ORIGINAL ARTICLE

Changing Trends in *P. falciparum* Burden, Immunity, and Disease in Pregnancy

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ABSTRACT

BACKGROUND

Prevention of reinfection and resurgence is an integral component of the goal to eradicate malaria. However, the adverse effects of malaria resurgences are not known.

METHODS

We assessed the prevalence of *Plasmodium falciparum* infection among 1819 Mozambican women who delivered infants between 2003 and 2012. We used microscopic and histologic examination and a quantitative polymerase-chain-reaction (qPCR) assay, as well as flow-cytometric analysis of IgG antibody responses against two parasite lines.

RESULTS

Positive qPCR tests for *P. falciparum* decreased from 33% in 2003 to 2% in 2010 and increased to 6% in 2012, with antimalarial IgG antibody responses mirroring these trends. Parasite densities in peripheral blood on qPCR assay were higher in 2010–2012 (geometric mean [±SD], 409±1569 genomes per microliter) than in 2003–2005 (44±169 genomes per microliter, P=0.02), as were parasite densities in placental blood on histologic assessment (50±39% of infected erythrocytes vs. 4±6%, P<0.001). The malaria-associated reduction in maternal hemoglobin levels was larger in 2010–2012 (10.1±1.8 g per deciliter in infected women vs. 10.9±1.7 g per deciliter in uninfected women; mean difference, −0.82 g per deciliter; 95% confidence interval [CI], −1.39 to −0.25) than in 2003–2005 (10.5±1.1 g per deciliter vs. 10.6±1.5 g per deciliter; difference, −0.12 g per deciliter; 95% CI, −0.67 to 0.43), as was the reduction in birth weight (2863±440 g in women with past or chronic infections vs. 3070±482 g in uninfected women in 2010–2012; mean difference, −164.5 g; 95% CI, −289.7 to −39.4; and 2994±487 g vs. 3117±455 g in 2003–2005; difference, −44.8 g; 95% CI, −139.1 to 49.5).

CONCLUSIONS

Antimalarial antibodies were reduced and the adverse consequences of *P. falciparum* infections were increased in pregnant women after 5 years of a decline in the prevalence of malaria. (Funded by Malaria Eradication Scientific Alliance and others.)

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The malaria parasite can persist and reappear in areas where infection is no longer circulating or is circulating at very low levels.[1] Since prevention of reinfection and resurgence is an integral component of the current goal to eradicate malaria,[2] understanding the determinants and clinical consequences of malaria declines and resurgences, as well as the timescales for gain and loss of antimalarial immunity, has become a priority.

Malaria is an infectious disease that requires boosting to maintain immunity over time[3]; a reduction in parasite exposure can lead to a loss of population-level immunity and an increase in the harmful effects of malaria infections during resurgences.[4] Because of the increased susceptibility to malaria during pregnancy,[5] the consequences of a reduction in malaria immunity among pregnant women could be particularly severe, especially in the context of the human immunodeficiency virus (HIV) pandemics in Africa,[6] which can impair the maintenance of effective immune responses.[7] However, declining malaria transmission among pregnant women[8-10] may have an effect on malaria-related clinical outcomes that has not yet been assessed.

In areas where malaria is endemic, antibodies against *Plasmodium falciparum* VAR2CSA[11] develop in pregnant women. VAR2CSA is a variant surface antigen that mediates placental accumulation of infected erythrocytes[12] and leads to the deleterious effects on the mothers and their offspring, especially in primigravid women.[13] Antibodies against VAR2CSA are acquired after exposure to placental parasites in a parity-dependent manner,[14] contribute to a reduction in the clinical consequences of malaria in pregnancy,[15] and are affected by variables that influence the risk of exposure to *P. falciparum*.[16-18] Therefore, decreases in this pregnancy-specific immunity after reductions in transmission might affect the disease burden during resurgences.

Although the relationship between the prevalence of malaria among pregnant women and the prevalence in the general community is still incompletely understood,[19] malaria testing in pregnant women at health care facilities can provide information about the effect of changes in malaria transmission on the disease burden. To better understand the consequences of malaria resurgences, we examined parasitologic, immunologic, and clinical trends among pregnant women delivering between 2003 and 2012 in the Manhiça District in southern Mozambique.

