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***Plasmodium falciparum* Infection is Associated with Epstein–Barr Virus Reactivation in Pregnant Women Living in Malaria Holoendemic Area of Western Kenya**

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Abstract

Background—The role of *Plasmodium falciparum* malaria in EBV transmission among infants early in life remain elusive. We hypothesized that infection with malaria during pregnancy could cause EBV reactivation leading to high EBV load in circulation, which could subsequently enhance early age of EBV infection.

Method—Pregnant women in Kisumu, where *P. falciparum* malaria is holoendemic, were actively followed monthly through antenatal visits (up to 4 per mother) and delivery. Using real-time quantitative (Q) – PCR, we quantified and compared EBV and *P. falciparum* DNA levels in the blood of pregnant women with and without *P. falciparum* malaria.

Results—Pregnant women that had malaria detected during pregnancy were more likely to have detectable EBV DNA than pregnant women who had no evidence of malaria infection during pregnancy (64% vs. 36%, $p=0.01$). EBV load as analyzed by quantifying area under the longitudinal observation curve (AUC) was significantly higher in pregnant women with *P. falciparum* malaria than in women without evidence of malaria infection ($p=0.01$) regardless of gestational age of pregnancy. Increase in malaria load correlated with increase in EBV load (p

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<0.0001). EBV load was higher in third trimester ($p=0.04$) than first and second trimester of pregnancy independent of known infections.

Conclusion—Significantly higher frequency and elevated EBV loads were found in pregnant women with malaria than in women without evidence of *P. falciparum* infection during pregnancy. The loss of control of EBV latency following *P. falciparum* infection during pregnancy and subsequent increase in EBV load in circulation could contribute to enhanced shedding of EBV in maternal saliva and breast milk postpartum, but further studies are needed.

Keywords

EBV load; mother; pregnancy; *Plasmodium falciparum* malaria

Introduction

Epstein–Barr virus (EBV) is a human gammaherpesvirus that infects and persists in >90% of the world adult population clinically manifesting either as an asymptomatic infection or as acute infectious mononucleosis (1, 2). However, EBV infection is also associated with malignant transformation of infected cells and is etiologically linked to endemic Burkitt's lymphoma (eBL), the most common childhood cancer in equatorial Africa (3, 4). Previous epidemiologic studies (5–7) as well as recent studies done in Kenya (7) reported a striking overlap between elevated incidence of malaria transmission and eBL, and confirmed that holoendemic malaria was associated with an increased risk for eBL.

Geographical differences in age of primary EBV infections exist with infants in sub-Saharan Africa getting infected early in life than their counterparts in the developed world where infections occur in adolescence and adulthood (8, 9). We have previously reported that there is a significantly earlier age of primary EBV infection in infants from Kisumu – a malaria holoendemic region of Kenya – compared to infants from Nandi, an area with little malaria transmission (9), suggesting a role for malaria in increasing EBV risk in early infancy. Strikingly, 35% of children in Kisumu were infected before 6 months of age (9), supporting the long-held hypothesis that EBV infection early in life and holoendemic malaria are risk factors for eBL (34).

Cellular immunity, particularly CD8⁺ cytotoxic T lymphocytes (CTL) play a pivotal role in the immunosurveillance of EBV infections (10–12). As pregnancy is thought to be a state of repressed cellular immune responses (13, 14), it is possible that the immunological control of EBV infections may be naturally altered. Using serology as a marker of EBV reactivation, early studies showed EBV reactivation during pregnancy (15–17). However, Meyohas et al (18) measured EBV DNA in pregnant women and found no significant differences in the prevalence of EBV DNA in pregnant women than non-pregnant women. Moreover, EBV has rarely been implicated in in utero infection and few studies reported the risk of mother-to-child transmission of EBV (18, 19). Pregnant women are at a greater risk of infection with *P. falciparum* with substantial adverse effects on both maternal and fetal health (20, 21). *P. falciparum* malaria is known to interfere with both EBV biology and EBV-specific immunity (11, 22, 23) and can induce EBV reactivation (24, 25). To the best of our knowledge, no study has been done on the impact of malaria on EBV reactivation in

pregnant women residing in malaria holoendemic areas where many women are exposed to malaria throughout the course of their pregnancies (20).

