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Malaria in the First Trimester of Pregnancy and Fetal Growth: Results from a Beninese Preconceptional Cohort

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Background. Malaria in early pregnancy occurs at a time when the placenta is developing, with possible consequences for placental function and fetal growth. We assessed the association between first trimester malaria and fetal growth documented through repeated ultrasound scans.

Methods. The RECIPAL preconceptional cohort included 411 Beninese pregnant women followed from 7 weeks' gestation (wg) until delivery. Among them, 218 had 4 scans for fetal monitoring at 16, 22, 28, and 34 wg. Multivariate seemingly unrelated regression models were used to assess association of microscopic malaria in the first trimester (<15 wg) with abdominal circumference, head circumference, biparietal diameter, and femur length throughout pregnancy.

Results. Of 39% (86/218) of women with at least 1 microscopic malarial infection during pregnancy, 52.3% (45/86) were infected in the first trimester. Most women (88.5%) were multiparous. There was no association between adjusted z-scores for fetal growth parameters and first trimester malaria. Parity, newborn sex, socioeconomic level, and maternal body mass index significantly influenced fetal growth.

Conclusions. In a context where malaria infections in pregnancy are well detected and treated, their adverse effect on fetal growth may be limited. Our results argue in favor of preventing and treating infections as early as the first trimester.

Keywords. malaria; epidemiology; fetal growth; Africa; modeling.

In sub-Saharan Africa, malaria in pregnancy is highly prevalent [1]. It is also one of the main risk factors for both low birthweight (LBW; defined as birthweight less than 2500 g) and small-for-gestational age (SGA; defined as a birthweight below the 10th centile for a given gestational age according to a reference chart). In 2017, it was estimated that malaria in pregnancy was responsible for 16% of all LBW babies in sub-Saharan Africa. Malaria-related LBW (and SGA) is due to fetal growth restriction (FGR), prematurity, or a combination of both [2]. It is generally believed that placental parasitization—and related inflammation—is the main underlying cause of FGR [3].

In recent years, the use of ultrasound scans (US) has made it possible to date pregnancies more accurately and to better determine the effect of malaria on fetal and perinatal outcomes according to its timing during pregnancy. Thus there is now evidence of the adverse effects of malaria in the first half of pregnancy on LBW [4, 5]. Because pregnant women usually attend their first antenatal care (ANC) visit at 4 or 5 months of pregnancy, however, there is a lack of data on the effect of malaria in the first trimester specifically. From a pathophysiological point of view, the first trimester corresponds to the period when the placenta is developing and malarial infections occurring at this time may be particularly harmful by impairing placentation and vascularization leading to placental dysfunction and FGR [6, 7]. While malaria in the first trimester has been associated with miscarriage [8, 9], fetal loss [10], and fetal growth alterations [11] in South East Asia, there is less evidence from sub-Saharan Africa. In studies conducted in Benin, Tanzania, and Burkina Faso, malaria in the first trimester was associated with growth alterations at the end of pregnancy [12, 13] as well as LBW [14]. In the absence of specific interventions against malaria

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in pregnancy, 65% of placental infection—and related morbidity such as FGR—is estimated to occur during this period of pregnancy [15].

The combined assessment of both malaria in the first trimester and fetal growth evaluated in utero requires data to accurately estimate gestational age and fetal growth, as well as longitudinal information on malaria and other maternal risk factors for FGR. In the present study, we assessed the association between malarial infections in the first trimester and fetal growth in Benin, using repeated US collected specifically to answer this question.

METHODS

Study Design, Population, and Procedures

We used data from the preconceptional Retard de Croissance Intrauterin et Paludisme (RECIPAL) study conducted in the districts of Sô-Ava and Abomey-Calavi, South Benin, between June 2014 and September 2017. In the area, malaria is hyperendemic and *Plasmodium falciparum* is the most common species [16]. RECIPAL's main objective was to assess the effect of malaria during the first trimester of pregnancy on fetal growth. The study protocol has been described elsewhere [17]. Briefly, a total of 1214 women of childbearing age were recruited at community level and followed monthly at home for a maximum period of 24 months until becoming pregnant. To be recruited, women had to meet the following criteria: negative urinary pregnancy test at inclusion, 18 to 45 years old, no current contraception, no previous fecundity issues, willingness to become pregnant, no planned travel for more than 2 months within the next 18 months, acceptance of RECIPAL protocol, and signed written informed consent. At each monthly visit, the first day of last menstrual period was recorded and a urinary pregnancy test was performed. Out of the 1214 women of childbearing age, 411 were identified as pregnant and followed monthly at the maternity clinic from the earliest days of pregnancy until childbirth.