Methods

Study Sites and Population

Pregnant women who provided written informed consent to participate in two clinical trials of antimalarial drugs between 2003 and 2005[20] and between 2010 and 2012[21,22] at antenatal clinics in the Manhiça District were included in the study. Biases due to pooling of data from these two clinical trials, as well as to changes from the earlier to the later period in the health care provided to the women, were minimized by the use of similar protocols and procedures during the two trials. The intensity of malaria transmission in this region used to be moderate[23] and has decreased in the past few years, as evidenced by a reduction in hospital admissions for malaria. The area has been under continuous demographic surveillance by the Centro de Investigação em Saúde da Manhiça (CISM) since 1996.[24]

All women included in the study received bed nets treated with long-lasting insecticide, as well as intermittent preventive treatment during pregnancy, which in 2003–2005 consisted of two doses of sulfadoxine–pyrimethamine[20] and in 2010–2012 consisted of two doses of mefloquine or sulfadoxine–pyrimethamine, if the women were HIV-negative,[22] or three doses of mefloquine or placebo, if they were HIV-positive and were receiving trimethoprim–sulfamethoxazole prophylaxis.[21] At delivery, maternal hemoglobin was measured with the use of a HemoCue or Sysmex KX21 analyzer, and newborns were weighed (with the use of weekly calibrated scales, which were either digital scales[20] or triple-beam balances[21,22]). Tissue samples from the maternal side of the placenta, as well as 50 μl of maternal peripheral-, placental-, and cord-blood samples on filter papers, were collected for parasitologic assessments. At delivery, peripheral blood was collected into EDTA vacutainers and centrifuged, with the plasma stored at −20°C. Clinical malaria episodes were treated according to national guidelines at the time of the study, and parenteral quinine was administered for severe malaria. The study protocols and informed-consent forms were reviewed and approved by the National Ethics Review Committee of Mozambique and the Ethics Review Committee of the Hospital Clinic of Barcelona.

PARASITOLOGIC ASSESSMENTS

Thick and thin blood films, as well as placental-biopsy samples in 10% neutral buffered formalin, were assessed for detection of plasmodium species according to standard, quality-controlled procedures.[25-27] A random selection of 50% of the peripheral-blood samples (958 samples) and placental-blood samples (941) on filter papers was used for detection of *P. falciparum* in duplicate by means of a real-time quantitative polymerase-chain-reaction (qPCR) assay targeting 18S ribosomal RNA (rRNA)[28] (see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org).

MEASUREMENT OF ANTIMALARIAL IGG ANTIBODIES

A random selection of 35% of the peripheral-blood plasma samples (654 samples) collected at delivery was used for flow-cytometric measurement of IgG[16] (expressed as mean fluorescence intensity [MFI]) against a chondroitin sulfate A–binding parasite line expressing VAR2CSA (CS2), indicating pregnancy-specific antimalarial immunity,[29] and a rosetting parasite line (R29), indicating general antimalarial immunity[30] (see the Methods section in the Supplementary Appendix). The *P. falciparum* CS2 (MRA-96) laboratory strain was obtained through the Malaria Research and Reference Reagent Resource Center (MR4) as part of the BEI Resources Repository, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

STUDY DEFINITIONS

Pregnant women were included in the analysis if they received sulfadoxine–pyrimethamine, mefloquine, or trimethoprim–sulfamethoxazole, alone or in combination with mefloquine, during pregnancy and if all information was available on the date of delivery, HIV status, age, and parity, as well as results of peripheral-blood microscopic examination and placental histologic assessment. Women were classified a priori as primigravid (first pregnancy) or multigravid (at least one previous pregnancy), and age was categorized as younger than 20 years, 20 to 24 years, or 25 years of age or older.[16,17] Maternal microscopic infection at delivery was defined as the presence of *P. falciparum* parasites in peripheral blood or in the placenta on either microscopic or histologic examination, qPCR-positive infection was defined as a positive result of qPCR testing in peripheral or placental blood, and submicroscopic infection was defined as the presence of malaria parasites on qPCR testing but not on microscopic examination. Past placental infection was defined by the presence of malaria pigment (i.e., hemozoin[27]) without parasite detection on placental histologic examination, and chronic placental infection was defined by the presence of malaria pigment in combination with the detection of parasites.

