Placental Malaria is Associated With Increased Risk of Nonmalaria Infection During the First 18 Months of Life in a Beninese Population

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Background. Several studies have shown that the risk of malaria infection increases for children born to a mother with placental malaria infection. An immune tolerance phenomenon has been hypothesized. We addressed whether *Plasmodium falciparum* placental infection could additionally be associated with the risk of nonmalaria fevers in infants.

Methods. From 2007 to 2009, 553 infants were followed up from birth to 18 months in Benin. The occurrence of fever was actively screened by trained community workers. Malaria fevers (temperature >37.5°C with positive results of rapid diagnostic test or thick blood smear) were excluded from analysis. The association between placental malaria infection and the number of total, gastrointestinal, and respiratory febrile episodes was explored using binomial negative regression, with adjustment for maternal age, parity, parents’ schooling, socioeconomic level, sex, village of birth, season of birth, prematurity, Apgar score and nutritional status.

Results. The prevalence of placental malaria infection was 11.2%. During a median follow-up of 17.8 months, 624 nonmalaria fevers were registered. Placental malaria infection was associated with a higher risk of nonmalaria fever episodes (adjusted incidence rate ratio, 1.4; 95% confidence interval, 1.1–1.8) as well as gastrointestinal (1.6; 1.1–2.5) and respiratory (1.5; 1.1–2.1) febrile syndromes. The same pattern was obtained when considering consultations after the age of 6 months.

Conclusions. These results suggest an association between placental malaria infection and nonmalaria infections in the first 18 months of life. Immune tolerance could lead to impaired immune development not specific to malaria infections in infants born to mothers with placental malaria infection, but further studies are needed.
immune tolerance phenomenon [11]. However, although the ability to construct a *P. falciparum*–specific immune response is acquired by the fetus very early in utero, to our knowledge, no clear hypothesis has been put forward to clarify the underlying mechanisms that could lead to immune tolerance [12]. The association between placental malaria infection and immune development has already been described [13, 14] as well as the fact that malaria infection can suppress immunity to a variety of viral infections, such as human immunodeficiency virus, Epstein-Barr virus, vaccinia virus, and lymphocytic choriomeningitis virus infections [15–17]. More recently, it has been shown that congenital cytomegalovirus infection was more frequent in children born to mothers with *P. falciparum*–infected placenta [18]. In this last study, this association was adjusted for maternal age, bed net use and other environmental risk factors; moreover, during the first year of life, mothers of congenitally infected children reported more health complaints for their child. Altogether, these results strengthen the potential implication of a tolerogenic environment caused by malaria placental infection in this phenomenon.

Therefore, the question to be answered is whether this tolerogenic environment could be responsible for an increased susceptibility to malaria only or, more generally, to infections. This study explores the potential effect of *P. falciparum* placental infection on the occurrence of nonmalaria fevers in infants from birth until 18 months of life.

**METHODS**

**Study Area and Population**

This study, forming a part of a larger project [19], was conducted in 9 villages of the Tori Bossito area (Southern Benin) and 3 health centers (Tori Avame, Tori Cada, and Tori Gare) providing birth attendance and primary healthcare. All women living in any of the 9 villages and attending health centers for prenatal care were recommended to participate in the study with their offspring after delivery. The birth cohort was initiated in June 2007, and subjects were recruited until July 2008. Figure 1 shows the flow chart of the study population. Of the 656 pregnancies registered, 26 multiple pregnancies and 23 stillbirths or neonatal deaths (<28 days) were excluded, as were children with identification number errors (n = 13) or missing birth date (n = 3), leaving 564 infants. Among those, maternal placental infection was missing in 11 (2%); these subjects were not considered in this analysis either. Finally, 553 infants were studied.

