

THE IMPACT OF MALARIA PARASITES ON THE PLACENTA AND PERINATAL OUTCOME AT KORLE BU TEACHING HOSPITAL, ACCRA, GHANA: A CASE CONTROL STUDY.

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Abstract

Objective: Malaria remains a complex and overwhelming health problem affecting vulnerable groups such as pregnant women and their infants in Ghana. Malaria during pregnancy does not only pose a threat to the mother but can cause serious structural damages to the placenta and subsequently affect the pregnancy outcome. The aim of the study was to investigate the impact of *Plasmodium* parasites on the placenta and perinatal outcome of women delivering at Korle Bu Teaching Hospital. A better understanding of the impact of malaria parasites on the placenta morphology and prenatal outcome is crucial for better management of pregnant women and their babies.

Methods: The study involved testing blood collected from postpartum placentas and examining the placental tissue for *Plasmodium* parasites, after which they were classified as study group (*Plasmodium* positive) or control (*Plasmodium* negative). The patients in the study group with similar gestational and maternal age were matched with patients from the control group. The morphological characteristics of the placenta and the perinatal outcome of the two patient groups were compared using an unpaired t-test.

Results: Sixteen (16, 13.6%) out of 118 women tested positive for *Plasmodium* parasites on the maternal side of the placenta by both rapid diagnostic test and microscopy and /or tested positive for malarial parasite during pregnancy, while the rest (102, 86.4%) had no history of malaria in the index pregnancy and tested negative. The mean placenta weight was significantly reduced in the study group (difference: -102.0g; 95% Confidence Interval [CI]: 424.4g, 486.6g) who delivered during early term (p=0.02). Patients in the study group, who delivered during late term, had a significantly reduced mean placenta diameter (difference: -2.5cm; 95% CI: 20.0cm, 21.4cm) (p=0.003) and delivered infants with lower mean birth weight (difference: -0.693kg; 95 CI: 3.268kg, 3.475kg) (p<0.001).

Conclusion: Malaria during pregnancy does not only pose a threat to the mother but to the fetus and our results add evidence that malaria parasites cause alterations to certain morphological characteristics of the placenta which subsequently affect the birth weight as the pregnancy progresses to late term.

Keywords: placental malaria, placenta morphology, perinatal outcome, birthweight

Introduction

Malaria remains a complex and overwhelming health problem known to contribute significantly to maternal and infant mortality. In Ghana, malaria is hyper endemic in pregnant women, accounting for 17.6% of out patients' department attendance, 13.7% of admissions and 3.4% of maternal deaths¹. Pregnant women are more susceptible than the general population to malaria². Studies attribute the increased susceptibility

to the lack of immunity to pregnancy-specific isolates that sequester in the placenta^{3,4}.

The placenta provides several functions during the different stages of pregnancy and has a sensitive morphology which can be altered. The physiology of the placenta permits it to perform respiratory, nutritive, and excretory functions, and it is also an important endocrine organ⁵. Although the detailed morphology of the placenta varies from patient to patient, a compilation of two studies reports the morphological characteristics (Quantitative variables) of the placenta at term to have a weight range of 470 to 530 grams, a diameter range of 18 to 22 centimeters, and a thickness range of 2.0 to 2.5 centimeters^{6,7}. A study reported that placenta examination could be used to explain fetal death in majority of their study cases⁸.

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Malaria parasites can affect the placenta directly by causing a mechanical compromise of the blood circulation or indirectly by interfering with the function and/or inducing pathological lesions. A study recorded that, the malaria parasites affect uterine and umbilical artery blood flows and suggested that this impairs the placental capacity to transport nutrients to the fetus⁹. It was reported in another study that the *Plasmodium* parasites indirectly interfere with the function of the placenta by disrupting its normal immune balance¹⁰. Other studies on placental malaria reported that the parasite causes histological changes to the placenta¹¹⁻¹³. Histological changes may in turn cause morphometric changes of the placenta structure both of which can cause placenta inefficiency and other serious complications during pregnancy¹³.

