0.26	0.13–0.53[d]	25/222 (11)	0.30	0.16–0.57[d]
HIV						
Neg	16/167 (10)			21/144 (15)		
Pos	23/192 (12)			29/165 (18)		
IPTp						
Placebo	28/172 (16)	1		32/146 (22)	1	
SP	11/187 (6)	0.30	0.14–0.63[d]	18/163 (11)	0.42	0.22–0.79[e]
qPCR						
Age (yr)						
≤20	44/130 (34)			45/111 (41)	1	
21–25	31/101 (31)			30/89 (34)	0.71	0.39–1.29
>25	30/128 (23)			23/109 (21)	0.37	0.20–0.69[d]
Parity						
PG	37/100 (37)	1		33/87 (38)		
MG	68/259 (26)	0.58	0.35–0.97[e]	65/222 (29)		
HIV						
Neg	48/167 (29)			44/144 (31)		
Pos	57/192 (27)			54/165 (33)		
IPTp						
Placebo	67/172 (39)	1		60/146 (41)	1	
SP	38/187 (20)	0.39	0.24–0.63[d]	38/163 (23)	0.42	0.26–0.70[d]
Submicroscopic infections[c]						
Age (yr)						
≤20	21/107 (20)			20/83 (24)		
21–25	22/92 (24)			21/80 (26)		
>25	23/121 (19)			14/96 (15)		
Parity						
PG	16/79 (20)			11/62 (18)		
MG	50/241 (21)			44/197 (22)		
HIV						
Neg	32/151 (21)			28/123 (23)		
Pos	34/169 (20)			27/136 (20)		
IPTp						
Placebo	39/144 (27)	1		31/114 (27)	1	
SP	27/176 (15)	0.49	0.28–0.85[e]	24/145 (17)	0.53	0.29–0.97[e]

[a] Included in the analysis are those women with data available for all variables considered in the model. OR, odds ratio; CI, confidence interval; PG, primigravidae; MG, multigravidae; IPTp, intermittent preventive treatment during pregnancy; SP, sulfadoxine-pyrimethamine; qPCR, quantitative PCR; Neg, negative; Pos, positive.
[b] Logistic regression model, mutivariate stepwise analysis.
[c] Microscopy negative and qPCR positive.
[d] $P < 0.005$.
[e] $P < 0.05$.

women are at risk of suffering the adverse effects that have been attributed to maternal malaria not only during gestation (5), but also in the postpartum period (45). Molecular detection of *P. falciparum* in the context of clinical trials evaluating tools to reduce malaria during pregnancy can provide valuable information on the burden and dynamics of maternal infections. This study shows that microscopy of peripheral blood fails to detect an important proportion of maternal infections (i.e., submicroscopic infections, 21%), as previously reported for pregnant women (19%) (28) and nonpregnant adults (35%) (26) from the same study area. Discrepancies are especially remarkable during the postpartum period, as 75% of the qPCR-detected parasitemias remained unnoticed under optical examination. Microscopic infections during the postpartum

TABLE 3. Descriptive and multivariate association between host factors and *P. falciparum* infection in peripheral blood of women during postpartum period

Parameter	Infection at postpartum[a]		
	No. of positive samples/total no. of samples (%)	OR[b]	95% CI
Microscopy			
Age (yr)			
≤20	8/95 (8)		
21–25	3/75 (4)		
>25	2/101 (2)		
Parity			
PG	7/73 (10)	1	
MG	6/198 (3)	0.29	0.10–0.91[e]
HIV			
Neg	6/133 (5)		
Pos	7/138 (5)		
IPTp			
Placebo	7/133 (5)		
SP	6/138 (4)		
Peripheral infection[c]			
Neg	5/190 (3)		
Pos	8/81 (10)		
Placental infection[c]			
Neg	6/185 (3)		
Pos	7/86 (8)		
qPCR			
Age (yr)			
≤20	25/95 (26)	1	
21–25	17/75 (23)	0.97	0.46–2.04
>25	9/191 (9)	0.34	0.15–0.81[e]
Parity			
PG	20/73 (27)		
MG	31/198 (16)		
HIV			
Neg	29/133 (22)		
Pos	22/138 (16)		
IPTp			
Placebo	24/133 (18)		
SP	27/138 (20)		
Peripheral infection[c]			
Neg	23/190 (12)		
Pos	28/81 (35)		
Placental infection[c]			
Neg	20/185 (11)	1	
Pos	31/86 (36)	4.20	2.19–8.08[f]
Submicroscopic infections[d]			
Age (yr)			
≤20	17/87 (20)		
21–25	14/72 (19)		
>25	7/99 (7)		
Parity			
PG	13/66 (20)		
MG	25/192 (13)		
HIV			
Neg	23/127 (18)		
Pos	15/131 (11)		
IPTp			
Placebo	17/126 (13)		
SP	21/132 (16)		
Peripheral infection[c]			
Neg	18/185 (10)		
Pos	20/73 (27)		
Placental infection[c]			
Neg	14/179 (8)	1	
Pos	24/79 (30)	5.14	2.49–10.63[f]