Though both EBV infection early in life and holoendemic malaria are known risk factors for eBL, why children are infected early in life with EBV, and what role malaria plays in EBV transmission remain elusive. We hypothesized that infection with malaria during pregnancy could cause EBV reactivation leading to high EBV load in circulation, which in turn enhance early age of EBV infection. In this prospective study in Western Kenya where malaria is endemic and the eBL risk is high, pregnant women were actively followed through delivery, to test the hypothesis that *P. falciparum* malaria during pregnancy could cause EBV reactivation. Our data describe the frequency and quantity of EBV in pregnant women with and without *P. falciparum* infection and suggest that women infected with malaria during pregnancy were more likely to have indicators of EBV reactivation compared to women without evidence of *P. falciparum* infection.

Methods

Study participants

Pregnant women, of all gravidities, attending the antenatal clinic (ANC) were recruited from Chulaimbo sub-district hospital, in Kisumu District of western Kenya. The hospital serves predominantly rural populations. Transmission of malaria in this area is holoendemic (e.g. perennial and intense malaria transmission) with two seasonal peaks, June to August and November to December (22, 26). *Plasmodium falciparum* malaria species is the predominant species in the study region (27). All pregnant women were tested for HIV as part of Maternal-to-Child-Transmission (MCTC) of HIV programs in accordance with the Kenya Ministry of Health national guidelines.

The inclusion criteria consisted of pregnant women of all gravidities, less than 30 weeks gestation, having a normal full blood count, HIV negative, residence within a 10km distance of the hospital and willing to return to the clinic for follow up clinical procedures and laboratory testing. Gestational age was evaluated by measurement of fundal height and history of the last menstrual period. All pregnant women were enrolled in a six-month period from June to November 2011. As part of the study participant's health care, the pregnant women were closely monitored for any illness and all cases were reported to the study clinicians for treatment as per Kenya Ministry of Health guidelines. A total of 200 pregnant women were screened for inclusion into the study. Of these, 25 were HIV positive and were excluded. The remaining 175 pregnant women were longitudinally evaluated in an active monthly follow-up visits through antenatal visits (up to 4 per mother) and delivery. Of these, there were 93 pregnant women that delivered at Chulaimbo sub-district hospital and had samples collected at delivery.

Pregnant women enrolled showed no signs of symptomatic malaria during the follow up visits

All pregnant women were provided with insecticide treated bed nets, intermittent preventive treatment and deworming during pregnancy in accordance with the Kenya Ministry of Health guidelines.

Approval of this study was obtained from both the Kenya Medical Research Institute and SUNY Upstate Medical University Ethical Review Boards. Written informed consent was obtained from the study participants before any sample collection.

Sample collection and processing—At enrolment, participants provided 2–4 ml of venous blood sample collected in EDTA tubes and measurements of hemoglobin (Hb) levels were determined using a portable β -hemoglobin photometer (Hemocue AB Angelholm, Sweden), while complete blood counts were performed with a Beckman coulter AcT diff2 (Beckman-coulter corporations, Miami, FL, USA), calibrated regularly according to the manufacturer's instructions and use of appropriate controls. Urine and stool samples were obtained and examined for *schistosoma hematobium* and for the presence of intestinal parasites (pathogenic protozoans and helminthes) respectively. During follow-up visits, finger-prick blood was collected in EDTA tubes for malaria surveillance and EBV load. Less than 24 hours after delivery, mothers provided 2–4 ml of venous blood sample collected in EDTA tubes for malaria diagnosis, and EBV load.