STATISTICAL ANALYSIS

Our primary hypothesis was that declines in the prevalence of malaria would be associated with reductions in antimalarial immunity, and our secondary hypothesis was that reduced immunity would lead to higher parasite densities, smaller differences in parasite densities and antibody levels between primigravid and multigravid women, and a larger adverse effect of malaria. Proportions were compared by means of Fisher's exact test, and continuous variables by means of Student's t-test. The primary outcome variables — maternal microscopic infection and level of IgG antibodies against CS2 — were compared between the two study periods (2003–2005 and 2010–2012) with the use of logistic- and linear-regression models, respectively, adjusted according to parity, age, and HIV infection status. Secondary variables included qPCR positivity for maternal infection, peripheral-blood and placental infections, submicroscopic infections, and levels of IgG antibodies against R29, as well as data according to year, with observations from 2003 and 2004 combined because of the small sample in 2003 (28 observations) and with the use of the first year of the second period (2010) as the reference year for comparisons. To test the secondary hypothesis, we used linear regression to analyze the effect of study period on log-transformed parasite densities, as well as the effect of malaria on maternal hemoglobin levels and newborn weight in each of the two periods. To determine whether a modification of the associations with parity was attributable to the study period, we included an interaction term in the regression models and assessed separately the effect of parity for each period. The data were analyzed with the use of Stata software, version 11.0 (Stata). P values of less than 0.05 were considered to indicate statistical significance for primary outcomes, with Bonfer-

roni corrections applied for the secondary outcome variables tested.

RESULTS

STUDY POPULATION

A total of 2259 women received bed nets treated with long-lasting insecticide and were given prophylaxis with sulfadoxine–pyrimethamine, mefloquine, or trimethoprim–sulfamethoxazole, alone or in combination with mefloquine, in the context of the two clinical trials of intermittent preventive treatment of malaria in pregnancy that were conducted in Manhiça.[20-22] Of these women, 440 were excluded because of missing data on HIV status, peripheral-blood microscopic examination, or placental histologic examination (Fig. S1 and Table S1 in the Supplementary Appendix). The 1819 women included in this study delivered between October 27, 2003, and August 14, 2012, and were similar in terms of baseline characteristics with all 2259 women participating in the randomized trials (Table S1 in the Supplementary Appendix), as were women in both study periods (Table 1).

TRENDS IN MALARIA DURING PREGNANCY

The prevalence of maternal microscopic infection at delivery (primary outcome) decreased from 11% in 2003–2005 to 2% in 2010–2012 (Table 1) (adjusted odds ratio, 0.19; 95% confidence interval [CI], 0.12 to 0.30; P<0.001). Similar decreases were observed in secondary parasitologic outcomes (P<0.001 in all cases) (Table 1). Microscopic examination of peripheral blood and histologic examination of the placenta, as compared with qPCR testing, are described in Table S2 in the Supplementary Appendix. The prevalence of peripheral-blood infections that were submicroscopic was higher during the 2003–2005 period than during the 2010–2012 period (70% [26 of 37 infections] vs. 47% [14 of 30]; adjusted odds ratio, 3.81; 95% CI, 1.17 to 12.46; P=0.03).

Secondary analysis by year showed that the prevalence of microscopic maternal infection decreased from 12% in 2003–2004 to 1% in 2010 (P<0.001, adjusted), and the prevalence of qPCR-positive maternal infection decreased from 33% to 2% (P<0.001, adjusted), with increases in 2012 to 4% for microscopic infections (P=0.003, adjusted) and to 6% for qPCR-positive infections (P=0.03, adjusted) (Fig. 1, and Fig. S2 in the Supplementary Appendix). Similar trends were observed after the exclusion of women who received mefloquine as intermittent preventive treatment of malaria during pregnancy in the 2010–2012 period (Table S3 in the Supplementary Appendix). Cord-blood microscopic infections were consistently low (≤1%) throughout the study period.

ANTIMALARIAL IMMUNITY

Geometric mean levels of IgG against CS2 (primary outcome) were lower in 2010–2012 than in 2003–2005 (Table 1) (adjusted ratio, 0.44; 95% CI, 0.36 to 0.54; P<0.001), with similar reductions observed for IgG antibodies against R29 (adjusted ratio, 0.43; 95% CI, 0.33 to 0.58; P<0.001). Secondary analysis by year showed that IgG levels decreased from 2003 to 2010 and gradually increased between 2010 and 2012 (Fig. 2). Maternal qPCR-positive infections at delivery were associated with an increase in IgG levels against CS2 (P<0.001, adjusted) but not against R29 (P=0.30, adjusted) (Fig. S3 in the Supplementary Appendix).

