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Maternal Placental Infection with *Plasmodium falciparum* and Malaria Morbidity during the First 2 Years of Life

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In areas endemic for malaria, pregnant women frequently present with a placenta that has been parasitized by *Plasmodium falciparum*, an infection associated with a reduction in the birth weight of the offspring. However, the impact of placental infection on malaria-related morbidity during the infant's first years of life has not been investigated. Between 1993 and 1995, 197 children in southern Cameroon were followed weekly clinically and monthly parasitologically. The dates of first positive blood smear and the evolution of the parasite prevalence rates were compared between infants born to mothers presenting with (n = 42) and without (n = 155) *P. falciparum* infection of the placenta. Infants born to placenta-infected mothers were more likely to develop a malaria infection between 4 and 6 months of age; then the difference progressively disappeared. Similarly, parasite prevalence rates were higher in placenta-infected infants from 5 to 8 months of age. Thus, malarial infection of the placenta seems to result in a higher susceptibility of infants to the parasite. This was not related to maternally transmitted antibodies, as specific antibody levels were similar in both groups of infants. A better understanding of the involved mechanisms may have important implications for the development of malaria control strategies. *Am J Epidemiol* 1997;146:826–31.

infant; malaria; placenta; *Plasmodium falciparum*

In areas endemic for *Plasmodium falciparum*, pregnant women are more likely to develop malaria than their nonpregnant counterparts (1). One of the most striking features of malarial infection during pregnancy is that, at delivery, these women frequently present with a parasitized placenta. Studies of placental parasite load have shown an association with maternal anemia and low birth weight in the offspring (2, 3). Low birth weight is one of the leading risk factors for neonatal mortality and morbidity in malaria-endemic areas (4, 5); thus, placental infection with *P. falciparum* poses a major public health challenge. Placental infection in the mother has also been associated with an increased prevalence of anemia in the infant during the first months of life (6). The mechanisms responsible for these malaria-related alterations at birth and the increased morbidity after birth have not been elucidated. Yamada et al. (7) suggested that the inflammatory process which occurs in the placenta in cases of malaria infection reduces maternal-fetal exchange and thus the growth of the fetus.

The consequences of maternal *P. falciparum* infection during pregnancy, especially of placental infection, on the child's health during the first years of life have been less frequently investigated. Infants born to a malaria-immune mother have reduced sensitivity to malaria during the first months of life, both clinically and parasitologically (8). This is thought to be related to the passage through the placenta of specific antibodies from the mother to the fetus (8). Alternatively, transplacental passage of parasitic antigens during pregnancy has been demonstrated (9), and the effect of in-utero stimulation of the fetal immune system by *P. falciparum* antigens is unknown. Studies of other parasitic diseases have shown that maternal infection during pregnancy may have a long-lasting influence on the child's capacity to eliminate (10) or tolerate (11) the parasites. In the case of *P. falciparum*, the effects of placental infection on the infant's immune system, and thus on his/her susceptibility to malaria during the first years of life, are poorly known. Therefore, we assessed the clinical and parasitologic malaria status of children from birth to 2 years of age, the

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Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M.

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mothers of some of whom had presented with *P. falciparum* placental infection at delivery.

MATERIALS AND METHODS

Study area

The study was carried out in Ebolowa, southern Cameroon, a city of 35,000 inhabitants 160 km south of Yaounde. The area is characterized by an equatorial climate. Rain falls year-round (1,700 mm), with two peaks from September to December and from April to June, allowing the persistence of anopheline breeding sites all year and perennial transmission of malaria parasites. The town is divided into three main physical areas: the residential area, located mainly in the center of the town; the slums around the town; and small villages scattered in the surrounding forest. In the inner city, houses are most often built of concrete. In the neighboring slums and villages, houses are built in the traditional style with mud walls and corrugated sheet metal roofs. The main ethnic groups are the Bulu people (a subgroup of the Bantu) and groups from western (Bamileke) and central (Eton, Ewondo, Bassa) Cameroon.