Women's demographic and socioeconomic characteristics, as well as reproductive history, were collected at enrolment in the cohort. Follow-up during pregnancy included clinical, malaria, nutritional, anthropometric, and US data monitoring. In particular, pregnant women had 5 Doppler US. The first one was performed between 9 and 13 weeks' gestation (wg) ±1 week for accurately dating the pregnancy. Dating was based on the crown-rump length (CRL) measurement using Robinson chart [18]. Gestational age was based on the last menstrual period if the difference between the last menstrual period and CRL was less than 7 days or on CRL if the difference was >7 days [19]. Then, 4 additional standardized US were performed every 6 weeks (±1week) for fetal growth monitoring, so that the possible ranges of gestational age were 15–20, 21–26, 27–32, and 33–38 wg. At each US, head circumference (HC), abdominal circumference (AC), biparietal diameter (BPD), and femur length (FL) were measured twice in 2 separate subsequent images. USs were performed by 4 skilled obstetrician-gynecologists (WA, AD, MM, TL) using a portable ultrasound system (high-resolution ultrasound system, 5–2 MHz C60 abdominal probe; Sonosite M-TURBO). Throughout the study, a random selection of 10% of the images was reviewed by a senior obstetrical sonographer to verify that the measurements fulfilled the INTERGROWTH-21st guidelines [19].

Women's anthropometric measurements including weight and height were collected every 3 months before pregnancy, and then monthly during pregnancy. Blood pressure, proteinuria, and urinary tract infection were monitored monthly during pregnancy.

Women were screened for malaria at each scheduled ANC visit (approximately every month) using a thick blood smear (TBS). In addition, they were encouraged to attend the maternity clinic anytime outside the scheduled visits in case of symptoms. In case of fever or symptoms suggestive of malaria, both a TBS and a rapid diagnostic test (*P. falciparum* + pan rapid test SD Bioline Ag, IDA Foundation; Biosynex) were performed. For TBS analysis, the Lambaréné technique was used to quantify parasitemia, with a detection threshold estimated to be 5 parasites/μL [20].

Women with uncomplicated malaria were treated immediately with oral quinine in the first trimester and artemether-lumefantrine in the second and third trimesters. Those with severe malaria received intravenous artesunate until oral medication could be tolerated. Anemic pregnant women were either treated with oral ferrous sulfate or transfused, depending on the severity. Intermittent preventive treatment with sulphadoxine-pyrimethamine was administered as per current national guidelines (moving from 2 to 3 doses during the RECIPAL study). Also, women received an insecticide-treated net at their first ANC visit, plus folic acid and iron supplementation every month. Newborns were weighed within 1 hour after birth on an electronic digital scale with an accuracy of 2 g (SECA).

The RECIPAL study received ethical approval from the Beninese Ethics Committee of the Institut des Sciences Biomédicales Appliquées and the Ministry of Health. All participants gave informed written consent before enrollment in the cohort.

Statistical Analysis

For each US and each set of fetal measurements, Bland-Altman plots were used to assess the intraoperator variability. Measurements that fell outside the acceptable ranges for each parameter were identified and checked [21]. These were mainly due to data entry errors and were corrected by returning to the source data. Then, the mean of the 2 measures of each parameter collected per US was used for the analysis.

Women who had a single live birth with no congenital malformation, and who had full US and malaria follow-up, were selected for the analysis. Our main exposure was malaria infection in the first trimester of pregnancy. Malaria in the first trimester was defined as at least 1 positive TBS before 15 wg corresponding to the period when the first US for fetal biometry was carried out. This cutoff has been used in other articles on malaria in pregnancy [22, 23]. Two positive TBS less than 3 weeks apart

were considered as a single infection. For the present analysis, we only considered malaria detected on TBS because the combined use of both TBS and RDT was only performed for a subgroup of women with symptoms suggestive of malaria. Finally, outcomes were fetal growth measurements throughout the pregnancy. Fetal growth measurements were transformed into z-scores according to INTERGROWTH-21st standards [24].