PARASITE DENSITIES AND CLINICAL OUTCOMES

Parasite densities in peripheral blood on qPCR assay were higher in 2010–2012 (geometric mean [±SD], 409±1569 genomes per microliter) than in 2003–2005 (44±169 genomes per microliter, P=0.02), as were parasite densities in placental blood on histologic examination (50±39% of infected erythrocytes vs. 4±6%, P<0.001) (Fig. 3). Similar increases in parasite densities, although not significant, were observed by means of microscopic examination in peripheral blood and qPCR assay in placental blood. Parasite densities quantified by means of microscopic examination in cord blood were also higher in 2010–2012 (geometric mean, 38,249±65,809 parasites per microliter) than in 2003–2005 (208±551 parasites per microliter; P=0.046; P=0.23 after Bonferroni correction).

The average maternal hemoglobin concentration increased slightly from 2003–2005 to 2010–2012, although no difference was observed in mean newborn weight (Table 1). A larger reduction in hemoglobin levels associated with microscopic maternal infections was observed in 2010–2012 (10.1±1.8 g per deciliter in infected women vs. 10.9±1.7 g per deciliter in uninfected women; mean difference, −0.82 g per deciliter; 95% CI, −1.39 to −0.25; P=0.005, adjusted; P=0.01 after Bonferroni correction) than in 2003–2005

Table 1. Characteristics of Study Participants and Parasitologic, Immunologic, and Clinical Outcomes, According to Study Period.*

Variable	2003–2005 (N=374)	2010–2012 (N=1445)	P Value
Year — no. (%)			
2003	28 (7)	0	
2004	232 (62)	0	
2005	114 (30)	0	
2010	0	358 (25)	
2011	0	803 (56)	
2012	0	284 (20)	
IPTp — no. (%)†			
Sulfadoxine–pyrimethamine	374 (100)	324 (22)	
Mefloquine	0	894 (62)	
Trimethoprim–sulfamethoxazole	0	227 (16)	
HIV status — no. (%)‡			
Negative	274 (73)	998 (69)	0.13
Positive	100 (27)	447 (31)	
CD4+ T-cell count at recruitment — per mm^3§	526.8±237.3	462.9±255.7	0.13
Parity — no. (%)			
Primigravid	113 (30)	422 (29)	0.70
Multigravid	261 (70)	1023 (71)	
Age — no. (%)			
<20 yr	129 (34)	458 (32)	
20–24 yr	106 (28)	358 (25)	0.08
≥25 yr	139 (37)	629 (44)	
Maternal hemoglobin — g/dl	10.5±1.5	10.8±1.7	0.02¶
Newborn birth weight — g	3055±473	3061±480	0.80
Primary outcomes			
Maternal microscopic infection — no. (%)‖	41 (11)	33 (2)	<0.001¶
IgG antibodies against CS2, geometric mean ±SD — MFI**	6055±8742	3001±3319	<0.001¶
Secondary outcomes			
Maternal qPCR-positive infection — no./total no. (%)††	50/165 (30)	33/753 (4)	<0.001¶
Peripheral-blood infection on microscopic assessment — no./total no. (%)	25/374 (7)	32/1445 (2)	<0.001¶
Peripheral-blood infection on qPCR testing — no./total no. (%)	37/169 (22)	30/789 (4)	<0.001¶
Active placental infection on histologic assessment — no./total no. (%)	37/374 (10)	18/1445 (1)	<0.001¶
Past or chronic placental infection on histologic assessment — no./total no. (%)	179/374 (48)	57/1445 (4)	<0.001¶
Placental-blood infection on qPCR testing — no./total no. (%)	39/165 (24)	27/776 (3)	<0.001¶
IgG antibodies against R29, geometric mean ±SD — MFI**	7820±9139	3610±5859	<0.001¶

* Plus–minus values are means ±SD unless otherwise noted. Dates of sample collection were as follows: 2003, October 27 through December 31; 2004, January 1 through December 26; 2005, January 1 through October 7; 2010, March 28 through December 31; 2011, January 1 through December 31; and 2012, January 1 through August 14. Percentages may not sum to 100 because of rounding. qPCR denotes quantitative polymerase chain reaction.

† IPTp denotes intermittent preventive treatment during pregnancy, with mefloquine given alone or in combination with trimethoprim–sulfamethoxazole.

‡ Maternal human immunodeficiency virus (HIV) status was determined with the use of the Determine HIV-1/2 test (Abbott Laboratories) and was confirmed by means of the Uni-Gold HIV Rapid Test (Trinity Biotech).