Health services are provided through one provincial government hospital (Ekombitie Hospital), one church hospital (Cameroonian Presbyterian Church Enongal Hospital), and governmental or church health centers. Recourse to health centers is usual, but self-medication is also frequent. Oral chloroquine is the current treatment for uncomplicated malaria, whereas intramuscular or intravenous quinine is used for severe malaria.

Study population

Between January and June of 1993, after informed consent was given by the parents, 197 newborn babies were enrolled at birth and subsequently followed for 2 years. A team member went to the infants' homes every week and asked each mother about the health condition of her child, measured the child's axillary temperature, and, in case of fever, obtained a blood smear for detection of a possible malaria attack. An attack was defined as an axillary temperature higher than 37.5°C, the presence of *Plasmodium* in the blood, and the absence of clinical signs that might denote a different pathologic infection. In addition, a blood smear was taken from each child every month to assess asymptomatic carriage of parasites.

For cultural and commercial reasons, Ebolowa residents regularly travel between the town and their home villages. They began to commute even more after their currency (the Communauté Financière Africaine or "CFA franc") devaluated in January 1994. Children were present for 60 percent of the household visits, on average. Children who were absent for a 6-month period or more were considered lost to follow-up. We assessed whether this absenteeism created any confusion and determined that, in the two groups of children, 1) the absenteeism rates over the entire duration of the study were similar; 2) the average durations of follow-up were similar; and 3) the children's absences occurred in the same way over time (table 1). Furthermore, each time a child was absent, even when he/she never came back into the study area, we were assured that this was not due to medical reasons.

Laboratory methods

At each child's birth, a small specimen of placenta was taken from the mother, and six wiped blood samples were applied to a slide. The slide was Giemsa-stained, and each smear was examined under a microscope. Thick blood smears were Giemsa-stained and examined against 200 leukocytes if positive or against 1,000 leukocytes prior to being declared negative. Parasite densities were recorded as the number of parasites per μl of blood, assuming an average leukocyte count of 8,000 cells per μl of blood.

Total and anti-*P. falciparum* levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) were measured in the mother's blood at delivery, as well as in

TABLE 1. Characteristics of children born to mothers with or without *Plasmodium falciparum* infection of the placenta, southern Cameroon, 1993

	Malaria-infected placenta	Noninfected placenta	*p* value
No. of children	42	155	
Gravidity of mother (%)			0.003
Primigravida	31	69	
Multigravida	14	86	
Geographic origin (%)			0.04
Residential area	19.0	81.0	
Slum	16.2	83.8	
Village	34.8	65.2	
Sex (%)			NS*
Male	16.2	83.8	
Female	22.6	77.4	
Birth weight (g)	2,971 (468)†	3,118 (428)	0.015
Absenteeism rate (%)	39	42	NS
Mean duration of follow-up (weeks)	73.6 (49)	67.8 (45)	NS
Mean age of children visited (weeks)	65.3 (40.4)	65.4 (40.0)	NS

* NS, not significant.
† Numbers in parentheses, standard deviation.

umbilical cord blood. Total IgG and IgM levels were measured by nephelometry. Specific anti-*P. falciparum* antibodies were measured by enzyme-linked immunosorbent assay, using a lysate from in vitro cultured parasites (Palo Alto strain), as described elsewhere (12). Positive and negative control serum pools were included in each plate, and results were expressed in arbitrary units, calculated from the following formula: $100 \times [\ln(\text{absorbance test serum}) - \ln(\text{absorbance negative pool})]/[\ln(\text{absorbance positive pool}) - \ln(\text{absorbance negative pool})]$.

Statistical analysis

Children born to mothers with *P. falciparum* infection of the placenta were compared with those born to noninfected mothers. The frequency and regularity of the follow-up procedure, as assessed by the mean duration of follow-up and the mean age of the children being followed, were compared between groups by analysis of variance. Groups were compared by Pearson's χ^2 or Fisher's exact test for qualitative variables and by analysis of variance for quantitative variables, using Epi-Info 5.0 software (Centers for Disease Control and Prevention, Atlanta, Georgia). The ages of first appearance of parasites in the blood were compared between groups by performing a lifetime estimate via the Kaplan-Meier method, using the EGRET program (Statistics and Epidemiology Research Corporation, Seattle, Washington). Any p values less than 0.05 were considered significant.