Low birth weight and intra-uterine growth restriction (IUGR) are frequently reported adverse perinatal outcomes associated with placenta malaria. Associations between placental malaria and birth weight have been reported in several studies^{9, 14, 15}. Other studies have attempted to explain the mechanisms behind this association. A study suggested that the association between the parasite and low birth weight is IUGR and/or preterm delivery¹⁶. Another study suggests that placental vascular insufficiency is a cause of low birth weight¹⁷. The primary mechanism responsible for the negative prenatal outcomes is not fully understood.

Knowing how the placenta is affected when infected with the *Plasmodium* parasite will contribute to understanding pregnancy outcomes and management in the presence of the parasite. Furthermore, focusing more research on the morphological changes in the placenta, caused by the malaria parasite, is crucial for an effective intervention of the highly vulnerable population of pregnant women.

The aim of the study was therefore, to investigate the impact of *Plasmodium* parasites on the placenta and perinatal outcome at the Korle Bu Teaching Hospital. The specific objectives were, to determine the prevalence of placental malaria in the sampled population, to compare the gross placenta morphology of the study and control group, and to compare the perinatal outcome of malaria infested placentas and the control placentas.

Methods

Study design and setting

This was a case control study which involved a purposive sampling of the postpartum placenta of 118 patients at the Korle Bu Teaching Hospital (KBTH); a tertiary referral hospital in Accra, Ghana, with approximately 10,000 births a year.

Participants

The study participants were mothers delivering at the two labor wards of the Department of Obstetrics and Gynecology of KBTH, Accra, Ghana, during the day time shift (9:00-17:00) with the exception of weekends. All mothers who have delivered live singleton babies of pregnancies between 37-42 weeks of gestation were

included in the study after signed informed consent. Women who did not give informed consent, had a medical history of HIV, were anemic (Hb record below 8g/dl in folder or had no Hb records) but tested negative for *Plasmodium*, sickle cell disease (SCD) or had an incomplete postpartum placenta were excluded from the study. Ethical approval to conduct the study was obtained from the Ethical and Review Committee of the College of Health Science, University of Ghana (Protocol Identification Number: CHS-Et/M.2 C/2017-2018).

Blood Examination

An immunoblot test kit (Immunetics Inc.) was used for the Rapid Diagnostic Test (RDT). A drop of whole blood collected from the maternal surface of the placenta and kept in K₂EDTA was placed on the sample column of the test kit after which four drops of the lysing buffer was added. The results were read within five minutes after application of the blood, following the manufacturers instruction and key for interpreting the results. The results were recorded as RDT positive or RDT negative. A grease free glass slide with frosted end was used for the thick and thin films. For thin film, an amount of 2µl of a thoroughly mixed whole blood sample (some of which has earlier been used for the RDT) was pipetted and put at the middle of the slide. Using a fine edge glass slide spreader, the blood was spread rapidly at an approximate angle of 45° to get a thin monolayer as recommended in previous studies¹⁸.

For thick film, 5 µl of the blood was pipetted and place closed to the frosted end of the same slide having the thin film. The 5µl blood drop was spread circularly to obtain between 1-2cm thick smear. The stain was thick enough so that when the blood stained slide is placed on a newspaper, the newspaper prints was barely visible. The films were air dried for three hours at room temperature, after which the thin film was fixed in absolute methanol. Rapid Giemsa solution was prepared by adding buffered solution (pH 7) with concentrated Giemsa to prepare a 10% Giemsa solution. Then both the thick and thin blood smears were stained for 5 minutes in the 10% Giemsa solution. Afterwards, the slide was rinsed gently for 1 or 2 seconds in a jar of tap water. Finally, the slides were dried and examined under the microscope.

