

***Plasmodium falciparum* Infection in Pregnant Cameroonian Women: An Assessment of Changes in the Placenta of Low Birth Weight Infants**

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ABSTRACT

During pregnancy, *Plasmodium falciparum*-infected erythrocytes often accumulate in the placenta. As a result, decreased numbers of parasites circulate in the peripheral blood, making diagnosis of malaria difficult. Our previous study reported that microscopic examination of peripheral blood smears failed to detect parasites in 21.1% of Cameroonian women with placental malaria. Unless accurately diagnosed and treated, placental malaria can affect fetal development. In the current study, infants born to malaria-positive mothers were found to have lower mean birth weights (averaged 184 grams less), and were more likely to be low birth weight (LBW; <2,500 grams), than those born to uninfected mothers ($p=0.004$). Histosections of the placenta of mothers with LBW infants demonstrated evidence of chronic placental infections with increased numbers of infiltrating macrophages. $TNF\alpha$ levels were also increased in placental plasma of these mothers. In response to malaria, a significant increase in both the number and diameter of fetal blood vessels was seen ($p<0.05$ and $p<0.001$, respectively), but these changes did not correlate with LBW. Neither did plasma levels of angiogenin, a cytokine involved in blood vessel formation. While placental malaria contributes to a remodeling of the micro-architecture of the placenta, angiogenesis was sufficient to support normal fetal growth.

Key words: *Plasmodium falciparum*, diagnosis, placenta, low birth weights

RESUME

Pendant la grossesse, les erythrocytes infectés par le *Plasmodium falciparum* s'accumulent souvent dans le placenta. Il en résulte un nombre réduit de parasites dans la circulation périphérique, rendant le diagnostic du paludisme difficile. Ceci a été prouvé dans une de nos études antérieures où 21.1% de Camérounaises présentant un paludisme placentaire avaient un examen du sang périphérique négatif. A moins d'être correctement diagnostiqué et traité, le paludisme placentaire peut affecter le développement du fœtus. Dans la présente étude, les enfants nés des mères avec paludisme placentaire ont présenté un poids de naissance plus faible (en moyenne 184g de moins) et étaient plus susceptibles à être de faible poids de naissance (FPN, < 2500g) que ceux nés des mères non-infectées ($P=0,004$), l'analyse histologique des placentas des mères avec FPN a démontré l'évidence de l'infection placentaire chronique avec un nombre élevé de macrophages infiltrés. Les taux de TNF étaient aussi élevés dans le plasma placentaire de ces patients. En réponse au paludisme, une augmentation significative du nombre et du diamètre des vaisseaux sanguins foetaux a été observée ($P<0,05$ et $0,001$ respectivement), mais ces changements ne correlaient pas avec le FPN, encore moins avec l'angiogenine, une cytokine impliquée dans la formation des vaisseaux sanguins. Bien que le paludisme placentaire contribue au remodelage de la micro architecture placentaire, l'angiogenèse était suffisante pour permettre une croissance foetale normale.

Mots clés : *Plasmodium falciparum*, diagnostique, placenta, Faible Poids de Naissance

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INTRODUCTION

P. falciparum during pregnancy is a risk factor for the mother and developing fetus (McGregor, 1984; Menendez, 1995). It is well-established that malaria infection during pregnancy has a negative effect on infant birth weight, especially in newborns of primigravid women (reviewed by Brabin 1983). Infants weighing less than 2,500 grams at birth are considered to be low birth weight (LBW) and have an increased mortality rate (McCormick, 1985). In the 1950's, an association between LBW and malaria was observed that has since been confirmed numerous times. Accordingly, the WHO currently recommends that women take antimalarial drugs during pregnancy. Indeed, early treatment of malaria in pregnant women has been shown to increase newborn birth weight (Steketee *et al.* 1996; Schultz *et al.* 1996; Bouvier *et al.* 1997; Goodman *et al.* 2001). Therefore, prompt and accurate diagnosis of malaria during pregnancy is very important.