First, we performed univariate analyses where mean z-scores were compared between women infected with malaria in the first trimester versus women who were not infected throughout the pregnancy. Then, we conducted 2 complementary analyses. The first series of analyses tested the effect of malaria in the first trimester on fetal parameters measured at each of the 4 US separately. The 4 fetal parameters were modelled simultaneously in each cross-sectional model to take into account their correlation. In these models, the effect of malaria was assessed within a limited window of time but on all parameters at the same time. The second series of analyses consisted of a longitudinal analysis testing the effect of malaria in the first trimester on the 4 z-scores of a single fetal parameter simultaneously. This latter analysis aimed to assess the short-term or long-term effects of malaria in the first trimester on each parameter.

For both analyses, we used a seemingly unrelated regression (SUR) model that combines a number of linear models to take into account the correlation between the error terms in each linear model [25, 26]. In a SUR model, the dependent variables can be different variables observed at the same time (hereafter called cross-sectional model) or the same variables observed at different times (hereafter called longitudinal model). Also, this model allows for the same or different covariables in each linear equation.

Each analysis was adjusted for potential confounders. Their selection was made a priori based on both biological plausibility and the scientific literature, and not on a cutoff for statistical significance in line with current recommendations [27]. The following covariables were selected: maternal body mass index (BMI), parity (0, 1 to 4, 5 and more previous deliveries), socioeconomic status, and newborn sex. We chose parity instead of gravidity because of the known association of parity with birthweight [28]; the association with gravidity on growth is less clear cut. BMI was calculated based on weight and height measurements before conception. Then, it was classified into low (<18.5 kg/m^2), normal (18.5–24.9 kg/m^2), and high (>25 kg/m^2) according to World Health Organization classification. Socioeconomic status was approximated using a synthetic score combining occupation and ownership of assets, which was then categorized according to tertiles. Malaria in the second (from 15 to 27 wg) and third (from 28 wg onwards) trimesters was also included in the models. Both variables were considered as time-dependent variables, so that each of them was coded specifically for each US; only malarial infections that occurred before a given US were considered as a source of exposure. Anemia and gestational weight gain were considered as intermediate factors for the association between malaria and fetal growth, and therefore were not included in the model. All covariables were kept in the final models whatever their level of statistical significance. As an example, the system of linear equations of 2 SUR models is presented in Supplementary Figure 1.

RESULTS

Selection and Characteristics of the Studied Population

Out of the 411 RECIPAL pregnant women, 88 were excluded from the present analysis because they did not have an US follow-up; most of them had a miscarriage before the first US for dating the pregnancy. Among the 323 women with an US follow-up, further exclusions were due to nonviable pregnancies for which there was no fetal biometry monitoring (n = 21), twin pregnancies (n = 7), migration from the study area or withdrawal of consent (n = 8), and performance of only 1 US for fetal biometry (n = 11). Among the remaining 266 pregnant

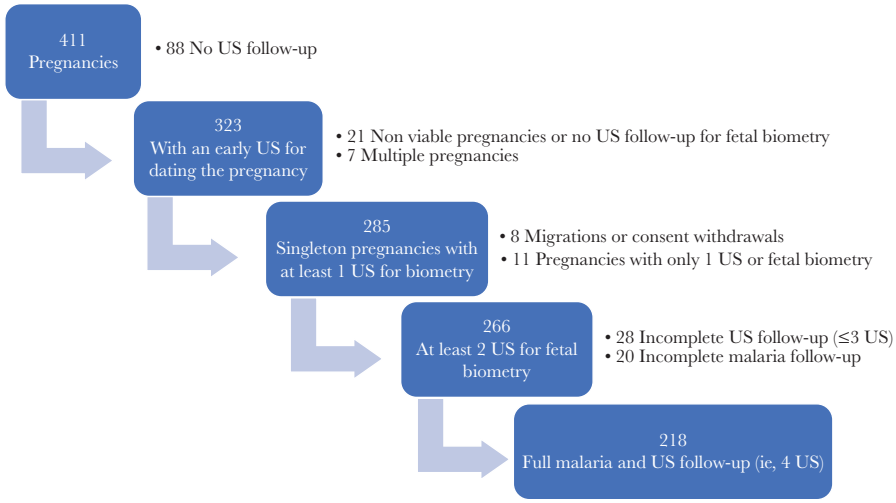

Figure 1. Flow chart of the studied population. Abbreviation: US, ultrasound.