All samples were transported to the SUNY Upstate research laboratory located at the Center for Global Health Research, KEMRI in Kisumu for processing within 1 hour of blood collection. The plasma fraction was separated from peripheral blood cells by centrifugation and aliquots were then stored at -80°C until use.

Detection of *P. falciparum* Infection—Because real-time quantitative (Q)-PCR has high sensitivity and allows for identification, quantification and speciation of malaria parasite(28, 29), we performed Q-PCR on DNA extracted from peripheral blood as described previously (28).

Measurement of EBV load—DNA was extracted from 200 μL of whole blood by use of a Qiagen DNAeasy kit (Qiagen, Valencia CA), in accordance with the manufacturer's protocol. DNA was eluted off the column in an equivalent volume of elution buffer and stored at -20°C . EBV DNA levels were determined using primers and probes designed to detect a 70 base pair region of the EBV BALF5 gene and β -actin gene as a control for DNA input using Q-PCR as described previously (9, 22). EBV load was normalized to the number of beta-actin DNA multiplexed and then calculated on the basis of copies of EBV genome per microgram of DNA.

Measurement of EBV-VCA antibodies—Plasma samples were analyzed for the presence and titers of antibodies to EBV-specific IgG Viral capsid antigen (VCA) using a synthetic peptide based enzyme-linked immunosorbent assay (ELISA) for immunodominant epitopes derived from VCA-p18 as described previously (23, 30). The cut-off value was defined as a mean of OD from EBV negative control, plus three standard deviations.

Statistical analysis—All statistical analyses were performed using Stata, IC software (13.1, StataCorp LP, College Station, TX) and setting 2-tailed alpha to reject the null hypothesis at 0.05. Differences in proportions were evaluated using Fisher's exact tests. Malaria and EBV viral load data were measured longitudinally during pregnancy, with each observation contributing to an overall area under the curve (AUC), (by trapezoidal methods) for statistical analysis (31). These AUC data were correlated using the Somer's D non-parametric measure of association for our primary hypothesis testing. Somer's D was also used for comparing EBV AUC between women with and without any positive malaria test results. Subsequent comparisons of repeated EBV viral load observations during the first and second *versus* third trimesters of pregnancy were log-transformed and compared via unpaired t-tests.

Results

Demographic, and other characteristics of study participants at enrolment

To investigate the impact of malaria on EBV reactivation during pregnancy, pregnant women from the Chulaimbo Sub-District Hospital in the Kisumu District of western Kenya were enrolled in this study. The hospital serves a predominantly rural population and malaria transmission in this area is holoendemic (26). The data obtained from 175 HIV-negative women were analyzed. The demographic, obstetric, clinical and laboratory characteristics of the study participants are presented in Table 1. The women had a mean age of 22.3 years (SD [5.6]) and 40% of the women were primigravidae. The majority (58%) of the women had at least upper primary level of education, with 82% being of the Luo ethnic group. Sixty five percent of the women were married.

To determine the prevalence of EBV in the study participants, we tested for EBV-VCA antibodies – a marker for past EBV infection – by ELISA and EBV DNA by Q-PCR. All (100%) participants were EBV seropositive, confirming results of previous studies that showed a high rate of EBV seroprevalence among adults in Africa (22). In addition, EBV DNA was detectable in blood in 44% of study participants at enrolment.

Because Q-PCR has high sensitivity and has allowed for identification, quantification and speciation of malaria parasite (28, 29), we next tested for *P. falciparum* infection using Q-PCR assay. We found that 33% of the pregnant women at enrolment were Q-PCR positive for *P. falciparum*. This is consistent with previous reported malaria PCR prevalence of 33.1% among pregnant women from Western Kenya (32). The prevalence of intestinal helminthes and pathogenic protozoans were 10% and 27% respectively.