[a] Included in the analysis are those women with data available for all variables considered in the model. OR, odds ratio; CI, confidence interval; PG, primigravidae; MG, multigravidae; IPTp, intermittent preventive treatment during pregnancy; SP, sulfadoxine-pyrimethamine; qPCR, quantitative PCR; Neg, negative; Pos, positive.

[b] Logistic regression model, multivariate stepwise analysis.

[c] Assessed at the time of delivery by qPCR.

[d] Microscopy negative and qPCR positive.

[e] $P < 0.05$.

[f] $P < 0.005$.

period, but not qPCR-detected infections, also tend to increase the risk of anemia, suggesting that patent infections during the postpartum period may be of clinical relevance.

In agreement with previous studies reporting an increased susceptibility to malaria during puerperium (34), this study shows that a notable percentage of Mozambican women (16%) were infected postpartum. Whereas the increased risk of malaria during pregnancy has been attributed to sequestration of *P. falciparum* in the placenta (13), little is known about mechanisms underlying susceptibility during the postpartum period (8, 34). Our results show that maternal age was independently associated with qPCR-detected infections in the peripheral blood of pregnant women during the postpartum period, as well as in their placentas at delivery. These data suggest that young women may be at increased risk of infection during pregnancy and the postpartum period, probably because they might still be in the process of acquiring natural immunity to malaria (35, 43). In contrast, HIV infection, which has been shown previously to increase the risk of malaria in the postpartum period (21) and during pregnancy (42), was not found to contribute to *P. falciparum* infection in this study, although discrepancies may be due to differences in study designs and patient characteristics.

Placental infection was found to be a significant risk factor for subsequent infection during the postpartum period, when parasites were detected by qPCR. Previous studies based on optical detection of infection (8, 34) might have missed this association because of the lower sensitivity of microscopy, pointing out the relevance of submicroscopic infections. The relationship between infections before and after delivery could be explained by similar risks of infection during pregnancy and the postpartum period due to environmental or host factors. Alternatively, maternal infections at the end of pregnancy (most of them untreated because parasitemias were under the detection threshold of microscopy) may persist after delivery and thus contribute to the increased risk of postpartum parasitemia. In agreement with this interpretation, molecular analysis of matched infections at delivery and during the postpartum period showed that half (12/24) of the women were infected postpartum with at least one parasite strain that was also present in their placentas. Of importance, our results suggest that parasites persisting after delivery may be of clinical relevance, as they tended to be associated with a lower hematocrit than the hematocrit in women with postdelivery infections by new parasites. These results are in contrast with a previous report suggesting a spontaneous postpartum clearance of *P. falciparum* parasitemia among African women (33); however, that study was based on microscopical detection of infections, which may have missed low-density parasitemias. Also supporting the concept of a persistence of infections after delivery, IPTp was shown to reduce the multiplicity of infections during the postpartum period. An IPTp-associated reduction of postpartum infections in the subsample of women included in this study was not observed, due either to a small sample size or to the decreasing antimalarial efficacy of SP. However, the postpartum risk of malaria infection was reduced by one-half in women who received SP compared to the placebo group in the IPTp trial (30), a finding that was also observed in previous IPTp trials (29).

Such a common persistence of *P. falciparum*, even once the placenta is removed, might be explained by a rapid shift of the parasite cytoadhesion from CSA to other binding specificities or by the availability of CSA in other organs apart from the placenta.