§ CD4+ T-cell counts were performed in BD Trucount tubes (BD Biosciences).

¶ The P value is significant according to multivariate analysis adjusted for HIV status, parity, and age.

‖ Maternal microscopic infection was defined by the presence of *P. falciparum* parasites in peripheral blood or in the placenta on microscopic or histologic examination, respectively.

** MFI (mean fluorescence intensity) was measured in arbitrary fluorescence units.

†† Maternal qPCR-positive infection was defined by a positive result on qPCR testing in peripheral or placental blood.

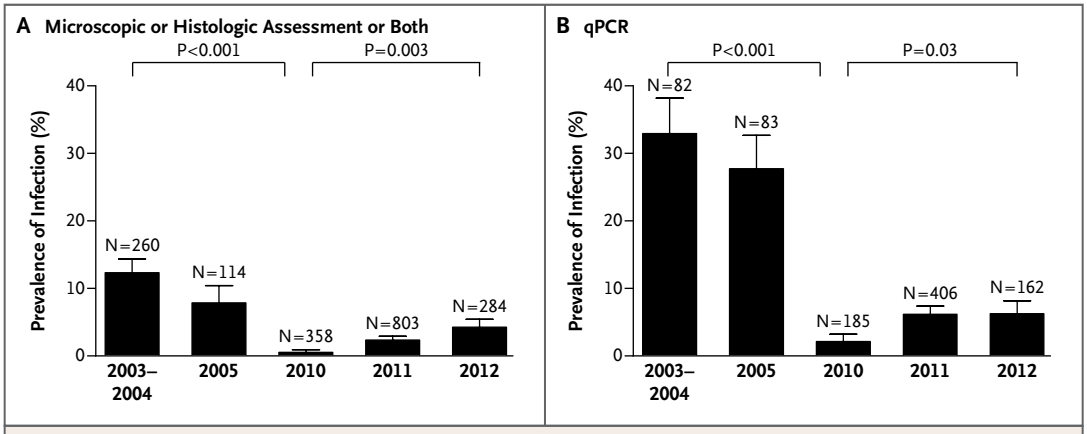

Figure 1. Prevalence of *Plasmodium falciparum* Infection among Pregnant Women at Delivery, According to Year.

Panel A shows the prevalence of infection on the basis of microscopic assessment, histologic assessment, or both, and Panel B shows the prevalence on the basis of quantitative polymerase-chain-reaction (qPCR) testing. Maternal infection was defined by the detection of *P. falciparum* in either peripheral blood or the placenta. Results of qPCR testing of both peripheral and placental blood were available for 918 women. Observations from 2003 (28 for microscopic assessment, histologic assessment, or both and 14 for qPCR testing) and 2004 (232 for microscopic assessment, histologic assessment, or both and 68 for qPCR testing) were combined because of the small sample in 2003. P values are based on a multivariate analysis adjusted for human immunodeficiency virus (HIV) status, parity, and age. T bars represent standard errors.

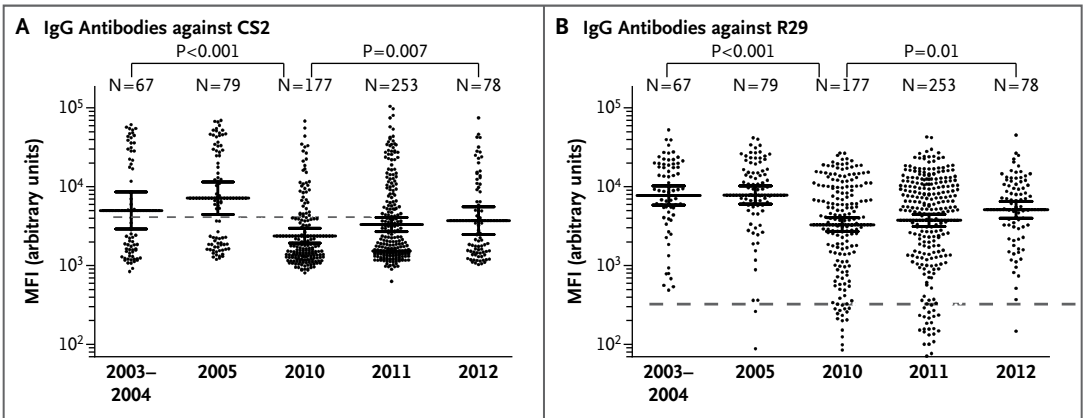

Figure 2. IgG Antibodies against Two *P. falciparum* Lines in Pregnant Women at Delivery, According to Year.