Comparisons

After the blood smears, an examination of the placenta was done in the labor ward within two hours of delivery. The umbilical cord was clamped and blood clots on the maternal surface of the placenta were removed. Quantitative data such as the weight (g), volume (ml), diameter (cm), thickness (cm), and surface area (cm²) of the placenta were recorded. The widest stretch of the placenta was measured as the placenta diameter whilst the thickest part of the placenta was measured as the placenta diameter whilst the thickest part of the placenta was recorded as the placenta thickness. The circumference was measured by tracing around the placenta edges with a string. The string was then stretched into a straight line and measured to obtain

the circumference which was then used to determine the surface area. Afterwards, the placenta was placed into a premeasured bowl and the bowl with the placenta was put onto a scale to measure the weight of the placenta. The volume of the placenta was measured by placing the placenta into a bowl filled with water to the brim. The water displaced out of the bowl was measured in a volumetric cylinder to obtain the volume of the placenta.

The perinatal outcome, birth weight (kg) and APGAR scores were also recorded and compared.

Statistical Analysis

The data from the patients' questionnaire and from the placenta examination were transferred and recorded digitally using excel (Microsoft company, USA). To analyze the data from the placenta examination, the data of the placentas were divided into two groups. Patients with confirmed laboratory results of parasitemia during gestation, malaria parasites seen under light microscopy, and/ or with positive RDT results from the placenta blood constituted the study group. Patients with no malaria parasites seen under light microscopy and had negative RDT results constitute the control group. Before comparing the data, each mother from the study group was paired with a mother from the control group that had similar age and gestational age at delivery. Quantitative variables were compared using Student T-test. The control and study groups were further selected and subdivided into those who delivered early term (37-39 weeks, 6 days gestation) and those who delivered late term (40-42 weeks gestation), based on record of early ultrasound scan or early ultrasound scan and self-reported LMP before performing the statistical test. Results of the analysis were considered statistically significant at 95% confidence interval (CI) with $p < 0.05$.

Results

Flow chart of patients throughout the study is shown in Figure 1. Sixteen (16, 13.6%) out of 118 women tested positive for *Plasmodium* parasites while the rest (102, 86.4%) had no history of malaria in the index pregnancy and tested negative (Fig. 1). Socio demographic characteristics of the patients in the control and study group were similar. Majority of the patients in the control group fell within the 26~30 age group whilst the majority of the patients in the study group were from the age group ≤ 25 . Majority (>57%) of patients in both the control and study group had blood group O. More than half ($\geq 50\%$) of patients in both the control and study group had gravidity equal to or greater than 3 (Table 1).