TABLE 4. *P. falciparum msp-1* and *msp-2* alleles detected in paired samples from 13 women with coincident genotypes at delivery and during postpartum period[a]

Patient no.	MSP-1				MSP-2				Diagnosis
	MOI		Shared		MOI		Shared		
	Delivery	Postpartum	Uncommon	Common	Delivery	Postpartum	Uncommon	Common	
Peripheral, postpartum									
1	3	1		1	5	1		1	IND
2	2	6	1		3	3	2		Persistent
3	5	4	2		3	5	1		Persistent
4	4	4		2	6	6	3		Persistent
5	1	1	1		1	1	1		Persistent
6	1	1	1		1	1	1		Persistent
7	2	4	1		2	4	2		Persistent
8	4	1		1	2	1	1		Persistent
Placenta, postpartum									
1	4	1		1	6	1		1	IND
2	1	6	1		1	3	1		Persistent
3	3	4	2		6	5	1		Persistent
4	3	4		2	7	6	3		Persistent
5	1	1	1		1	1	1		Persistent
6	1	1	1		1	1	1		Persistent
7	2	4	1		1	4	1		Persistent
8	5	1		1	2	1	1		Persistent
9	5	2		2	2	1	1		Persistent
10	1	3		1	1	4	1		Persistent
11	2	2	1	1	3	1		1	Persistent
12	6	1	1		6	2	1		Persistent
13	1	2		1	2	4	1		Persistent

[a] MOI, multiplicity of infection; IND, indeterminate because infections at delivery and during postpartum period share only high-frequency *msp-1* and *msp-2* genotypes (>10%). Common alleles are those found at frequencies of 10% or higher in the parasite population, and uncommon alleles are those present at frequencies lower than 10%.

Alternatively, the placenta may not be the only focus of infection in pregnant women if maternal parasites can adhere to other receptors apart from CSA, such as CD36 (12). In support of the latter scenario, we found that a significant number of women (19%) had qPCR-detected malaria infection in their peripheral blood in the absence of placental infection. Moreover, 43% of the women had peripheral parasitemia with genotypes that were not found in their placental blood. Although discrepancies between genotypes (18, 19, 25, 38) might also be explained by synchronicity of infections (10) or by inconsistent detection of parasites at low densities (27), these results suggest that parasites adhering to other receptors apart from those specifically displayed in the placenta might be the ones that persist after delivery. Further studies evaluating the adhesive phenotype of persisting parasites would be of great utility to better understand the pathophysiology of maternal infections.

In summary, the results of this study point out the need of improving diagnostic tools for a more effective management of malaria during pregnancy and the postpartum period, as well as for the measurement of the impact of interventions such as IPTp. This study also shows that younger women and those with a placental infection are at increased risk of malaria postpartum and that some of the parasites infecting pregnant women might not be spontaneously cleared after delivery. This persistence of infections might contribute to the increased risk of malaria during the postpartum period. Interventions such as IPTp that reduce malaria during pregnancy may also translate into a lower postpartum burden of infection (29, 30).

ACKNOWLEDGMENTS

We are grateful to the women participating in the study, the staff of the Manhiça District Hospital, clinical officers, field supervisors, and data manager, and Cleofé Romagosa, Laura Puyol, Alfons Jiménez, Lázaro Mussacate, Nelito Ernesto José, Ana Rosa Manhiça, and Vania Simango for their contribution to the collection and analysis of samples.

The study received financial support from Banco de Bilbao, Vizcaya, Argentaria Foundation (grant BBVA 02-0) and Instituto de Salud Carlos III (grant PS09/01113). The Manhiça Health Research Centre receives core support from the Spanish Agency for International Cooperation. A.M. (contract CP-04/00220) and E.S.C. (grant BF-03/00106) were supported by the Instituto de Salud Carlos III, and C.D. (RYC-2008-02631) was supported by the Ministerio de Ciencia e Innovación.

We do not have any commercial or other association that might pose a conflict of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. **Adegnika, A. A., J. J. Verweij, S. T. Agnandji, S. K. Chai, L. P. Breitling, M. Ramharter, M. Frolich, S. Issifou, P. G. Kremsner, and M. Yazdanbakhsh.** 2006. Microscopic and sub-microscopic Plasmodium falciparum infection, but not inflammation caused by infection, is associated with low birth weight. Am. J. Trop. Med. Hyg. **75:**798–803.
2. **Alonso, P., F. Saute, J. J. Aponte, F. X. Gomez-Olive, A. Nhacolo, R. Thomson, E. Macete, F. Abacassamo, P. J. Ventura, X. Bosch, C. Menendez, and M. Dgedge.** 2001. Manhica DSS, Mozambique, p. 189–195. *In* Population and health in developing countries, vol. 1. Population, health, and survival at INDEPTH sites. International Development Research Centre, Ottawa, Ontario, Canada.
3. **Alonso, P. L., T. Smith, J. R. Schellenberg, H. Masanja, S. Mwankusye, H. Urassa, I. Bastos de Azevedo, J. Chongela, S. Kobero, C. Menendez, et al.** 1994. Randomised trial of efficacy of SPf66 vaccine against Plasmodium falciparum malaria in children in southern Tanzania. Lancet **344:**1175–1181.
4. **Beeson, J. G., E. J. Mann, S. R. Elliott, V. M. Lema, E. Tadesse, M. E. Molyneux, G. V. Brown, and S. J. Rogerson.** 2004. Antibodies to variant