RESULTS

A total of 197 children were enrolled in the study at birth. Forty-two infants (21.3 percent) were born to a mother with malarial infection of the placenta (table 1). Thirty-seven children (18.8 percent) were lost to follow-up during the 2-year follow-up period, including seven children born to placenta-infected mothers. The prevalence rate of placental infection was higher among primigravidae than among multigravidae (31 percent vs. 14 percent). However, *P. falciparum* malaria prevalence rates during follow-up were similar in children born to primigravidae and those born to multigravidae (29.7 percent vs. 27.1 percent). Similarly, women originating from villages were more likely to present with a placental infection than other women (table 1), but *P. falciparum* malaria prevalence rates during follow-up were similar in children from villages and those from slums (27.5 percent and 32.6 percent, respectively) and were higher than the rate seen in children from the residential areas of the city (19.5 percent) ($\chi^2 = 31$, $p < 0.00001$). Contrary to gravidity, which is unlikely to represent a confounding factor in the relation between maternal placental infection and child's parasite prevalence rate, location of residence may be a confounding factor in this relation. Indeed, area of residency reflects the level of anopheline malaria transmission, as well as the socioeconomic status of the family.

Figure 1 shows the age-related evolution of the parasite prevalence rates in children born to mothers presenting with and without placental malarial infection. To reduce the fluctuations from one month to the next, we smoothed the curves by centered moving average with a span of 1. From the age of 4 months onward, and up to the age of 18 months, the parasite prevalence rate was consistently higher in the children born to placenta-infected mothers. This difference was significant between the ages of 5 and 8 months. After 18 months of age, prevalence rates were comparable in both groups and averaged 35 percent. To take into account the potentially confounding role of area of residency, we conducted the same analysis for each area separately. Despite reduced numbers of children, the difference in the evolution of the prevalence rate curves remained.

Figure 2 compares the rates of not having had a positive blood smear since birth in the two groups of children. Up to the age of 17 weeks (4 months), the two curves overlapped. Then, from age 17 weeks to age 26 weeks, the "survival rate" of the children born to placenta-infected mothers tended to decrease rapidly. From 26 weeks to 35 weeks, the slope of the curve for children born to placenta-infected mothers was smoother than that of the other group. Consequently, after the age of 35 weeks, the two curves met

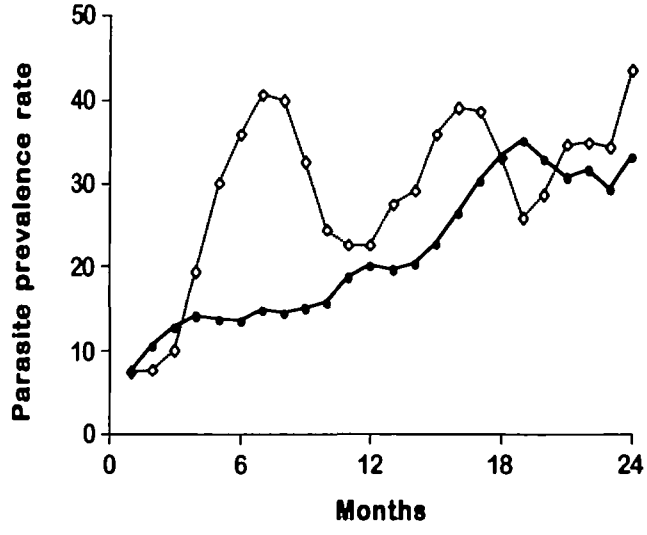

FIGURE 1. Monthly evolution of *Plasmodium falciparum* prevalence rates (percent) in infants born to mothers presenting with (◇) or without (●) placental malaria infection at delivery, southern Cameroon, 1993–1995.