Unfortunately, diagnosis of malaria is not always straightforward during pregnancy as *P. falciparum* infected erythrocytes sequester in the placenta. It is currently thought that ligands expressed in the placenta bind to receptors on infected erythrocytes, resulting in parasite sequestration. The most documented interaction is between placentally-expressed chondroitin sulfate A (CSA) and a product of the *var* gene family (PfEMP1) produced by the parasite (Fried and Duffy, 1996). Accordingly, trophozoites, schizonts and perhaps some ring-stage parasites are retained in the placenta. As a result, high numbers of parasites may be present in the placenta with few or no parasites circulating in the peripheral blood.

Malaria infections are diagnosed based on clinical symptoms and detection of parasites in peripheral blood smears by light microscopy. Previously, it was reported that 20.1% of women with placental malaria were peripheral blood smear negative by microscopy (Leke *et al.* 1999). Even women with placental parasitemias as high as 10% parasitemias were misdiagnosed. Thus, diagnosis of malaria during pregnancy by microscopy is difficult. Physicians should keep this in mind when examining pregnant women.

The role of innate and acquired immune cells in placental pathology, and how pathological changes impact on fetal development, remain unclear. The sequestration of parasites is thought to be important. In response to sequestered parasites, maternal leukocytes are attracted to the placenta and produce an inflammatory-type response (Galbraith and Fox, 1980; Leopardi *et al.*, 1996). Elevated levels of pro-inflammatory

cytokines, including IL-6, IL-1, TNF α , and INF γ have been found at the maternal-fetal barrier (Fried *et al.*, 1998; Moorman *et al.*, 2000). In addition, pathological changes in villus tissue have been reported, including increased numbers of hemozoin-laden macrophages, deposition of hemozoin pigment in the intervillous spaces and within syncytiotrophoblasts, thickening of the basement membrane, syncytial knotting, and focal necrosis (Galbraith and Fox, 1980; Walter *et al.* 1982; Yamada *et al.*, 1989; Bulmer *et al.*, 1993; Leopardi *et al.* 1996).

Because of the high correlation between placental malaria and the risk of delivering a LBW baby, we sought to identify malaria-associated changes in the placenta of LBW infants. Initially, data collected between April 1996 to July 1997 from 740 women delivering at the Biyem Assi Hospital, Yaounde were evaluated. The prevalence of placental malaria due to *P. falciparum* infection was determined, as well as the effect of placental malaria on infant birth weight. Next, results from women with and without placental malaria who delivered either LBW or average birth weight infants were compared. Since malaria can induce an inflammatory response, sections of placentas from these women were examined for the presence of parasites, extent of mononuclear cell infiltration, and level of TNF α present in placental plasma. It is thought that sequestered malarial parasites might also place a stress on maternal-fetal exchange and alter placental architecture. Accordingly, the mean diameter and number of fetal vessels per villus were determined, as well as levels of angiogenin, a cytokine important in blood vessel growth. Changes in the placenta of women in the different groups were compared.

MATERIALS AND METHODS

Study Population Between April 1996 and July 1997, clinical histories and samples were obtained from 740 women delivering at the Biyem Assi Hospital, Yaounde. Prior to delivery, the nature of the study was explained to the women and verbal informed consent was obtained. Ethical clearance for the research was obtained from the Ethical Committee, Ministry of Health, Cameroon and the Institutional Review Board, Georgetown University. The project was covered by single project assurance number, S-9601-01. Clinical histories included maternal age, gravidae, length of gestation, and use of antimalarial drugs. Information from the 740 women included in this study was compared with data obtained from a larger group (n=1,700) collected between Sept. 1994 and March 1999. Paired group comparisons (*e.g.*, maternal age, number of pregnancies, infant birth weights, number of multiple

births, percent low birth weight infants) revealed no statistically significant differences between the two data sets. Thus, we conclude that the women in this study are representative of those living in the Biyem Assi area of Yaounde.

Collection and Processing of Samples for Parasitological and Immunological Evaluation

At delivery, samples of maternal venous (8 cc) and placental (5 cc) heparinized blood were collected by the attending physician/mid-wife. Infant birth weight was recorded, and a 2x2x2 cm section of the placenta was obtained, a portion of which was fixed in 10% buffered formalin. In the laboratory, thick and thin blood films were prepared of maternal blood and impression smears were made of placental tissue. Slides were fixed, stained with Diff-Quick (Baxter Scientific, Franklin Lakes, NJ), and examined for the presence of parasites. Parasitemia was scored as the number of parasitized erythrocytes divided by the total number of erythrocytes counted (usually 2,000) based on thin film and impression smears. If parasites were not detected in 200 oil immersion fields of thick films, the slide was declared to be negative. Blood samples were centrifuged, the packed cell volume was recorded, and plasma samples were frozen at -28° until used for detection of cytokines.