Panel A shows IgG antibodies, expressed as mean fluorescence intensity (MFI, measured in arbitrary fluorescence units), against a chondroitin sulfate A–binding parasite line (CS2), and Panel B shows MFI for IgG antibodies against a rosetting parasite line (R29). I bars represent geometric means plus 95% confidence intervals. Dashed lines indicate the mean plus 3 SD from the negative control values. P values are based on a multivariate analysis adjusted for HIV status, parity, and age.

(10.5±1.1 g per deciliter vs. 10.6±1.5 g per deciliter; mean difference, −0.12 g per deciliter; 95% CI, −0.67 to 0.43; P=0.67, adjusted). Similarly, past or chronic placental infections, which have been shown to lead to low birth weight because of fetal growth restriction,[5] were associated with larger reductions in the weight of newborns during the 2010–2012 period (2863±440 g in infected women vs. 3070±482 g in uninfected women; mean difference, −164.5 g; 95% CI, −289.7 to −39.4; P=0.01, adjusted; P=0.02 after Bonferroni correction) than during the 2003–2005 period (2994±487 g vs. 3117±455 g; mean difference, −44.8 g; 95% CI, −139.1 to 49.5; P=0.35, adjusted).

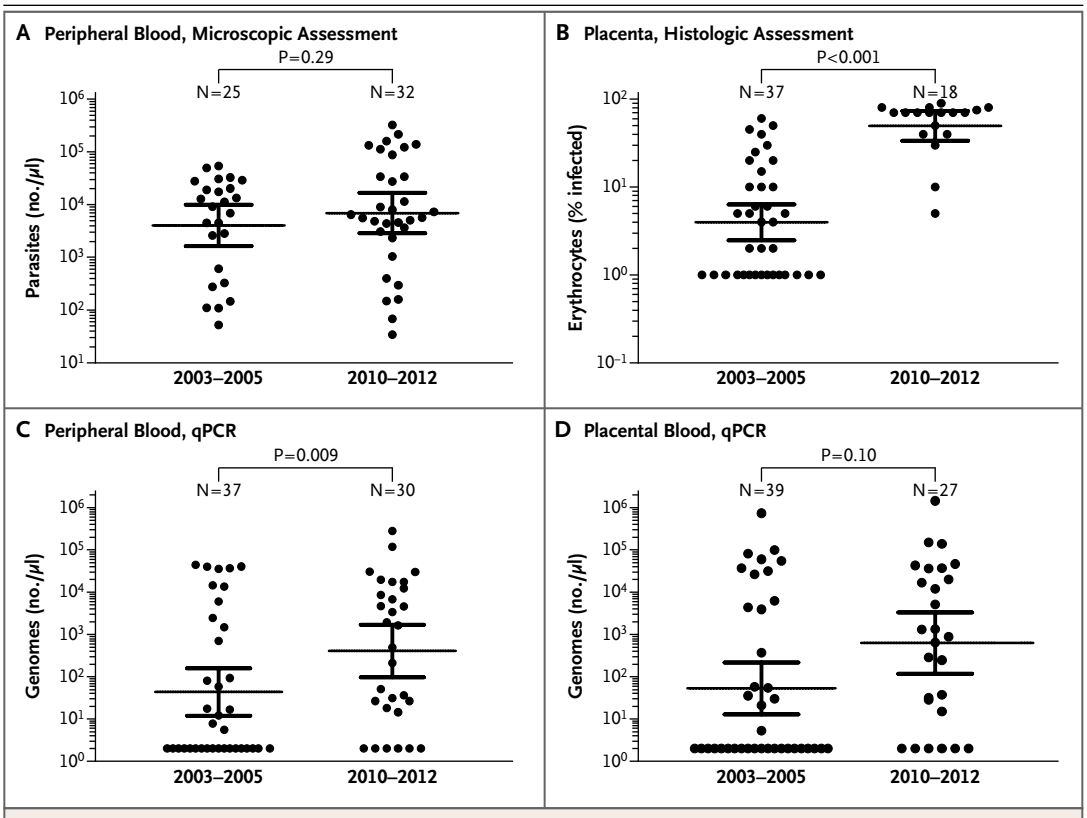

Figure 3. *P. falciparum* Densities in Pregnant Women Infected at Delivery, According to Study Period.