women, 238 had a complete fetal biometry monitoring (ie, 4 US); of these, 218 had a full malaria follow-up and constituted our study population (Figure 1). These 2 groups of women (266 vs 218) appeared to have similar characteristics, particularly in terms of malaria exposure and z-scores for fetal parameters.

Among the 218 pregnant women, 86 (39.4%) had at least 1 microscopic malarial infection during pregnancy versus 132 (60.6%) for whom no microscopic malaria was detected. Among the infected women, 45, 35, and 29 were infected at least once in the first, second, and third trimester, respectively; 21 of them were infected twice or more during pregnancy. Fifty-two percent (45/86) of the infected women had at least 1 malaria infection in the first trimester; of these, 17 were infected both in the first trimester and later on. Most women were multiparous (Table 1). Mean gestational age at inclusion was 6.7 wg (SD 2.1 wg). Very few women (fewer than 1%) were infected with human immunodeficiency virus (HIV) or presented high blood pressure during pregnancy, or declared smoking or consuming alcohol during pregnancy. The median BMI was 21.9 kg/m^2 (interquartile range [IQR], 20.2–24.5), and the median gestational weight gain was 9.3 kg (IQR, 6.8–11.6). Baseline characteristics of women infected with malaria in the first trimester versus those not infected in the first trimester are presented in Table 1. Infected and noninfected women had similar characteristics except for socioeconomic level (P = .03) and Intermittent Preventive Treatment in Pregnancy (IPTp) coverage (P = .02), which were higher in noninfected compared to infected women.

USs were performed at a mean of 16, 22, 28, and 34 wg. Table 2 presents the mean values and mean z-scores of AC, HC, FL, and BPD parameters at each US. For all parameters except BPD, the z-scores were positive, meaning that RECIPAL values were higher than those from INTERGROWTH-21st.

Table 1. Characteristics of the 218 Pregnant Women According to Their Malaria Status in the First Trimester of Pregnancy

Variable	At Least 1 Microscopic Malarial Infection at 1st Trimester (n = 45)	No Microscopic Malarial Infection at 1st Trimester (n = 173)	All Women (n = 218)	P[a]
Age, y, median (IQR)	25 (23–29)	27 (23–30)	26 (23–30)	.19
Height, cm, median (IQR)	157.5 (154.1–161.5)	158.6 (155.3–161.8)	158.6 (154.9–161.8)	.24
Weight at 1st ANC visit, kg, mean (SD)	57.9 (13.0)	57.2 (10.3)	57.3 (10.9)	.83
BMI, kg/m^2, median (IQR)	21.9 (20.3–25.7)	21.9 (20.2–24.3)	21.9 (20.3–24.7)	.70
BMI class				
Normal	62.2	69.3	67.9	.61
Underweight	8.9	8.7	8.7	
Overweight/obesity	28.9	22.0	23.4	
GWG, kg, median (IQR)	8.9 (4.9–11.2)	9.2 (7.1–11.5)	9.1 (6.8–11.38)	.08
HIV status				
Positive	4.4	1.1	1.5	…
Negative	95.6	96.0	95.5	
Not known	0.0	2.9	3.0	
Parity				
No previous delivery	7.8	9.8	11.5	.32
1–4 deliveries	60.0	67.1	65.6	
5 or more deliveries	22.2	23.1	22.9	
Gestational age at 1st US for dating pregnancy, wk, mean (SD)	11.3 (1.4)	11.3 (1.3)	11.3 (1.4)	.82
Education				
Literate	20.0	33.5	30.7	.12
Illiterate	80.0	66.5	69.3	
Number of IPTp doses				
0	6.7	2.3	3.2	.05
1	26.7	16.2	18.3	
2	62.2	65.3	64.7	
3	4.4	16.2	13.8	
SES				
Higher tertile	51.1	30.6	34.9	.04
Intermediate tertile	26.7	39.3	36.7	
Lower tertile	22.2	30.1	28.4	

Data are percentages except where indicated.