Defining Outcome Groups In order to evaluate the influence of placental malaria on birth weight, four groups of women were constructed. A total of 65 LBW infants were available for study, 39 who were born to malaria-negative mothers (Group 1: $n=39$, MAL⁻/LBW) and 26 whom were born to malaria-positive mothers (Group 2: $n=26$, MAL⁺/LBW). For comparison, a total of 73 average (ABW) infants were selected, 50 of who were born to malaria-negative (Group 3: $n=50$, MAL⁻/ABW) and 23 who were born to malaria-positive mothers (Group 4: $n=23$, MAL⁺/ABW). ABW was defined as birth weights within one standard deviation of the population mean birth weight.

Histological Evaluation of Placental Tissues Formalin-fixed placental samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Each slide was coded and examined microscopically for pathology. Based on a modification of the classification system devised by Bulmer *et al.* (1993), slides were scored as 1) active new infection (parasites present, but little or no evidence of cellular infiltration or hemozoin pigment deposition), 2) chronic (parasites plus sizable amounts of hemozoin pigment deposition and increased numbers of leukocytes), 3) past infection (same as 2 but no parasites were seen), and 4) negative (no evidence of parasites or pigment). It

should be noted that improperly buffered formalin produces formalin crystals that are indistinguishable from hemozoin, including size, shape and location. It is likely that formalin crystals were present in some of our samples. However, since the slides were coded, background levels of this artifact should be equally distributed among the four groups.

Morphometric Analysis of Placental Tissues Histological sections of placentas were examined using the mean linear intercept technique described by Weibel and Elias (1967) and expanded upon by Aherne and Dunnill (1966, 1982). In brief, slides were examined using an Olympus BX-40 light microscope at 400X with a 1 mm² reticule imposed on the image (surface area 250 μm²). On each slide, villi were selected that fit within the confines of the imposed reticule. Since villi diameter increases as one ascends the villus tree, cross sections were selected to fit within the 250 μm² area. Once properly positioned, the number of times parallel lines within the reticules intersected with fetal capillaries and the surrounding syncytiotrophoblasts was recorded. The measurement was repeated with the reticule rotated 45 degrees from the first observation. A third measurement was made after rotating the reticule an additional 45 degrees. The three values for each villus were averaged, and the perimeter of each vessel was calculated. A total of 10-15 villi were measured for each placenta.

Histological sections were also examined to determine the average number of fetal vessels per villus. Only villi that fit within the 250 μm² reticule were included to ensure uniformity. The number of fetal vessels present per villus was recorded for 10-15 villi per slide.

For comparison, sections from 20 formalin-fixed placentas obtained from normal, full-term deliveries at the Georgetown University Medical Center were examined.

Assay for TNFα Plasma samples obtained from intervillous placental blood were assayed for TNFα using a Quantikine kit (R&D Systems, Minneapolis, MN). In this antigen-capture assay, 200 μl of undiluted placental plasma were used. Amounts (pg/ml) were determined by comparing O.D. values for the test plasma with a standard curve of known concentrations of human TNFα provided in the kit.

The Assay for Angiogenin Levels of angiogenin present in maternal peripheral plasma were determined by quantitative ELISA (Quantikine, R&D Systems, Minneapolis, MN). This antigen-capture assay uses a 1:200 dilution of plasma. Amounts are deter-

Table 1: Comparison of Malaria-Positive and Malaria-Negative Women at Delivery

	Malaria-Positive Women	Malaria-Negative Women
Maternal age	24.7 ± 5.0 years	25.6 ± 5.3 years
Weeks of gestation	39.3 ± 2.7 weeks	39.5 ± 2.5 weeks
Number of pregnancies	3.0 ± 1.9 pregnancies	3.1 ± 2.0 pregnancies
% women with PCV < 30%	62%	16%
Mean hematocrit	31 ± 5%	35 ± 5%

mined by comparing O.D. values for the test plasma with a standard curve of known concentrations of human angiogenin (78-5,000 pg/ml).