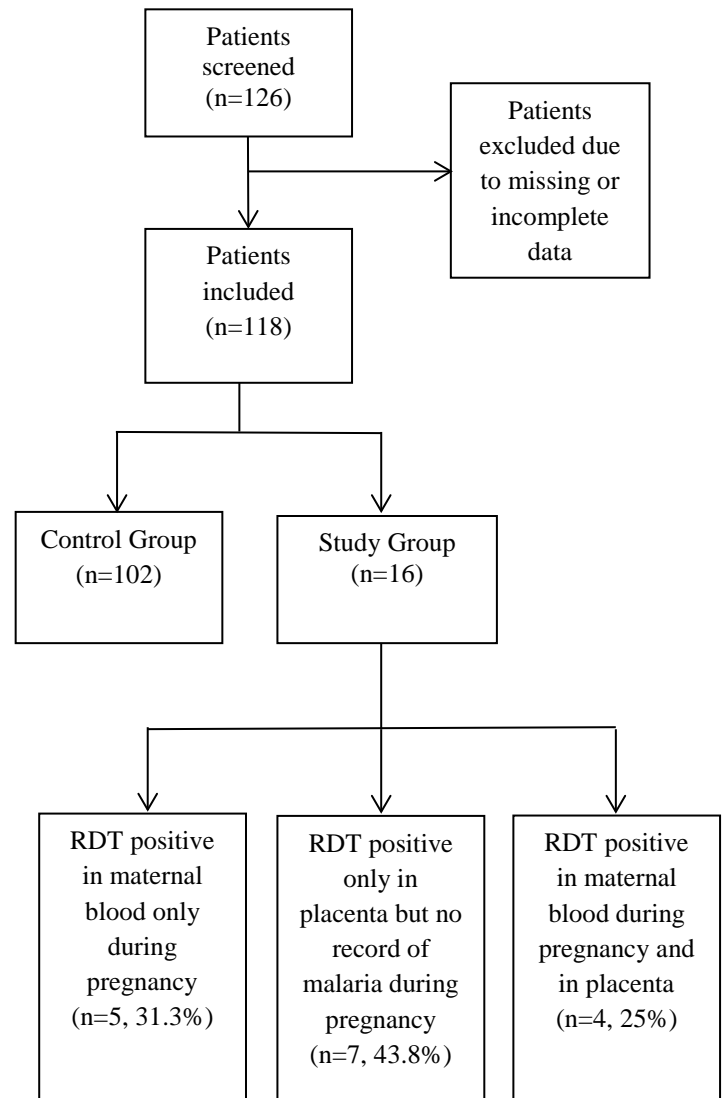


Fig.1 Flow of patients in the study

Placenta Examination

Among the various morphological comparisons with an unpaired t-test, the placenta weight, the placenta diameter, and the perinatal outcome (birth weight) revealed statistically significant differences (95% CI, $p < 0.05$) between the two patient groups.

The mean placenta weight of study group patients who delivered early term (Mean standard Error of the mean; SEM) (455.5g SEM 31.1) was significantly lower than the mean placenta weight of the control group (557.5g SEM 30.4, $n=10$ in each group, $p=0.02$) (Fig. 2). However, in the case of patients who delivered late term, the mean placenta weight of the study group (563.3g SEM 43.0) was not significant different from the controls (548.8g SEM 40.4, $n=6$ in each group, $p=0.67$).

Table 1 Socio demographic characteristics of patients

| Variables | Control N (%) | Study N (%) | Total N=118 |
|-------------|---------------|-------------|-------------|
| Age Group | | | |
| ≤25 | 15 (14.71) | 6 (37.50) | 21 |
| 26~30 | 38 (37.25) | 4 (25.00) | 42 |
| 31~35 | 29 (28.43) | 5 (31.25) | 34 |
| ≥36 | 20 (19.61) | 1 (6.25) | 21 |
| Blood Group | | | |
| A | 18 (17.65) | 2 (12.5) | 20 |
| B | 17 (16.67) | 1 (6.25) | 18 |
| AB | 4 (3.92) | 1 (6.25) | 5 |
| O | 59 (57.84) | 11 (68.75) | 70 |
| Unknown | 4 (3.92) | 1 (6.25) | 5 |
| Gravidity | | | |
| 1 | 22 (21.57) | 4 (25.00) | 26 |
| 2 | 27 (26.47) | 4 (25.00) | 31 |
| ≥3 | 53 (51.96) | 8 (50.00) | 61 |

n: number of patients in a group.

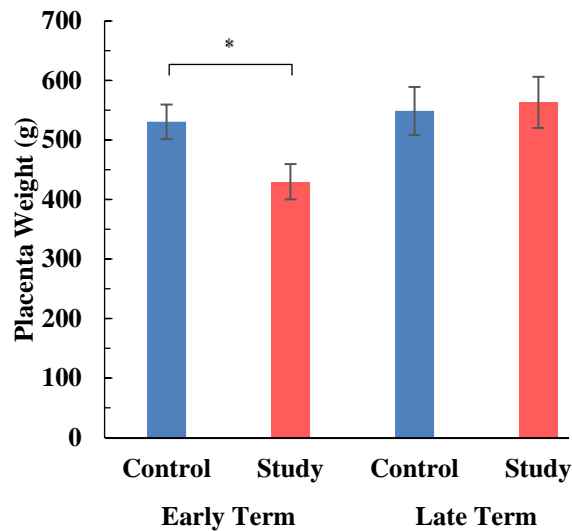


Fig. 2 The mean placenta weight (Columns) and error bars (\pm SEM) of the babies delivered to mothers infected with *Plasmodium* parasite during pregnancy (study group) was less than control at early term (n=10 each, $p<0.05$), but the difference in means was not significant at late term (n=6 each)