Abbreviations: ANC, antenatal care visit; BMI, body mass index; GWG, gestational weight gain; IPTp, Intermittent Preventive Treatment in pregnancy; IQR, interquartile range; SES, socioeconomic status.

[a] P indicates the P value either of a t test (comparison of 2 means) for quantitative variables or a Pearson χ^2 test for categorical variables.

Table 2. Mean (SD) Values and Mean Z-Scores for AC, HC, FL, and BPD at Each Ultrasound Scan

	US1	US2	US3	US4
Gestational age, wk	16.92 (1.3)	22.59 (1.2)	28.48 (1.2)	34.41 (1.1)
Fetal parameters				
Raw values				
AC, mm	113.8 (14.6)	176.7 (14.9)	239.9 (16.7)	299.4 (16.1)
HC, mm	136.6 (16.1)	204.0 (14.1)	266.9 (12.9)	310.4 (11.7)
FL, mm	23.0 (4.2)	39.5 (3.5)	53.9 (3.3)	65.8 (3.2)
BPD, mm	38.2(4.5)	56.2 (4.1)	73.6 (3.9)	86.4 (3.9)
Z-scores				
AC	0.09 (1.5)	0.09 (1.1)	0.16 (1.2)	0.14 (1.0)
HC	0.35 (1.3)	0.14 (1.1)	0.27 (1.2)	0.02 (1.1)
FL	0.60 (1.5)	0.82 (1.3)	1.16 (2.0)	1.66 (2.4)
BPD	−0.20 (1.3)	−0.24 (1.1)	−0.38 (1.2)	−0.62 (1.1)

Analyzed population, n = 218. Z-scores were calculated using INTERGROWTH 21st standards.

Abbreviations: AC, abdominal circumference; BPD, biparietal diameter; FL, femur length; HC, head circumference.

Effect of Malaria in the First Trimester of Pregnancy on Fetal Growth

In univariate analysis, z-scores for all parameters were globally higher in infected than in uninfected women in the first trimester, but the difference was only statistically significant for AC at the fourth US in the third trimester (Table 3).

In multivariate analysis, using the longitudinal SUR model, we did not find any significant association between malaria in the first trimester and z-scores throughout the pregnancy, for all the fetal parameters considered (Table 4). Only malaria in the third trimester was associated with a significantly higher z-score for AC at the fourth US. The other covariables significantly associated with the z-scores for fetal parameters were the following: increasing BMI was consistently associated with higher z-scores for all the fetal parameters and US considered; male sex was associated with higher z-scores for AC, HC, and BPD, in particular in the second trimester; there was no consistent association between socioeconomic status and z-scores; and multiparous women had higher z-scores than nulliparous in the third trimester, although

Table 3. Mean Differences in Unadjusted Z-Scores for AC, FL, HC, and BPD Parameters According to Microscopic Malaria in the First Trimester

	Mean Z-Score			
Fetal Parameter	Women Not Infected With Malaria Throughout the Pregnancy	Women Infected With Malaria in the 1st Trimester	Mean Difference Between Uninfected and Infected Women[a,b]	*P*[c]
AC_1	0.04	0.01	0.03	.90
AC_2	0.03	0.28	−0.25	.16
AC_3	0.05	0.28	−0.23	.20
AC_4	**0.03**	**0.37**	**−0.34**	**.05**
FL_1	0.60	0.57	0.03	.91
FL_2	0.82	1.13	0.31	.17
FL_3	1.00	1.40	0.40	.22
FL_4	1.65	1.65	0.00	1.00
HC_1	0.33	0.32	0.01	.97
HC_2	0.05	0.31	−0.26	.16
HC_3	0.15	0.24	−0.09	.62
HC_4	−0.07	0.03	−0.10	.61
BPD_1	−0.23	−0.17	−0.06	.77
BPD_2	−0.35	−0.03	−0.32	.09
BPD_3	−0.47	−0.43	−0.04	.84
BPD_4	−0.67	−0.70	0.03	.89

The notations in the first column indicate the z-scores at the 1st (16 wg), 2nd (22 wg), 3rd (28 wg), and 4th (34 wg) US; eg, AC_2 is the mean z-score for AC measured at the 2nd US. Z-scores were calculated using INTERGROWTH-21st standards. Values in bold indicate statistically significant results at 5% level.