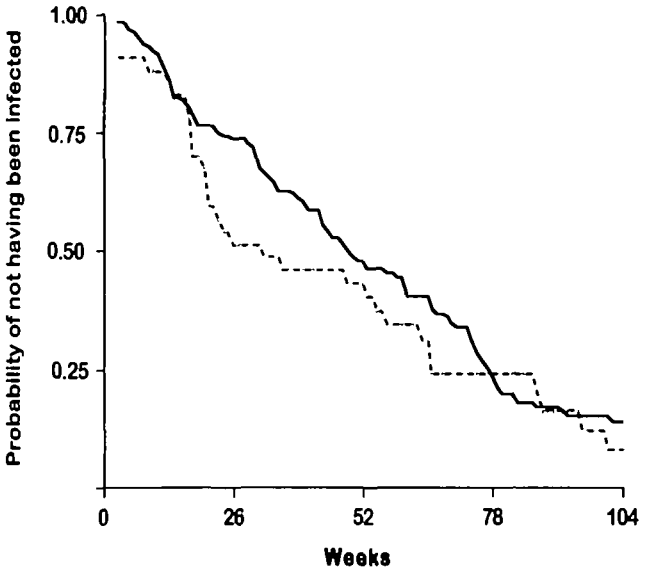

FIGURE 2. Probability of not having had a *Plasmodium falciparum* infection since birth in infants born to mothers presenting with (- - -) or without (—) placental malaria infection at delivery, southern Cameroon, 1993–1995.

and showed similar decreases up to the age of 104 weeks (24 months).

As a consequence of the study design (weekly home visits), the number of clinical malaria attacks identified was small. Forty percent of the children had at least one malaria attack during follow-up—46.5 percent in the placenta-infected mother group and 38.5 percent in the non-placenta-infected mother group ($\chi^2 = 0.24$, $p = 0.60$). In the two groups of children, the survival curves for malaria attacks were parallel during the entire follow-up period. This is in contrast to the parasite prevalence curve and the survival curve of parasite infection, which both diverged between 4 and 6 months of age.

Immunoglobulins were measured in peripheral maternal blood and umbilical cord blood from 128 mother-child pairs, in 34 of which *P. falciparum* malarial infection of the placenta was detected. Total IgG levels were higher in the peripheral blood of women with placental infection than in the other women (table 2). Total levels of IgM, as well as levels of anti-*P. falciparum* IgG and IgM, were similar in the two groups of mothers. Similarly, total and anti-*P. falciparum* IgG levels were similar in umbilical cord blood from the two groups of newborns. As expected, the prevalence rates of total and anti-*P. falciparum* IgM were low in both groups of cord blood, and, in positive samples, the levels were consistently low. IgM was detected in 11 of 34 and 14 of 94 cord blood samples corresponding to infected and noninfected placentas, respectively ($\chi^2 = 5.25$, $p = 0.028$). For anti-*P. falciparum* IgM, these prevalence rates were 3/34 and 2/94 ($p = 0.13$).

DISCUSSION

In this study, the prevalence rates of *P. falciparum* infection of the placenta varied between areas of residency. These areas differ mainly by two parameters: the malaria transmission level and the socioeconomic background of the inhabitants. These two factors may affect the occurrence of malaria parasitemia in both pregnant women and children. Hence, women with a malaria-infected placenta may have been more exposed to malaria during pregnancy than women without a placental infection. The children of the former women, living in the same environment as their mothers, may on average be more exposed than children born to the latter women. Since individual levels of exposure cannot be determined in an epidemiologic study such as this one, we cannot definitely rule out the influence of an exposure factor in the differences observed between the two groups. However, most likely, there was no difference in exposure between the two groups of children. Indeed, the parasite prevalence rates increased similarly in 5- to 8-month-old children from the different parts of the study area. Similarly, in all parts of the study area, the incidence of malarial infection was higher in 4- to 6-month-old