RESULTS

Prevalence of Malaria in Pregnant Women: The 740 women ranged in age from 16 to 38 years old; 26% were primigravidae, 22% secundigravidae, and 52% had had three or more pregnancies (multigravidae) (Table 1). Among the deliveries, 89.8% were full-term, 6.2% premature, and 4.0% resulted in spontaneous abortions or stillbirths.

At delivery, 22.8% of the women were infected with *P. falciparum*. Among the malaria-positive women, 96% had placental malaria, 26.1% of whom were peripheral blood smear negative. Only 4% of the women had parasites in peripheral blood but not in the placenta. Thus, approximately 1 out of 4 women with placental malaria would not have been correctly diagnosed based on routine microscopic examination of their peripheral blood smears. These results confirm those we reported previously (Leke *et al.*, 1999) and emphasize the need for improved methods of diagnosis.

Primigravidae mothers were more likely to be infected than multigravidae at the time of delivery (24.9% compared to 16.4%, respectively) (Chi-Square, $p=0.04$). They were also more likely to suffer from anemia (<30.0% PCV) (Table 1). The distribution of peripheral and placental parasitemias in all malaria-positive women is shown in Figure 1. Peripheral parasitemias ranged from 0-5.6%, with most of the women having peripheral parasitemias between 0.001-1.0%. As noted above, 26.1% of the women with placental malaria were blood smear negative. Placental parasitemias were clearly higher than peripheral parasitemias, and ranged from 0.01-70.0% (Fig.1). There was a strong correlation between peripheral and placental parasitemias (lin-

ear regression, $r = 0.604$, $p < 0.001$). Although the prevalence of malaria was higher in primigravidae, no statistical difference was found between primi- and multi-gravid women with respect to peripheral or pla-

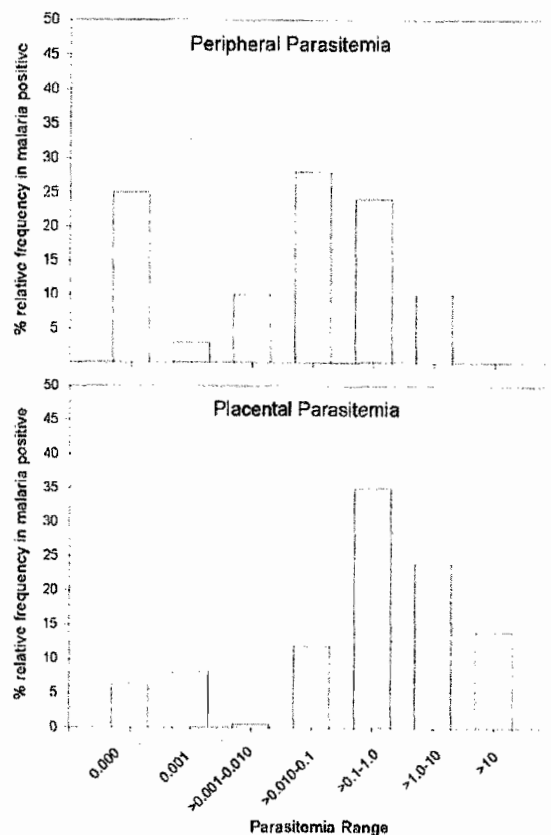


Figure 1: Distribution of Parasitemias in Malaria-Positive Women.

- A. Maternal Peripheral Blood
B. Maternal Placenta Blood

The top figure shows the distribution of peripheral parasitemia among malaria-positive women. Peripheral parasitemias ranged from 0.001-5.5%. The bottom figure displays the distribution of placental parasitemias. Approximately 5% of mothers who were peripheral blood smear-positive did not have detectable levels of parasites in the placenta. Placental parasitemias ranged from <0.001 to 70%.

Table 2: Comparison of Pregnancy Outcome in Women who were Malaria-Positive and Malaria-Negative at Delivery

	Malaria-positive Women	Malaria-Negative Women	P value
Mean Birth Weight	3,077 ± 539 gm	3,261 ± 466 gm	0.001
Mean Placental Weight	571 ± 147 gm	608 ± 172 gm	0.0
% LBW Infants	12.1%	5.2%	0.004

cental percent parasitemias.