Abbreviations: AC, abdominal circumference; BPD, biparietal diameter; FL, femur length; HC, head circumference; US, ultrasound scan; wg, weeks' gestation.

[a]Numbers of women included in the analysis at the 1st, 2nd, 3rd, and 4th US (for all fetal parameters, ie, AC, FL, HC, and BPD) were 45 women infected in the 1st trimester vs 132 women not infected throughout the pregnancy.

[b]A positive value corresponds to a higher z-score in uninfected women, while a negative value corresponds to a higher z-score in infected women.

[c]*P* value of *t* test comparing the mean z-score between infected and not infected women.

Table 4. Factors Associated With Z-Scores for AC, HC, FL, and BPD Parameters: Longitudinal Multivariate Analyses 1 Single Fetal Parameter at a Time Measured Throughout the Pregnancy (Seemingly Unrelated Regression Model)

		Coefficient (SE) $_{Category}$						
Model	Fetal Parameter	BMI	Sex, Male	SES[a]	Parity[b]	Malaria 1st Trimester	Malaria 2nd Trimester[c]	Malaria 3rd Trimester[c]
Model AC	AC_1	**0.05** (3)*	0.11 (20)	−0.05 (24)$_2$ −0.19 (27)$_3$	0.07 (33)$_2$ 0.05 (38)$_3$	−0.13 (25)		
	AC_2	0.03 (2)	**0.30** (14)*	0.25 (17)$_2$ **0.48** (19)$_3$*	−0.01 (23)$_2$ −0.28 (27)$_3$	0.30 (18)	0.02 (17)	
	AC_3	**0.04** (2)*	**0.37** (16)*	−0.08 (20)$_2$ −0.12 (22)$_3$	−0.18 (26)$_2$ −0.05 (30)$_3$	0.10 (20)	−0.04 (20)	
	AC_4	**0.05** (2)**	0.06 (13)	0.20 (15)$_2$ −0.20 (17)$_3$	**0.44** (20)$_2$* 0.23 (24)$_3$	0.28 (16)	−0.154 (16)	**0.41** (16)**
Model HC	HC_1	**0.07** (2)**	0.17 (18)	−0.16 (21)$_2$ −0.28 (24)$_3$	−0.21 (29)$_2$ −0.28 (34)$_3$	−0.12 (22)		
	HC_2	0.03 (2)	**0.40** (14)**	−0.05 (17)$_2$ **0.05** (19)$_3$*	−0.37 (23)$_2$ −**0.66** (27)$_3$*	0.17 (18)	0.01 (15)	
	HC_3	**0.05** (2)*	**0.59** (16)***	−0.28 (19)$_2$ −0.30 (21)$_3$	−**0.51** (26)$_2$* −0.34 (30)$_3$	−0.16 (20)	0.12 (19)	
	HC_4	**0.05** (2)**	**0.32** (14)*	−0.09 (17)$_2$ −**0.48** (19)$_3$**	**0.50** (23)$_2$* **0.72** (27)$_3$**	−0.06 (18)	0.20 (18)	0.20 (17)
Model LF	LF_1	**0.10** (3)***	0.12 (20)	0.06 (24)$_2$ −0.23 (26)$_3$	−0.29 (31)$_2$ −0.47 (37)$_3$	−0.09 (24)		
	LF_2	**0.06** (2)**	−0.16 (18)	0.38 (21)$_2$ 0.14 (24)$_3$	−0.43 (29)$_2$ −**0.75** (34)$_3$*	0.41 (22)	−0.24 (20)	
	LF_3	**0.10** (4)**	0.15 (28)	−0.007 (33)$_2$ −0.17 (37)$_3$	−0.01 (44)$_2$ 0.07 (30)$_3$	0.25 (34)	0.02 (33)	
	LF_4	**0.08** (4)*	0.11 (33)	0.40 (40)$_2$ −0.07 (44)$_3$	0.51 (53)$_2$ 0.06 (62)$_3$	0.02 (41)	−0.23 (41)	0.30 (38)
Model BPD	BPD_1	**0.05** (2)*	0.13 (18)	−0.06 (21)$_2$ −0.09 (23)$_3$	−0.44 (28)$_2$ −0.55 (32)$_3$	−0.02 (22)		
	BPD_2	0.02 (2)	**0.38** (5)**	0.03 (18)$_2$ 0.18 (20)$_3$	−**0.53** (23)$_2$* −**0.79** (27)$_3$**	0.24 (18)	−0.001 (16)	
	BPD_3	0.04 (2)	**0.54** (16)***	−0.36 (20)$_2$ −0.26 (22)$_3$	−0.37 (26)$_2$ −0.27 (30)$_3$	−0.18 (20)	0.004 (19)	
	BPD_4	0.04 (2)	0.25 (15)	−0.23 (18)$_2$ −**0.50** (20)$_3$**	0.42 (23)$_2$ **0.68** (27)$_3$**	−0.15 (18)	−0.04 (18)	0.11 (17)