surface antigens of Plasmodium falciparum-infected erythrocytes and adhesion inhibitory antibodies are associated with placental malaria and have overlapping and distinct targets. J. Infect. Dis. **189:**540–551.
5. **Brabin, B. J.** 1983. An analysis of malaria in pregnancy in Africa. Bull. World Health Organ. **61:**1005–1016.
6. **Cox, S. E., T. Staalsoe, P. Arthur, J. N. Bulmer, L. Hviid, K. Yeboah-Antwi, B. R. Kirkwood, and E. M. Riley.** 2005. Rapid acquisition of isolate-specific antibodies to chondroitin sulfate A-adherent plasmodium falciparum isolates in Ghanaian primigravidae. Infect. Immun. **73:**2841–2847.
7. **Dellicour, S., A. J. Tatem, C. A. Guerra, R. W. Snow, and F. O. ter Kuile.** 2010. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. PLoS Med. **7:**e1000221.
8. **Diagne, N., C. Rogier, C. S. Sokhna, A. Tall, D. Fontenille, C. Roussilhon, A. Spiegel, and J. F. Trape.** 2000. Increased susceptibility to malaria during the early postpartum period. N. Engl. J. Med. **343:**598–603.
9. **Duffy, P. E., and M. Fried.** 2003. Antibodies that inhibit Plasmodium falciparum adhesion to chondroitin sulfate A are associated with increased birth weight and the gestational age of newborns. Infect. Immun. **71:**6620–6623.
10. **Farnert, A., G. Snounou, I. Rooth, and A. Bjorkman.** 1997. Daily dynamics of Plasmodium falciparum subpopulations in asymptomatic children in a holoendemic area. Am. J. Trop. Med. Hyg. **56:**538–547.
11. **Fievet, N., J. Y. Le Hesran, G. Cottrell, S. Doucoure, I. Diouf, J. L. Ndiaye, G. Bertin, O. Gaye, S. Sow, and P. Deloron.** 2006. Acquisition of antibodies to variant antigens on the surface of Plasmodium falciparum-infected erythrocytes during pregnancy. Infect. Genet. Evol. **6:**459–463.
12. **Fried, M., G. J. Domingo, C. D. Gowda, T. K. Mutabingwa, and P. E. Duffy.** 2006. Plasmodium falciparum: chondroitin sulfate A is the major receptor for adhesion of parasitized erythrocytes in the placenta. Exp. Parasitol. **113:**36–42.
13. **Fried, M., and P. E. Duffy.** 1996. Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta. Science **272:**1502–1504.
14. **Fried, M., F. Nosten, A. Brockman, B. J. Brabin, and P. E. Duffy.** 1998. Maternal antibodies block malaria. Nature **395:**851–852.
15. **Garnham, P.** 1938. The placenta in malaria with special reference to reticulo-endothelial immunity. Trans. R. Soc. Trop. Med. Hyg. **32:**13–34.
16. **Ismail, M. R., J. Ordi, C. Menendez, P. J. Ventura, J. J. Aponte, E. Kahigwa, R. Hirt, A. Cardesa, and P. L. Alonso.** 2000. Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. Hum. Pathol. **31:**85–93.
17. **Jafari-Guemouri, S., N. T. Ndam, G. Bertin, E. Renart, S. Sow, J. Y. Le Hesran, and P. Deloron.** 2005. Demonstration of a high level of parasite population homology by quantification of Plasmodium falciparum alleles in matched peripheral, placental, and umbilical cord blood samples. J. Clin. Microbiol. **43:**2980–2983.
18. **Kamwendo, D. D., F. K. Dzinjalamala, G. Snounou, M. C. Kanjala, C. G. Mhango, M. E. Molyneux, and S. J. Rogerson.** 2002. Plasmodium falciparum: PCR detection and genotyping of isolates from peripheral, placental, and cord blood of pregnant Malawian women and their infants. Trans. R. Soc. Trop. Med. Hyg. **96:**145–149.
19. **Kassberger, F., A. Birkenmaier, A. Khattab, P. G. Kremsner, and M. Q. Klinkert.** 2002. PCR typing of Plasmodium falciparum in matched peripheral, placental and umbilical cord blood. Parasitol. Res. **88:**1073–1079.