Effect of Malaria on Birth Weight The distribution of birth weights of Cameroonian infants ($n=708$) born to malaria-positive and malaria-negative mothers is shown in Figure 2. Infants of malaria-negative mothers averaged 3,261 grams (Table 2) and their weights

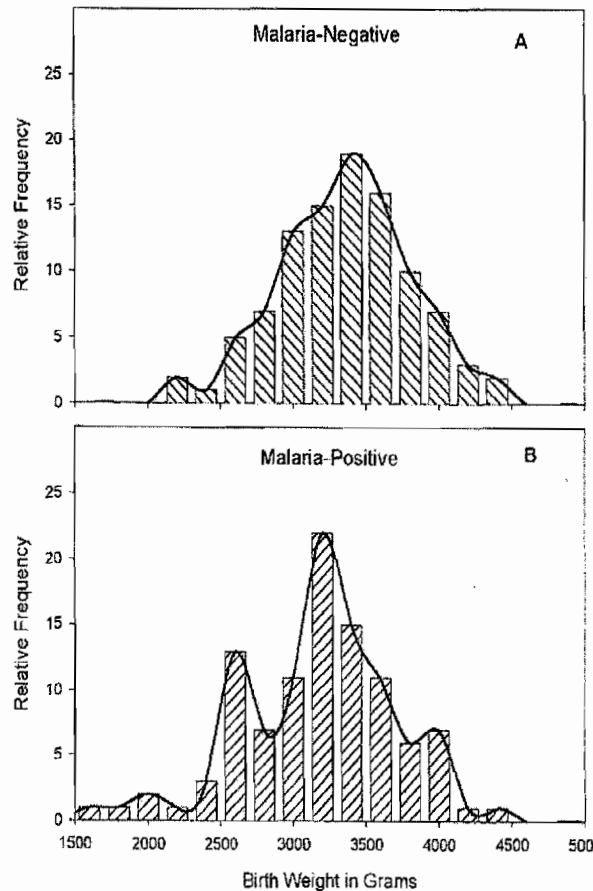


Figure 2. Distribution of Infant Birth Weights ($n=708$).
 A. Malaria-negative women
 B. Malaria-positive women

The top figure shows the distribution of birth weights for infants born to malaria-negative mothers. Note that weights were normally distributed with mean, median and mode within 1% of each other. The mean birth weight was 3,261 grams. The bottom figure shows the distribution of birth weights for infants born to malaria-positive mothers. The average was 3,077 grams and the distribution curve exhibited broad central tendencies with a central peak flanked by minor peaks.

were normally distributed with mean, median and mode within 1% of each other. In comparison, infants born to malaria-positive mothers averaged 3,077 grams and the distribution curve exhibited broad central tendencies with a central peak flanked by minor peaks (Fig. 2). The mean birth weight was significantly lower in infants born to malaria-positive mothers, with a mean depression of 184 grams ($p<0.001$). In addition, the frequency of LBW infants was significantly higher in women who were malaria-positive at delivery (12.1% compared to 5.2%, respectively), (Chi Square, $p = 0.004$) (Table 2).

Figure 3 shows that about half of the LBW cases were due to prematurity (<37 weeks), and the other half were due to intrauterine growth reduction (IUGR). Therefore, *P. falciparum* has a demonstrated effect on infant birth weight and risk of LBW deliveries in Yaounde.

Comparison of Placental Changes in LBW and Average BW Infants: Table 3 summarizes important parameters for the four groups of MAL⁺/MAL⁻ women who delivered LBW and ABW infants. Women in the four groups were similar with respect to age and parity, but malaria-positive mothers (both Groups 2: MAL⁺/LBW and Group 4: MAL⁺/ABW) suffered more from anemia than their malaria-negative counterparts (*i.e.*, Groups 1 compared to 2: 35% vs. 31%; Group 3 compared to 4: 34% vs. 34.6%; $p<0.05$).

Examination of coded slides revealed significant differences between the LBW malaria-positive group (Group 2) and the other 3 groups (Table 4). Seventy-five percent of the placentas in the LBW group showed evidence of chronic infection compared to only 11% of ABW deliveries (Table 4). In addition, there was a significantly larger number of macrophages in the MAL⁺/LBW group compared to the number of macrophages in the MAL⁻/ABW group ($p<0.05$). Thus, there was evidence that an on-going inflammatory-type response was occurring at the time of delivery in malaria-positive mothers who had LBW infants.