Analyzed population, n = 218. Four distinct multivariate models were run, 1 for each fetal parameter. The notations in the second column indicate the z-scores at the 1st, 2nd, 3rd, and 4th US; eg, AC_2 is the z-score for AC measured at the 2nd US. Z-scores were calculated using INTERGROWTH-21st standards. In each cell, each line contains 2 parameters: the coefficient of regression and its standard error (×100): eg, large multiparous women (ie, 5 or more previous deliveries) had fetuses with a significantly (**) higher z-score for BPD (+0.68 [0.27]) at the 4th US than nulliparous women. Values in bold indicate statistically significant results at 5% level. *** $P \leq .001$, ** $0.001 < P \leq .01$, * $0.01 < P \leq .05$.

Abbreviations: AC, abdominal circumference; BMI, body mass index; BPD, biparietal diameter; FL, femur length; HC, head circumference; SE, standard error; SES, socioeconomic status; US, ultrasound scan.

[a]SES was categorized according to tertiles: the lower tertile corresponds to the reference group, the intermediate tertile is coded 2, and the upper tertile is coded 3.

[b]Parity was categorized into 3 classes: no previous delivery corresponds to the reference group, 1–4 previous deliveries is coded 2, and 5 and more deliveries is coded 3.

[c]Malaria in the 2nd and in the 3rd trimesters were considered as time-dependent variables, so that only malarial infections that occurred before a given US were considered as an exposure.

this was the opposite in the second trimester. Globally, FL was less sensitive to the covariables than the other fetal parameters.

The results of the 4 multivariate cross-sectional analyses using a SUR model are presented in Supplementary Table 1. These were similar to those obtained with the longitudinal SUR model. Whatever the US considered, we did not find any significant association between malaria in the first trimester and z-scores for AC, HC, FL, and BPD. Paradoxically, malaria in the third trimester was associated with significantly higher z-scores for AC, HC, and BPD at the fourth US.

DISCUSSION

Women infected with microscopic malaria represented 39.6% of the study population; 52% of them were infected at least once in the first trimester of pregnancy. There was no association between malaria in the first trimester and z-scores for fetal growth parameters in adjusted models. Unexpectedly, there was a positive association between malaria in the third trimester and fetal parameters values at the end of pregnancy. In addition, maternal BMI preconception, parity, and newborn sex influenced fetal growth. AC, HC, and BPD were more likely to be impacted than FL regardless of the GA and the risk factor considered.

There is a lack of studies from sub-Saharan Africa countries investigating the consequences of malaria in the first trimester of pregnancy on fetal and birth outcomes [4, 5, 12, 13, 29]. RECIPAL was specifically designed to address this question. For that purpose, US and parasitological data were collected prospectively from the very beginning of pregnancy by