20. **Kwiek, J. J., A. P. Alker, E. C. Wenink, M. Chaponda, L. V. Kalilani, and S. R. Meshnick.** 2007. Estimating true antimalarial efficacy by heteroduplex tracking assay in patients with complex Plasmodium falciparum infections. Antimicrob. Agents Chemother. **51:**521–527.
21. **Ladner, J., V. Leroy, A. Simonon, E. Karita, J. Bogaerts, A. De Clercq, P. Van De Perre, and F. Dabis.** 2002. HIV infection, malaria, and pregnancy: a prospective cohort study in Kigali, Rwanda. Am. J. Trop. Med. Hyg. **66:**56–60.
22. **Mackintosh, C. L., J. G. Beeson, and K. Marsh.** 2004. Clinical features and pathogenesis of severe malaria. Trends Parasitol. **20:**597–603.
23. **Malhotra, I., A. Dent., P. Mungai, E. Muchiri, and C. L. King.** 2005. Real-time quantitative PCR for determining the burden of Plasmodium falciparum parasites during pregnancy and infancy. J. Clin. Microbiol. **43:**3630–3635.
24. **Mankhambo, L., M. Kanjala, S. Rudman, V. M. Lema, and S. J. Rogerson.** 2002. Evaluation of the OptiMAL rapid antigen test and species-specific PCR to detect placental Plasmodium falciparum infection at delivery. J. Clin. Microbiol. **40:**155–158.
25. **Mayengue, P. I., H. Rieth, A. Khattab, S. Issifou, P. G. Kremsner, M. Q. Klinkert, and F. Ntoumi.** 2004. Submicroscopic Plasmodium falciparum infections and multiplicity of infection in matched peripheral, placental and umbilical cord blood samples from Gabonese women. Trop. Med. Int. Health **9:**949–958.
26. **Mayor, A., J. J. Aponte, C. Fogg, F. Saute, B. Greenwood, M. Dgedge, C. Menendez, and P. L. Alonso.** 2007. The epidemiology of malaria in adults in a rural area of southern Mozambique. Malar. J. **6:**3.
27. **Mayor, A., F. Saute, J. J. Aponte, J. Almeda, F. X. Gomez-Olive, M. Dgedge, and P. L. Alonso.** 2003. Plasmodium falciparum multiple infections in Mozambique, its relation to other malariological indices and to prospective risk of malaria morbidity. Trop. Med. Int. Health **8:**3–11.
28. **Mayor, A., E. Serra-Casas, A. Bardaji, S. Sanz, L. Puyol, P. Cistero, B. Sigauque, I. Mandomando, J. J. Aponte, P. L. Alonso, and C. Menendez.** 2009. Sub-microscopic infections and long-term recrudescence of Plasmodium falciparum in Mozambican pregnant women. Malar. J. **8:**9.
29. **Mbaye, A., K. Richardson, B. Balajo, S. Dunyo, C. Shulman, P. Milligan, B. Greenwood, and G. Walraven.** 2006. A randomized, placebo-controlled trial of intermittent preventive treatment with sulphadoxine-pyrimethamine in Gambian multigravidae. Trop. Med. Int. Health **11:**992–1002.
30. **Menéndez, C., A. Bardaji, B. Sigauque, C. Romagosa, S. Sanz, E. Serra-Casas, E. Macete, A. Berenguera, C. David, C. Dobano, D. Naniche, A. Mayor, J. Ordi, I. Mandomando, J. J. Aponte, S. Mabunda, and P. L. Alonso.** 2008. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. PLoS One **3:**e1934.
31. **Miller, L. H., D. I. Baruch, K. Marsh, and O. K. Doumbo.** 2002. The pathogenic basis of malaria. Nature **415:**673–679.
32. **Mockenhaupt, F. P., B. Rong, H. Till, T. A. Eggelte, S. Beck, C. Gyasi-Sarpong, W. N. Thompson, and U. Bienzle.** 2000. Submicroscopic Plasmodium falciparum infections in pregnancy in Ghana. Trop. Med. Int. Health **5:**167–173.