Results are shown for microscopic assessment of peripheral blood (Panel A), histologic assessment of the placenta (Panel B; lower limit of quantification, ≤1%), and qPCR testing of peripheral and placental blood (Panels C and D, respectively). For Panels C and D, the lower limit of quantification was 2 genomes per microliter, which was the number assigned if qPCR amplification was observed outside the lower range of the standard curve (i.e., 5 genomes per microliter). I bars represent geometric means plus 95% confidence intervals. P values are based on a multivariate analysis adjusted for HIV status, parity, and age.

EFFECT OF PARITY ON PARASITOLOGIC AND IMMUNOLOGIC OUTCOMES

The relationship of parity to parasite density, submicroscopic infections, and IgG levels differed between the two study periods. In the 2003–2005 period, higher parity was associated with a decrease in parasite densities in peripheral blood on microscopic examination and qPCR testing, as well as in placental blood on qPCR testing (Table 2). In contrast, parasite densities were similar between primigravid and multigravid women in the 2010–2012 period. The prevalence of peripheral-blood infections that were submicroscopic was higher among multigravid women (87% [20 of 23 infections]) than among primigravid women (43% [6 of 14], P=0.008) in 2003–2005 but was similar in primigravid women and multigravid women (38% [3 of 8 infections] and 50% [11 of 22], respectively; P=0.55) in 2010–2012. Finally, the increase in levels of pregnancy-specific IgG antibodies (i.e., IgG antibodies against CS2 parasites) with increasing parity was more marked among women delivering in 2003–2005 (adjusted ratio of mean MFI in multigravid women to mean MFI in primigravid women, 4.38; 95% CI, 2.30 to 8.36; P<0.001) than among those delivering in 2010–2012 (adjusted ratio of mean MFI, 1.49; 95% CI, 1.11 to 2.01; P=0.009) (Table 2), with similar results for the level of IgG antibodies against DBL3X and DBL5ε from VAR2CSA (Table S4 in the Supplementary Appendix). No parity effect was observed for levels of IgG antibodies against R29.

Table 2. Densities of *P. falciparum* and Levels of IgG Antibodies against *P. falciparum* Lines, According to Study Period and Parity.*

Outcome	2003–2005					2010–2012					P Value for Interaction†
	Primigravid		Multigravid		P Value	Primigravid		Multigravid		P Value	
	no. of patients	*geometric mean*	*no. of patients*	*geometric mean*		*no. of patients*	*geometric mean*	*no. of patients*	*geometric mean*		
Parasite density in the placenta											
Histologic assessment (% of infected erythrocytes)	20	5.9±8.4	17	2.5±3.2	0.06	9	47.7±43.8	9	51.8±33.9	0.83	0.18
qPCR testing (no. of genomes/μl)	13	857.3±4146.1	26	13.2±44.4	0.003‡	12	200.9±786.2	15	1548.4±6787.2	0.22	0.004
Parasite density in peripheral blood											
Microscopic assessment (no. of parasites/μl)	15	13,825.6±17,879.4	10	631.9±1228.5	<0.001	15	4269.0±13,248.4	17	10,647.9±18,047.6	0.30	0.001
qPCR testing (no. of genomes/μl)	14	546.6±2540.5	23	9.4±21.7	<0.001‡	8	988.3±4271.4	22	296.3±1098.3	0.46	0.14
IgG antibodies (MFI)§											
CS2 parasite line	45	2307.8±2097.3	101	9306.1±13,336.6	<0.001‡	131	1763.4±1198.7	376	3611.2±4206.7	<0.001‡	0.003
R29 parasite line	45	8390.4±8545.1	101	7579.1±9346.3	0.63	131	3071.0±5157.8	376	3819.4±6115.9	0.19	0.13

* Plus–minus values are geometric means ±SD.
† P values are for the interaction between study period and parity. P values for the interaction after Bonferroni correction were as follows: P=0.02 for placental parasite density on qPCR testing, P=0.005 for peripheral parasite density on microscopic assessment, and P=0.02 for IgG antibodies against the CS2 parasite line.
‡ The P value is significant according to a multivariate analysis adjusted for HIV status, parity, and age.
§ Mean fluorescence intensity (MFI) was measured in arbitrary fluorescence units.