: Mean Values for the Malaria-Positive and Malaria-Negative Women Who Delivered LBW and ABW Infants

Group	n	Maternal Age (yrs)	# pregnancies	Gestation (weeks)	Birth weight		Parasitemia (%)	
					(grams)	% premature	Peripheral	Placental
MAL ⁺ /LBW	39	24.8 ± 6.6	2.8 ± 2.4	36.7 ± 4.0	2,233 ± 442	42.9%	0	0
MAL ⁻ /LBW	26	23.0 ± 4.5	2.4 ± 1.7	36.6 ± 4.5	2,286 ± 378	50.0%	0.18 ± 0.39	5.8 ± 12.6
MAL ⁻ /ABW	50	24.7 ± 4.5	2.9 ± 1.8	40.2 ± 2.5	3,498 ± 328	7.0%	0	0
MAL ⁺ /ABW	23	23.8 ± 4.5	3.0 ± 1.9	40.6 ± 2.0	3,429 ± 282	9.10	0.16 0.32	5.2 ± 11.5

To confirm this observation, placental plasma samples were tested for TNF α , a pro-inflammatory cytokine. The 138 plasma samples tested were classified as either negative for TNF α (*i.e.*, had levels below the sensitivity of the test, <10 pg/mL) or positive for TNF α (*i.e.*, had \geq 10pg/mL). Results showed that 70% of placental plasma samples from MAL⁺/LBW infants (Group 2) had >10 pg/ml of TNF α , compared to 40% of those in Groups 1 (MAL⁻/LBW) and 4 (MAL⁺/ABW), and about 25% of those in Group 3 (MAL⁻/ABW). Thus, placentas of malaria-positive with LBW infants showed signs of long-term malaria infection, an increase in the number of macrophages, and a higher rate of TNF α production.

To evaluate the potential of malaria-induced stress on placental architecture, the number of fetal vessels per villus was determined (Table 4). Pair-wise ANOVA analysis showed that none of the four groups differed significantly from each other, however, both malaria-positive groups (MAL⁺/LBW and MAL⁺/ABW) had a larger number of fetal vessels per villus compared to that found in placentas collected in the U.S. from nor-

mal full-term deliveries (p<0.05) (Table 4). No association between number of fetal vessels and LBW was found.

The ratio of fetal capillary absorptive surface to the villus absorptive surface is usually <1.0 (Laga, 1973). In the current study, the value for placentas collected in the U.S. averaged 0.74 (Table 4), and those for the MAL⁻/LBW and MAL⁻/ABW averaged 0.76 and 0.79, respectively. There was however, a significant increase in the ratio in both the MAL⁺/LBW and MAL⁺/ABW groups (p<0.001). The changes in placental architecture in the malaria-positive women were similar to those reported for women with various types of anemia (Bernishke and Kaufman, 1995). Since malaria-positive women had a significantly higher level of anemia (Table 1), it is likely that malaria-associated anemia contributed to the restructuring of the placenta.

Because of the trend of increased number and diameter of fetal vessels in malaria-positive women, plasma angiogenin levels were measured. Plasma angiogenin ranged from 104 to 767 pg/mL. No difference in the

Table 4: Summary of Histological Results for the Four Groups

Group	Placental Malaria ^(a)				Macroph. Score ^(b)	No. Fetal Vessels per Villus ^(c)	Ratio FV/V ^(d)
	None	Acute	Chronic	Past			
1-MAL ⁻ /LBW	80%	0	0	20	1.6	6.5	0.76
2-MAL ⁺ /LBW	0	10	75	15	2.2	7.4	1.10
3-MAL ⁻ /ABW	76%	4	0	20	1.7	5.7	0.79
4-MAL ⁺ /ABW	0	47	11	42	1.7	7.7	1.13
Placentas from US	0	0	0	0	1.5	4.4	0.74

(a)- See Materials and Methods for description of acute, chronic, and past.

(b)- Macrophage score: 1= 0 per 50 WBC; 2= 1-2 per 50 WBC; 3= >2 per 50 WBC.