Malaria status of study participants during pregnancy

We actively followed the pregnant women monthly through antenatal visits (up to 4 per mother) and delivery and assessed whether they had *P. falciparum* malaria infection at any time during their antenatal visits or at delivery. We observed that 94 (54%) of the pregnant women had malaria, i.e. at least one positive Q-PCR *P. falciparum* infection test during follow-up. One hundred and fifty-three of 617 (25%) total follow-up visits had *P. falciparum* malaria infection as detected by Q-PCR.

Because of the variation in maternal age, parity, bed net use and gestational age influencing the prevalence of malaria among pregnant women (33, 34), we tested whether these variables had an impact on the prevalence of *P. falciparum* infection in our cohort. We found no statistically significant associations between pregnant women who had *P. falciparum* malaria detected *versus* those who had no evidence of *P. falciparum* infection at anytime during pregnancy relative to their age, hemoglobin level, parity, bed net use and gestational age at enrolment (Table 2).

Prevalence of EBV DNA is higher in pregnant women with evidence of *P. falciparum* infection compared to those without *P. falciparum* infection

To assess whether the frequency of EBV DNA detection differs with *P. falciparum* infection status at enrolment, we performed EBV DNA detection by Q-PCR. We observed that pregnant women who had detectable *P. falciparum* infection were more likely to have a detectable EBV DNA at enrolment than pregnant women who had no evidence of *P. falciparum* infection during pregnancy (64% vs. 36%, $p=0.01$), Table 2.

EBV load is higher in the third trimester among pregnant women without known infections

To investigate the dynamics of EBV load in pregnancy independent of known infections, we measured EBV load in whole blood by Q-PCR. We included in the analysis only pregnant women with no known infections: *P. falciparum*, helminthes and pathogenic protozoans ($n=44$). Because the resultant data had few counts at first semester, we combined the data for first and second trimester (≤ 26 weeks of gestation). We next categorized the data according to gestational age, ≤ 26 weeks *versus* >27 weeks, and compared the log transformed EBV load data among groups using unpaired t-tests. We found higher EBV load at >27 weeks of gestation ($p=0.04$) relative to the ≤ 26 weeks of gestation (figure 1).

EBV viral load is not affected by presence of anemia

A significant portion of the pregnant women in the study were identified as having mild anemia (Table 2). Because anemia could be caused by a number of factors that potentially modulate EBV viral load, pregnant women were categorized into non-anemic (Hb ≥ 11) and anemic Hb <11 g/dl (anemic) as per WHO classification of anemia (35). None of the study participants had Hb <7.5 g/dl (for severe anemia), hence the anemia documented in our participants were all mild to moderate anemia. Next, we compared the EBV load of pregnant women with ($n=87$) and without anemia ($n=88$) at enrolment. We found no significant difference in EBV load between pregnant women with and without anemia ($p=0.88$, by Mann–Whitney U test). Furthermore, to control for the effect of infections during pregnancy on anemia, we excluded data of pregnant women with known infections: malaria, helminthes and pathogenic protozoan and then compared EBV load of pregnant women with anemia ($n=47$) and those without anemia ($n=50$). Again we found no significant difference in EBV load between women with and without anemia, independent of known infections ($p=0.73$, by Mann–Whitney U test).

EBV load is higher in pregnant women with *P. falciparum* infection compared to those without *P. falciparum* infection

To determine whether there were differences in levels of EBV load between pregnant women with or without *P. falciparum* infection during pregnancy, we measured EBV viral load and *P. falciparum* in whole blood by Q-PCR. We then compared levels of EBV load of pregnant women that had *P. falciparum* detected during pregnancy, i.e., at least one positive Q-PCR test during pregnancy, to those that had no evidence of *P. falciparum* infection during pregnancy using EBV load area under the longitudinal observation curve (AUC). AUC analysis is a method for evaluating the overall response of a continuously scaled outcome that can increase and decrease over time. Repeated observations of detectable EBV load were recorded throughout the course of study, and these values for any given individual may increase or decrease given their exposure and immune response. In order to “capture” the effect of their exposure and immune response in an overall sense, we literally calculate the areas under the curve that would be drawn for each individual given all of their recorded viral loads, and used this one number (their area under the curve) to represent the entirety of their viral challenge (31). We found that EBV load as analyzed by quantifying AUC was significantly higher in women that had malaria detected than in women that had no evidence of malaria infection during pregnancy ($p=0.01$).