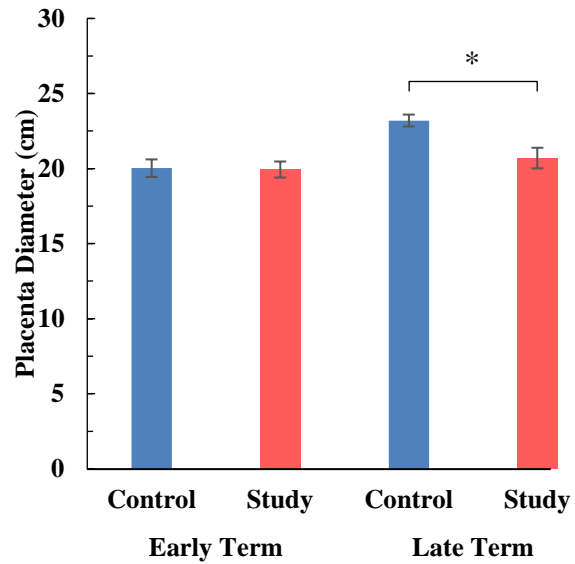


Fig. 3 The mean placenta diameter (Columns) and error bars (\pm SEM) of the babies delivered to mothers infected with *Plasmodium* parasite during pregnancy (study group) was less than control at late term (n=6 each, $p<0.05$), but the difference in the means was not significant at early term (n=10 each)

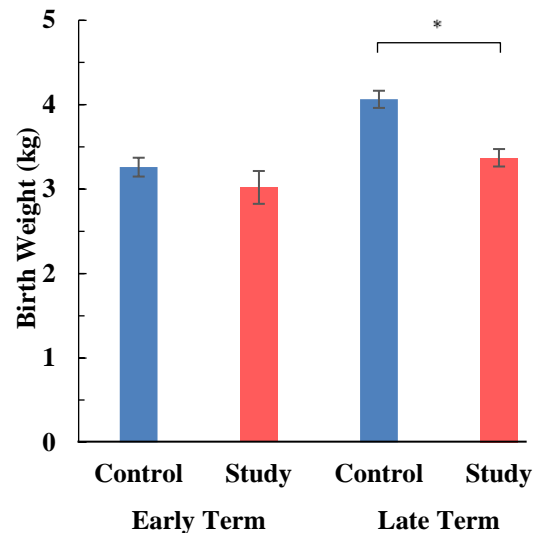


Fig. 4 The mean birth weight (Columns) and error bars (\pm SEM) of the babies delivered to mothers infected with *Plasmodium* parasite during pregnancy (study group) was less than control at late term (n=6 each, $p<0.05$), but the difference in means was not significant at early term (n=10 each)

The mean placenta diameter of study group patients who delivered early term was similar to that of the control group (19.9cm SEM 0.5 and 20.2cm SEM 0.6

respectively, $n=10$ in each group, $p=0.67$). For the late term patients, however, the difference in the mean placenta diameter of the study group (20.7cm SEM 0.7) and the control group (23.2cm SEM 0.4) was of statistical significance, $p=0.003$ (Fig. 3).

The mean birth weight of babies in the study group who were delivered early term, did not significantly differ from that of the control group who delivered early term (2.99kg SEM 0.1 and 3.34kg SEM 0.1 respectively, $n=10$ in each group, $p=0.05$). For the late term babies, however, the mean birth weights of the study group and the control group (3.37kg SEM 0.1, 4.06kg SEM 0.1 respectively) were statistically different, $p<0.001$ (Fig. 4).