recruiting women in the preconception period. We did not evidence a negative association between microscopic malaria in the first trimester and z-scores for fetal parameters in multivariate SUR models. There are several possible explanations for these findings. Malaria infections in the first trimester have been associated with placental vascular development alterations [6] as well as dysregulation of angiogenesis, metabolism, and inflammation [7], which both contribute to placental dysfunction [30]. These effects may partly be mediated by the adhesion to extravillous trophoblasts of *P. falciparum* parasites, which have been shown to express VAR2CSA as early as 8 weeks of gestation [31]. VAR2CSA profile of these very early infections are currently being assessed using RECIPAL data. Their effect on placental blood flow is another important research question to address. However, these early infections might be a necessary but not sufficient condition for growth abnormalities. Previous findings from RECIPAL have suggested a cumulative rather than a punctual effect of malaria infections starting from the first trimester on the risk of LBW. Indeed, we showed that women infected both in the first trimester and later in the pregnancy were more likely to have a LBW baby compared to women uninfected during the whole pregnancy; this effect was not found in women infected in the first trimester only [32].

Another explanation may be that microscopic malarial infections were detected monthly and treated immediately in the RECIPAL study, thereby mitigating adverse effects. In RECIPAL, we did not control for parasitemia during or after treatment, but women were followed carefully from a clinical point of view. There were only a few women who remained or became symptomatic while treated with quinine in the first trimester. The very close follow-up of women in RECIPAL from the beginning of the first trimester may explain the difference in malaria-related effect between our study and previous studies carried out in Benin and Tanzania [12, 13, 29]. Furthermore, in these studies, malaria-related effects on birth weight and fetal growth were mainly shown in primi- and secundigravidae. Our study population consisted mainly in multigravidae, which may partly explain the lack of association between malaria and fetal growth in the present analysis.

In contrast, our analysis suggested a positive association between malaria in the third trimester and AC, HC, and BPD measurements in the mid-third trimester. This association may reflect a negative effect of submicroscopic infections in women in the "control" group (ie, women for whom no microscopic malaria was detected) and were not treated during the pregnancy. Indeed, malaria exposure was defined based on microscopy results only. It is likely that women in the "control" group were infected with submicroscopic infections, which are 2 to 3 times more frequent than microscopic infections [33], particularly at the end of pregnancy when women are no longer protected with IPTp [34]. It then becomes difficult to discriminate between the effect of treated microscopic malarial infections and untreated submicroscopic infections, which have been associated with poor birth outcomes [33, 35]. This issue clearly deserves to be addressed in future analyses and studies.

We acknowledge some limitations to the present study. First, our sample was restricted in this longitudinal study of fetal growth to women who had 4 US during their pregnancy. Because of the small differences in z-scores observed, we cannot exclude a lack of statistical power to demonstrate an association with malaria in the first trimester, although parameter estimates were not negative in adjusted models. In addition, nulliparous women, who are the most likely to have adverse events due to malaria, represented only 12% of our study sample. Because of the low number of nulliparous women, we were not able to assess the interaction between malaria and parity on fetal growth, which is an area for further study. Second, a high proportion of women were excluded from the analysis. Among them, a high number of women were excluded because the pregnancy was not viable; early miscarriages were reported in more than 70 women. In a previous analysis, we did not find any association between malaria and miscarriage (data not shown). Pregnant women were also excluded because of missing US or malaria data. While these women had similar baseline and malaria characteristics compared with included women, a selection bias cannot be excluded. Third, very few women had their last US at the end of the third trimester—most USs were performed around 34 wg before the peak growth velocity. This may have hindered the full assessment of the effect of malaria on fetal growth.

In conclusion, in a context where malarial infections in pregnancy are well detected and treated, their adverse effects on fetal growth may be mitigated. Our results argue in favor of preventing and treating infections as early as in the first trimester of pregnancy, as witnessed by the high proportions of infections occurring in the first trimester [36]. Although there have been concerns about the artemisinin drug class because of embryotoxic effects in rodents [37], there is increasing evidence of the efficacy [38] and safety [9, 39] of artemisinin-based combinations in pregnant women in the first trimester of pregnancy, reinforcing the idea of their use as first-line treatment for malaria [40, 41]. Also, there is a need to better prevent malaria during pregnancy by improving IPTp coverage, in particular at the end of the pregnancy [42]. Finally, preconceptional strategies such as vaccination against parasites expressing VAR2CSA [43] or drug-related strategies administered before conception might contribute to reducing the overall burden of malaria during pregnancy [34, 44].

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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