Increase in *P. falciparum* load is associated with increase in EBV load

We next assessed whether *P. falciparum* load correlated with EBV load AUC. Since these data are highly skewed and zero-inflated we used a non-parametric measure of association (Somers d) (31). We found a highly significant positive association: increase in *P. falciparum* load correlated with increase in level of EBV load ($p<0.0001$).

EBV viral load is higher in pregnant women with *P. falciparum* infection Independent of gestation age of pregnancy

Because pregnancy associated malaria morbidity is gestational age dependent where the prevalence of malaria infection and parasite density are highest during the first half of pregnancy with gradual decline during the second half (33, 34); we assessed whether there was gestational age-related difference in EBV load between pregnant women with and without evidence of *P. falciparum* infection during pregnancy. To eliminate the confounding effect of known infections e.g. helminthes and pathogenic protozoans, we excluded from analysis data of pregnant women with any known infections besides *P. falciparum* malaria. We then categorized the data according to gestational age, 26 weeks *versus* >27 weeks relative to *P. falciparum* infection status. Median EBV load was higher at both 26 weeks ($p=0.02$) and >27 weeks ($p=0.05$) of gestation in pregnant women with malaria in comparison to women without malaria.

Discussion

Despite the well-established link and our recent support of the long-held hypothesis that EBV infection early in life and holoendemic malaria being risk factors for eBL (9, 36), little is known about why children are infected early in life with EBV, and what role malaria plays in EBV transmission. We hypothesized that infection with malaria during pregnancy

could cause EBV reactivation leading to high EBV load in circulation, which could subsequently enhance early infant age of EBV infection. To test the first part of our hypothesis, we conducted a cohort study, which actively followed up pregnant women during pregnancy, and categorized these women according to their malaria history during pregnancy. We observed that significantly higher frequency and elevated EBV loads were found in pregnant women with malaria compared to women without malaria during pregnancy independent of gestational age of pregnancy.

Plasmodium falciparum malaria has been linked to EBV reactivation in children following acute malaria infection(24). In addition, in vitro studies show that *P. falciparum* can induce reactivation of EBV latently infected B cells (37). Thus, the correlation of elevated EBV loads in woman infected with *P. falciparum* during pregnancy could be due to a direct effect of *P. falciparum* malaria on EBV infected B cells. Alternatively, immune control of EBV is critical in maintaining stable viral loads (38) and because pregnancy itself could modulate immune function especially T-cell functions, which appear to be down-regulated during pregnancy (13, 14), it is plausible that the synergistic effect of *P. falciparum* malaria and pregnancy could significantly alter and shift the dynamic balance between EBV and the host immunity in favor of the virus. Moreover, increased malaria load was associated with increased EBV load, suggesting that the level of EBV load is proportional to the parasite density. This dovetails with our previous findings of increased viral load in peripheral blood of children living in a malaria holoendemic area, an area characterized by high parasite densities, compared to children living in malaria sporadic area (22).

Consistent with our findings, previous studies (24, 39, 40) albeit in children, reported significantly elevated peripheral blood EBV DNA loads – indicative of viral reactivation – following malaria infection, suggesting that malaria has inherent effects on EBV-host balance. The possible mechanisms through which *P. falciparum* malaria induce EBV reactivation is through a multiplicity of pathways: an interaction between a specific malaria antigen, the cysteine rich interdomain 1 alpha (CIDR1 α) of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) protein, and B cells (41); leading to activation of memory B cells, which in turn results in increased production of EBV in virus-positive cell line (37). Secondly, expansion of latently infected B cells during acute malaria has also been postulated (12, 39). Thirdly, malaria parasite is also known to possess unmethylated CpG DNA, a ligand for Toll like receptor (TLR) 9 (42), which can potentially enhance EBV induced proliferation and activation of B cells (43).