Although the other morphological comparisons, namely: the mean placenta volume, surface area, thickness, as well as the Apgar's score revealed differences between the two groups, however, these differences were not of statistical significance (95% CI, $p>0.05$).

Discussion

Our study found 13.6% prevalence of malaria in the study population reflecting the endemic nature of the disease in the study population in spite of the use of intermittent preventive treatment with sulphadoxine-pyrimethamine during pregnancy.

The results of the comparison between the placenta weights of two groups were in agreement with previous studies which also observed a decrease in the mean weight of the malaria infected placentas^{19, 21}. These changes may cause placenta insufficiency and adverse prenatal outcome. The results of this study give insight that the weight change in the placenta is more pronounced during early term. The cause for the significant differences recorded between the mean placenta weights in early term but not in late term is not clear, we can only hypothesize that the immature placenta is more susceptible to the malaria infection but as the pregnancy matures, there could be a compensatory role to rebuild itself, this might also depend on the time of infection and how effective the treatment was. We also observed a reduction in the mean birth weight of malaria associated pregnancies as reported in other studies^{15,22}. The change in birth weights is more pronounced during late term and concurs with other studies which also reported a correlation between the placenta weight and the birth weight^{23,24}. The mechanism responsible for the difference between early term (in placenta weight only) and late term (in birth weight only) is not certain. A possible explanation for this observation is that the placenta uses much of the energy for its compensatory reaction at the expense of the fetal metabolism as the pregnancy progresses to late term.

To our knowledge, this study represents the first instance in which multiple gross morphological characteristic (diameter, thickness, & surface area) have been compared between malaria-infected placentas and

malaria free placentas. Past studies, however, established morphological changes such as macrophage concentration in the intervillous spaces, syncytiotrophoblastic damage, and trophoblastic basal lamina thickening as common features associated with placental malaria^{19,20}. These changes observed in previous studies could explain the observation of significant decrease in the mean diameter of the placentas affected with malaria parasites in the present study. It can be inferred, that the effects of syncytiotrophoblastic damage, and trophoblastic basal lamina thickening are significant enough to reduce the placenta diameter. These changes could also explain the slight differences observed in the comparison of the means of placenta volume, surface area, and thickness in the two groups. This reflects on the intrauterine study of *Rijken et al.* who found that most placenta volumes in the *Plasmodium* infected women at 14-24 weeks' gestation were below the 50th centile for gestational age and in particular most of those with *Plasmodium falciparum* were below the 10th centile²⁵.

The strength of our study is the comparison between cases and controls at two timelines (early and late terms) and this reveals possible compensatory mechanism in the body's effort to protect the growing fetus during infection with the malaria parasite. This comparison was lacking in previous studies which put all term gestation as one group. One limitation worth mentioning is the fact that, our method of detecting placenta malaria could not identify when and how long the parasites were in the placenta. Morphological changes on the placenta are believed to be significantly greater if the placenta malaria occurred during the early stages of pregnancy compared to the changes if placenta malaria occurred later during pregnancy. However, our study focused on changes in the placenta that could only be examined post-partum without differentiation on the time of infection.

Conclusion

Among the various morphological and perinatal outcome comparisons, the placenta weight (for early term), the placenta diameter and the birth weight (for late term) revealed statistically significant differences (95% CI, $p<0.05$) between the two patient groups. The results add evidence that malaria parasites cause alterations to certain characteristics of the placenta which subsequently affects the birth weight as the pregnancy progresses to late term. Future studies could be focused on the follow up of the infants in the study group to determine the effect of the intrauterine infection on their developmental mile stones as compared with control infants.

List of Abbreviations

CHS - College of Health Sciences

CI - Confidence interval

Hb - Hemoglobin

HIV - Human immunodeficiency virus

IUGR - Intra-uterine growth restriction
 KBTH - Korle Bu Teaching Hospital
 NICU - Neonatal intensive care unit
 NMCP - National Malaria Control Program
 OPD - Out patients' department
 RDT - Rapid diagnostic test
 SEM - Standard Error of the Mean

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