33. **Nguyen-Dinh, P., R. W. Steketee, A. E. Greenberg, J. J. Wirima, O. Mulenda, and S. B. Williams.** 1988. Rapid spontaneous postpartum clearance of Plasmodium falciparum parasitaemia in African women. Lancet **ii:**751–752.
34. **Ramharter, M., M. P. Grobusch, G. Kiessling, A. A. Adegnika, U. Moller, S. T. Agnandji, M. Kramer, N. Schwarz, J. F. Kun, S. Oyakhirome, S. Issifou, S. Borrmann, B. Lell, B. Mordmuller, and P. G. Kremsner.** 2005. Clinical and parasitological characteristics of puerperal malaria. J. Infect. Dis. **191:**1005–1009.
35. **Rogerson, S. J., N. R. van den Broek, E. Chaluluka, C. Qongwane, C. G. Mhango, and M. E. Molyneux.** 2000. Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey. Am. J. Trop. Med. Hyg. **62:**335–340.
36. **Salanti, A., T. Staalsoe, T. Lavstsen, A. T. Jensen, M. P. Sowa, D. E. Arnot, L. Hviid, and T. G. Theander.** 2003. Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. Mol. Microbiol. **49:**179–191.
37. **Saute, F., C. Menendez, A. Mayor, J. Aponte, X. Gomez-Olive, M. Dgedge, and P. Alonso.** 2002. Malaria in pregnancy in rural Mozambique: the role of parity, submicroscopic and multiple Plasmodium falciparum infections. Trop. Med. Int. Health **7:**19–28.
38. **Schleiermacher, D., J. Y. Le Hesran, J. L. Ndiaye, R. Perraut, A. Gaye, and O. Mercereau-Puijalon.** 2002. Hidden Plasmodium falciparum parasites in human infections: different genotype distribution in the peripheral circulation and in the placenta. Infect. Genet. Evol. **2:**97–105.
39. **Serra-Casas, E., C. Menendez, A. Bardaji, L. Quinto, C. Dobano, B. Sigauque, A. Jimenez, I. Mandomando, V. S. Chauhan, C. E. Chitnis, P. L. Alonso, and A. Mayor.** 2010. The effect of intermittent preventive treatment during pregnancy on malarial antibodies depends on HIV status and is not associated with poor delivery outcomes. J. Infect. Dis. **201:**123–131.
40. **Snounou, G., X. Zhu, N. Siripoon, W. Jarra, S. Thaithong, K. N. Brown, and S. Viriyakosol.** 1999. Biased distribution of msp1 and msp2 allelic variants in Plasmodium falciparum populations in Thailand. Trans. R. Soc. Trop. Med. Hyg. **93:**369–374.
41. **Staalsoe, T., C. E. Shulman, J. N. Bulmer, K. Kawuondo, K. Marsh, and L. Hviid.** 2004. Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated Plasmodium falciparum malaria. Lancet **363:**283–289.
42. **Steketee, R. W., J. J. Wirima, P. B. Bloland, B. Chilima, J. H. Mermin, L. Chitsulo, and J. G. Breman.** 1996. Impairment of a pregnant woman's acquired ability to limit Plasmodium falciparum by infection with human immunodeficiency virus type-1. Am. J. Trop. Med. Hyg. **55:**42–49.
43. **Tako, E. A., A. Zhou, J. Lohoue, R. Leke, D. W. Taylor, and R. F. Leke.** 2005. Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. Am. J. Trop. Med. Hyg. **72:**236–242.
44. **Uneke, C. J.** 2008. Diagnosis of Plasmodium falciparum malaria in pregnancy in sub-Saharan Africa: the challenges and public health implications. Parasitol. Res. **102:**333–342.
45. **Vallely, L., Y. Ahmed, and S. F. Murray.** 2005. Postpartum maternal morbidity requiring hospital admission in Lusaka, Zambia—a descriptive study. BMC Pregnancy Childbirth **5:**1.
46. **Yamada, M., R. Steketee, C. Abramowsky, M. Kida, J. Wirima, D. Heymann, J. Rabbege, J. Breman, and M. Aikawa.** 1989. Plasmodium falciparum associated placental pathology: a light and electron microscopic and immunohistologic study. Am. J. Trop. Med. Hyg. **41:**161–168.

Editor: J. H. Adams