Although induction of EBV reactivation during pregnancy following *P. falciparum* infection seen in our cohort may not result in apparent EBV-related diseases in pregnant women, the significant increase of EBV load in the maternal circulation may have ramifications for their infants early in life. One possible consequence of this is the increased shedding of infectious EBV in maternal saliva and possibly breast milk postpartum, the probable routes of EBV transmission. Moreover, pregnancy associated *P. falciparum* malaria not only influences pregnancy outcomes but also the development of neonatal immune responses (44-47) suggesting that the ability of infants – born to mothers that had malaria during pregnancy – to effectively control EBV and other infections early in life may be impaired. We previously found that 35% of children in this area were infected before 6 months of age and that there

was evidence of repeated infections with EBV during the first year of life, implying a failure to control the virus in infants living in malaria holoendemic area (9).

We observed higher EBV load during the third trimester of pregnancy independent of known infections, suggesting that the dynamics of EBV load during pregnancy is affected by gestational age of pregnancy. A rather intriguing report of increase of CD4⁺ CD25⁺ T regulatory cells (Tregs) at early pregnancy, dramatically peaking during the second and third trimesters, with subsequent decline postpartum (48), may perhaps explain the observed phenomenon. Tregs are known potent suppressors of T-cell functions (49), and T cells particularly CD8⁺ cytotoxic T lymphocytes (CTL) play a pivotal role in maintenance of persistent EBV infections (10-12). Thus, elevated EBV load observed during the last phase of pregnancy could have been secondary to increased levels of Tregs, which in turn compromised host control of EBV persistence.

Previous studies reported pregnancy associated malaria morbidity being dependent on covariates such as, parity, bed net use and gestational age of pregnancy (33, 34), however, we found no significant association between these variables and malaria in our cohort. This lack of finding is not surprising as we were investigating the impact of malaria on EBV reactivation and we, therefore, accordingly selected for only pregnant women with normal blood count and no apparent known infections besides malaria. The rather stringent inclusion criteria we employed may have consequently circumvented the prospects of finding any significant association with the foregoing variables.

Earlier studies to examine the effects of pregnancy on EBV persistence only focused on healthy pregnant women from the developed world, often correlating EBV reactivation with adverse pregnancy outcomes (50, 51), seldom examining in the context of infectious diseases such as malaria. Though using different approach in determining EBV reactivation, some studies (15-17) but not others (18, 51) reported increased EBV reactivation during pregnancy among pregnant women.

The potential strength of this study is that we actively followed up pregnant women monthly during pregnancy through to delivery, and categorized them according to their malaria history. We were therefore able to document accurate malaria infection histories for each pregnant woman. Secondly, because pregnancy itself could modulate immune functions, we compared one group of pregnant women with malaria with another without malaria, with a view to control for the possibility of pregnancy itself playing a confounding role and having an influence on the results. Lastly, we used Q-PCR for malaria detection, which in addition to detecting submicroscopic parasitaemia, also allows for accurate quantification as well as speciation of malaria parasites.

In summary, significantly higher frequency and elevated EBV loads were found in pregnant women with malaria than in women without malaria during pregnancy. It remains to be determined whether the loss of control of EBV latency following *P. falciparum* malaria during pregnancy and subsequent increase of EBV load in circulation could contribute to enhanced shedding of EBV in saliva and breast milk postpartum; probable conduits of EBV transmission in infants early in life. In addition, because malaria during pregnancy may alter

the development of neonatal immune responses, it remains to be determined whether the ability of infants to control EBV early in life is impaired in infants born to mothers that had malaria during pregnancy.

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Figure 1.