(c)- Average number of fetal blood vessels per villus

(d)- FV/V= average ratio of fetal vessel perimeter to trophoblast perimeter.

(e)- Percent of women in this category

level of angiogenin was found among the four groups (mean MAL⁻/LBW 435 pg/mL; MAL⁺/LBW 425 pg/mL; MAL⁻/ABW 450 pg/mL; and MAL⁺/ABW 550 pg/mL).

DISCUSSION

In Yaounde, the general picture of *P. falciparum* malaria in pregnant women is similar to that reported in many African settings. Approximately 22.8% of women delivering at the Biyem Assi Hospital were infected with *P. falciparum*. Results showed that 96% of infected women had placental malaria, *i.e.*, parasites were sequestered in the placenta. Only 4% had peripheral parasitemia without evidence of placental infections. As reported previous, approximately 1 out of 4 women with placental malaria was diagnosed as malaria-negative based on examination of peripheral blood smears. Since placental malaria has a effect on infant birth weight, diagnosing and adequately treating women with placental malaria is of extreme importance.

Based on results from 740 women residing in Yaounde, a statistically significant decrease in mean birth weight of 184 grams was observed in infants born to malaria-positive women compared to uninfected women ($p < 0.001$). Approximately 12 comparable epidemiological studies of malaria in pregnant women have been conducted in West (Nigeria, Ghana, Gambia, Zaire) and East (Uganda, Tanzania) Africa (Reviewed in Brabin 1983; Jelliffe 1968; Meuris *et al.* 1993; Morgan 1994). In all but one (Bulmer, *et al.* 1993a), mean birth weights were decreased in malaria-positive deliveries (range 55-310 grams) based on samples sizes ranging from 50-

576 women. In some of these studies, mean birth weight differences were not significant between malaria-positive and malaria-negative women, probably because of the small sample size. In addition, our results demonstrate an increased risk of LBW infants ($p = 0.004$) and reduced placental weights in mothers with placental malaria at time of delivery (Table 2).

There are two major causes for LBW of infants, namely prematurity and intrauterine growth reduction (IUGR). Based on information on expected date of delivery, half of the LBW infants were due to prematurity (Fig. 3). The cytokine TNF α has a demonstrated role in termination of pregnancy (Wegmann *et al.* 1993; Hill and Choi, 2000; Gucer *et al.* 2001; Aidoo *et al.* 2001). Clark and Chaudhri (1988) demonstrated that TNF α levels during rodent malaria resulted in fetal adsorption (abortion). Many pathogens, in addition to malaria, induce TNF α production (Raghupathy, R. 1997). It is thought that TNF α and accompanying fever lead to induction of premature labor (Gucer *et al.* 2001). TNF α promoter polymorphism has been shown to influence the amount of TNF α produced. Individuals homozygous for TNF2 allele have a significantly higher risk of pre-term delivery following malaria infection (Aidoo *et al.*, 2001). The impairment of transport of nutrients and oxygen between the mother and fetus can result in a decrease in fetal growth rate (*i.e.*, IUGR, or small for gestational age). IUGR may result from slow placental developing early in pregnancy, resulting in a small placenta with reduced transport capabilities or from damage to syncytiotrophoblasts. Inflammation within the placenta, including the production of TNF α , is likely to result in