TABLE 2. Total and anti-*Plasmodium falciparum* levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) in the peripheral blood of mothers and in umbilical cord blood, according to the presence of malarial infection of the placenta, southern Cameroon, 1993

	Mother's blood					Cord blood				
	Malaria-infected placenta		Noninfected placenta		*p* value	Malaria-infected placenta		Noninfected placenta		*p* value
	Mean	SD*	Mean	SD		Mean	SD	Mean	SD	
Total IgG (mg/ml)	24.7	1.7	22.3	1.1	0.03	19.1	1.4	17.8	0.7	0.1
Specific IgG (AU*)	84.3	3.7	83.2	3.5	0.7	85.7	3.3	83.9	3.4	0.8
Total IgM (mg/ml)	2.8	0.4	2.8	0.3	0.9					
Specific IgM (AU)	61.5	7.9	60.0	5.6	0.8					

* SD, standard deviation; AU, arbitrary units (see text).

children born to placenta-infected mothers. Moreover, if longer or higher exposure to the parasite in some areas had caused a difference between the two groups of children, this difference should have been recorded throughout the entire duration of the study. That was not the case. Furthermore, a difference in exposure between the two groups might account, at least partially, for the discrepancy in the two prevalence curves between 9 and 17 months of age.

The curve of the dates of first positive blood smear in children born to placenta-infected mothers suggests that the increased susceptibility to malaria between 4 and 6 months of age diminishes or vanishes above a given age. Children born to infected mothers have been largely excluded during the increased susceptibility period. Thus, afterward, the number of never-positive subjects born to noninfected mothers decreases proportionally faster than the number of children born to infected mothers. Hence, overall, survival rates are comparable in both groups, which reduces the likelihood of a significant result in the log rank test. Taken together, the parasite prevalence rate curves and the survival curves both suggest the existence of an additional and transient phenomenon that increases the incidence of malaria between 4 and 6 months of age in children born to placenta-infected mothers. Whether this phenomenon has an actual impact on resistance to *P. falciparum* infection or on the control of parasite density below the detection threshold of the thick blood smear (10 parasites per μl of blood) remains to be determined. Indeed, when the children were 6 months old, the prevalence rate of anemia was higher among children born to placenta-infected mothers than in the other children (13). Similarly, in Malawi, placental malaria infection in the mother was the strongest risk factor for an infant's exhibiting anemia during the first months of life (6). Placental damage related to malaria infection may allow exposure of the fetal immune system to a variety of malaria antigens; this exposure may render the newborn infant more susceptible to immunologically mediated hemolysis or to dyserythropoiesis.

An infant has a reduced sensitivity to malaria during his or her first months of life. This is related to both the persistence of fetal hemoglobin (14) and transplacental passage of the mother's antibodies (8). Fetal hemoglobin limits the growth of parasites, but its action is likely to be similar in all children. Maternal antibodies persist in child blood for a few months, but this does not seem to be related to the increased susceptibility to malaria of children born to placenta-infected mothers. Indeed, the umbilical cord blood levels of total and specific anti-*P. falciparum* IgG were similar in both groups of children.

Alternatively, in-utero exposure to malaria antigens may induce immunologic tolerance (15) and potentially modify future susceptibility to malaria infection and/or disease. The impact of tolerance has already been observed with other parasites; a higher mortality rate occurs in offspring of mice chronically infected with *Trypanosoma cruzi* (16). In-utero exposure to filarial antigens renders offspring immunologically tolerant to parasites, and increases susceptibility to infection (11). Conversely, this exposure can prime the immune system and improve defenses against future infections (17). Because umbilical cord blood lymphocytes may be induced to differentiate into effector cells producing predominantly Th1 or Th2 cytokines (18), malaria might direct the functional capacity of fetal T cells to respond to further infection. Although the mechanism involved is not specified, the current study demonstrates that the offspring of mothers presenting with *P. falciparum* infection of the placenta are more susceptible to malaria infection during their first 2 years of life. A better understanding of the mechanisms underlying immune responses to *P. falciparum* in newborns and infants may have important implications for the development of malaria control strategies.

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