Comparison of the levels of EBV load in ≤ 26 weeks of gestation *versus* > 27 weeks of gestation ($n=44$), independent of known infections. We excluded EBV load data of pregnant women with known infections during pregnancy. Then we categorized the data according to gestational age (first and second, *versus* third trimesters) and compared the log transformed EBV load data among groups using unpaired t-tests. P value of unpaired t-test test is indicated in the figure.

Table 1
Demographic, and other characteristics of the participants (n=175) at enrolment

Characteristics	Summary
Mean age in years ($\pm$ S.D.) at enrolment	22.3 (5.6)
Education	
Lower Primary	20%(35)
Upper Primary	58%(101)
Secondary	16%(28)
Village Polytechnic	1% (1)
Other	5%(10)
Ethnic group	
Luo	82% (144)
Luhya	14% (26)
Kalenjin	1% (1)
Other	2% (4)
Marital Status	
Single	31% (55)
Married	65% (114)
Separated	1% (1)
Widowed	2% (3)
Widowed, inherited	1% (1)
Parity	
Multiparous	60%(105)
Primiparous	40%(70)
Gestation Age at enrolment	
1 st trimester (<14 weeks)	15%(27)
2 nd trimester (14-27 weeks)	61%(107)
3 rd trimester (28 weeks)	24%(41)
Mean hemoglobin (g/dl) ($\pm$ S.D) at enrolment	10.9(1.7)
EBV seroprevalence by ELISA VCA positive at enrolment	100%(175)
EBV DNA prevalence by q-PCR at enrolment	44%(77)
<i>P. falciparum</i> malaria prevalence at enrolment by qPCR	33% (57)
Prevalence of helminthes infections at enrolment *	10%(18)
Prevalence of pathogenic protozoan Infections at enrolment **	27%(47)

Characteristics	Summary
<i>P. falciparum</i> and helminthes co-infections	7% (13)

* Prevalence of specific helminthes infections: Hookworm ($n=9$ [5%]); *Ascaris lumbricoides* ($n=4$ [2%]); *Trichuris trichiura* ($n=3$ [2%]); *Schistosoma mansoni* ($n=2$ [1%]).

** Pathogenic protozoan Infections: *Trichomonas vaginalis* ($n=5$ [3%]), *Entamoeba histolytica* ($n=29$ [17%]), and *Giardia lamblia* ($n=13$ [7%]).

Table 2
Malaria Status of Pregnant Women During Pregnancy Versus Demographic, obstetric, clinical and laboratory history at follow-up

Variable	Had malaria detected during pregnancy, 94(54%)	Had no evidence of malaria infection during pregnancy, 81(46%)	P value *
Mean Gestation Age, weeks ($\pm$ SD) at enrolment	21.3 $\pm$ 6.4	21.4 $\pm$ 6.3	0.93
Parity			
Primiparous	41(58.6%)	29(41.4%)	0.35
Multiparous	53(50.4%)	52(49.5%)	
Hemoglobin (Hb) level at enrolment **			
Moderate Anemia (Hb <11g/dl)	49(56.3%)	38(43.7%)	0.54
No Anemia (Hb $\geq$ 11g/dl)	45(51.1%)	43(48.9%)	
Age at enrolment			
<20 years	46(57.8%)	36(43.9%)	0.66
20 – 30 years	38(50.0%)	38(50.0%)	
>30 years	10(58.8%)	7(41.2%)	
Bed net use at enrolment			
Yes	71(53%)	64(47%)	0.71
No	23(57%)	17(43%)	
EBV DNA Prevalence at enrolment			
EBV DNA Positive	50(64%)	28(36%)	0.01
EBV DNA Negative	44(45%)	53(55%)	

* All estimated using Fisher's exact test except gestation age, which was estimated by unpaired t-test. All tests of statistical significance are two sided.

** Cut-off as defined by the World Health Organization (WHO) for pregnant women(35)