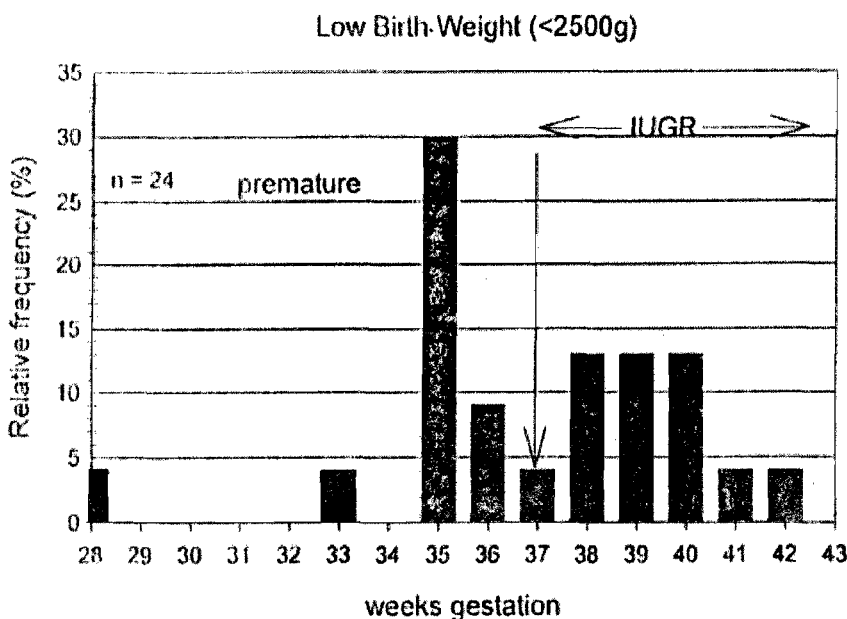


Figure 3: Gestational Age of Infants Weighing Less than 2,500 grams at Birth (LBW). Approximately, half of LBW infants were premature (<38 weeks) and half were small for gestational age (IUGR). *n* = 24.

placental damage. Thus, several different mechanisms are responsible for LBW. The presence of malarial parasites sequestered among fetal villi could invoke all of the above responses.

All results in this study are based on detection of placental malaria at the time of delivery. It was not possible to determine when during pregnancy the mother became infected and placental malaria became established. Examination of histological sections gives some indication about events that occurred during pregnancy. Bulmer *et al.* (1993b) developed a classification system that allows investigators to separate placental pathologies into four categories: namely no infection, a new acute infection, a chronic infection, or past infections as demonstrated by the presence of hemozoin pigment. Unfortunately, improperly buffered formalin produces crystals with the same morphological characteristics as hemozoin. Thus, in this study, we cannot be absolutely certain that what appears as hemozoin really constitutes a past infection. Taking this caveat into consideration, results obtained from reading "coded slides" show pathological evidence of chronic malaria infection with an increase in macrophages in 75% of malaria-positive placenta of LBW deliveries (MAL⁺/LBW) compared to only 10.6% of those that resulted in average birth weight (MAL⁺/ABW) infants. Examination of placental plasma for TNF α from these two groups revealed that 80% of samples from the MAL⁺/LBW group had measurable levels of TNF α compared to only 40% of women in the MAL⁺/ABW group. Since a variety of microbes and pathological situations stimulate TNF α production, it was not surprising to find TNF α in 30-40% of malaria-negative women (MAL⁻/LBW and MAL⁻/ABW) (Table 4). Overall, these results suggest that placental malaria infections that have been on-going for an extended period of time, increased the risk of LBW infants due to both prematurity and IUGR.

To determine if sequestration of *P. falciparum* parasites and subsequent inflammatory-type responses occurring among the intervillous trees lead to remodeling of placental architecture, the number and absorptive surface area of fetal blood vessels and angiogenin were measured. Angiogenin is thought to be responsible for both placental growth early in pregnancy and structural changes late in pregnancy (Kolben *et al.* 1997). As the name implies, angiogenin is a growth factor for blood vessels. Histological studies found an increase in the number and diameter of fetal blood vessels in the placentas of malaria-positive women, but there was no association with these changes and newborn birth weights. In addition, there was no difference in amount of angiogenin among the four groups. Accordingly,

changes in the micro-architecture were correlated with placental malaria, but not LBW deliveries. Therefore, it appears that angiogenesis occurs during pregnancy that is sufficient to prevent a decrease in fetal development and infant birth weight.

In summary, the pattern of malaria in pregnant women in Yaounde is similar to that reported in other parts of Africa, with approximately one-quarter of women having placental malaria at the time of delivery. The presence of malaria was associated with a significant decrease in infant birth weight. Changes in the placenta of women who delivered LBW infants included evidence of chronic malaria infection, infiltration of macrophages, and increased prevalence of TNF α production. Placental malaria also induced changes in the micro-architecture of the placenta, including increased number of fetal blood vessels and diameter of fetal vessels, but these changes were not correlated with decreased birth weight. Clearly, prompt and accurate diagnosis of placental malaria is important, but microscopic examination of peripheral blood smears fails to detect placental malaria in approximately 1 out of every 4 women with this condition. Improved diagnosis of placental malaria is needed to improve the health of pregnant women and their developing children.

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