DISCUSSION

Substantial declines in the prevalence of malaria among Mozambican pregnant women (from 11% in 2003–2005 to 2% in 2010–2012 on the basis of peripheral-blood microscopic assessment or placental histologic assessment, and from 30% to 4% on the basis of qPCR assay) were paralleled by reductions in levels of antimalarial IgG antibodies. These changes over a 10-year period were associated with increasing parasite densities and a larger adverse effect of infection on maternal hemoglobin levels and on the weight of newborns during the period of low malaria prevalence. Taken together, the data suggest that a weakening of antimalarial immunity as a result of infrequent parasite exposure can increase the occurrence of high-density infections and their harmful effect among women who become infected, as well as among their newborns.

The decline in the prevalence of malaria between 2003 and 2010, which was associated with a slight increase in the mean maternal hemoglobin level, is consistent with reductions in other areas of sub–Saharan Africa over similar time frames,[9,10,31,32] suggesting a continent-wide trend during this period. Intermittent preventive treatment with mefloquine during pregnancy in a number of the women between 2010 and 2012 was not responsible for the decrease in the prevalence of malaria, since similar declines were observed when these women were excluded from the analysis. Also, the numbers of malaria-susceptible primigravid women, rates of HIV infection, and CD4+ T-cell counts in HIV-infected women were relatively constant throughout the study period, and all the women received bed nets treated with a long-lasting insecticide, suggesting that these factors do not fully account for the changes observed. The introduction of artemisinin-based combination therapies in Mozambique in 2009, together with increasing use of intermittent preventive treatment with sulfadoxine–pyrimethamine during pregnancy and insecticide-treated bed nets, as recommended by the World Health Organization in 2005, may have contributed to the observed decline in the prevalence of malaria, together with socioeconomic development, improved accessibility to health care facilities, and continuous conduct of clinical trials and other investigations in the study area. The increases in prevalence between 2010 and 2012 without evident changes in malaria-control efforts suggest that climate factors, as well as increasing mosquito and parasite resistance to insecticides and antimalarial agents, respectively, may have played a role. Although similar trends have been observed in malaria-related hospital admissions in the area,[33] it remains unclear whether health care facility–based trends in malaria among pregnant women who are targets of intensive preventive measures can be extrapolated to the general community.

Levels of IgG antibodies against CS2 parasites (pregnancy-specific immunity) and R29 parasites (general immunity) mirrored changes in the prevalence of malaria infection. Moreover, levels of IgG antibodies against CS2 were increased in women with qPCR-positive *P. falciparum* infection at delivery.[16,17] These results indicate a close relationship between antibody levels and the intensity of malaria transmission[16-18,34-36] and suggest that antibodies against VAR2CSA may be a marker for cumulative exposure to the parasite during pregnancy.[19] More important, this study shows that 5 years of a marked decline in the prevalence of malaria was accompanied by reductions in levels of IgG antibodies against CS2 parasites by a factor of 2.8 and by reductions in IgG antibodies against antigens that are not specific to pregnancy, as well as by an increase in the geometric mean of parasite densities and a larger adverse effect of these infections on maternal hemoglobin levels and the weight of newborns. Reduced opportunities to acquire immunity during pregnancy because of low infection rates may render multigravid women as susceptible to malaria as primigravid women, as suggested by the similar parasite densities and levels of IgG antibodies against CS2 in the two groups of women during the period of low malaria prevalence. In accordance with previous reports on pregnant women[9] and children[37,38] residing in areas where malaria has declined substantially, these observations suggest that infrequent parasite exposure can weaken immune regulation of parasite density and increase the occurrence of adverse clinical outcomes among women who become infected, as well as among their newborns. However, the increase in antibody levels between 2010 and 2012 after relatively modest increments in the prevalence of infection suggests that immunity may be regained as exposure increases.

Our study has several limitations. Although similar procedures were followed in both study periods, possible confounding factors, such as changes in health care (i.e., access to antiretroviral agents and prenatal care) and in other vectorborne infections, were not measured, and we did not control for pooling of data from separate cohorts in this study. Moreover, estimates of the prevalence of malaria among women undergoing malaria-prevention measures at the hospital may not be representative of the prevalence in the community at large.

In conclusion, this study shows a rise in malaria with increased parasite densities and a larger adverse clinical effect after a marked fall in the prevalence of malaria and antimalarial immunity among pregnant women. These data reinforce the importance of sustaining efforts to avoid rebounds of malaria associated with reductions in naturally acquired immunity.[39]

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