**Data Collection**

At delivery, a questionnaire was administered to gather information on women’s sociodemographic, gynecological, and obstetric background and on the course of the current pregnancy. At the end of this questionnaire, and before delivery, anthropometric measurements (weight and height) were taken. After delivery, thick and thin blood smears were performed from the maternal side of the placenta to determine the existence of a malaria placental infection. Gestational age at delivery was determined using Ballard’s method [20]. Newborns were listed and received an identification card, giving them access to free treatment in health centers during the 18 months of follow-up.

Each child was visited once a week and axillary temperature was measured with a digital thermometer by community health workers at the infant’s home (active fever screening). In case of temperature higher than 37.5°C, mothers were told to bring their children to the health center where a questionnaire was filled out. Mothers were also invited to bring their infants to the health center at any time for free care in case of fever (suspected by the mother) or clinical signs. To test for malaria infection, both a rapid diagnostic test (RDT) and a thick blood smear (TBS) were performed; the TBS was stained with Giemsa stain and read by 2 laboratory technicians (with <1% disagreement). Leukocytes and parasites were counted simultaneously. A TBS was declared negative if no parasite was found after 500 leukocytes had been counted. Symptomatic malaria infection was defined as the presence of fever (>37.5°C) and positive findings at either TBS and/or RDT. In case of symptomatic malaria infection, infants were treated with an artemisinin-based combination therapy (artemether and lumefantrine) as recommended by the Benin National Malaria Control Program. All health expenditures, whatever the cause, were assumed by the program.

**Measured Variables**

**Definition of the Outcomes**

The outcome of interest was the nonmalaria fever episodes occurring during the first 18 months of life. They were defined...
by the presence of a measured axillary temperature $\geq 37.5^\circ$C, negative results of malaria RDT, and negative TBS during the consultation. When only the RDT or the TBS result was available, we considered its result.

In the second step, 2 types of nonmalaria febrile syndromes were considered: gastrointestinal and respiratory. The first was defined as the association of nonmalaria fever and $\geq 1$ gastrointestinal symptom (vomiting, diarrhea, constipation or abdominal pain). The second was defined as the association of nonmalaria fever and 1 respiratory (cough, dyspnea or abnormalities on auscultation) or 1 otorhinolaryngological clinical sign (mainly otitis or rhinitis). One given febrile episode could match both definitions of gastrointestinal and respiratory syndromes; in this case it was included in both categories.

\textbf{Explicative Variables}

Explicative variables concerning the mother, the family and the child were collected at inclusion and during follow-up.

\textbf{Mother and Family Variables}. The following variables were collected: (1) age of the mother at delivery (in years); (2) parity (primiparae vs multiparae); (3) educational status of the parents ($\geq 1$ parent schooled vs neither parent schooled); (4) and socioeconomic level evaluated according to the number of durable goods (radio, motorbike, flashlight, bicycle, car, pirogue, electric generator, etc) owned by the family estimated on a declaration basis ($< 4$ or $\geq 4$ goods).

\textbf{Child Variables}. Concerning the child, we collected the following variables. (1) sex, (2) village of birth, (3) season of birth (during rainy season or not), (4) prematurity (defined as a gestational age $< 37$ weeks), (5) the 5-minute Apgar score ($\leq 7$ vs $> 7$), and (6) nutritional status based on World Health Organization (WHO)/UNICEF indicators (characteristics of breast-feeding and infant and young child feeding) [21]. These indicators are based on the practice of exclusive breast-feeding from birth to 6 months and on the number of food groups consumed up to 18 months (assessed by individual 24-hour dietary recall of the mother) as recommended by WHO.

\textbf{Statistical Analysis}

As the dependent variables are nonnegative counts, we first used Poisson regression models. However, goodness-of-fit tests revealed that the dependent variables were not consistent with a Poisson-like process because of an overdispersion (data not shown). We then used negative binomial regression models, which allowed us to take this overdispersion into account. Results were expressed as incidence rate ratios (IRRs) rather than the underlying coefficients, and the potential variability of the duration of follow-up for each individual was taken into account.

The association between \textit{P. falciparum} placental infection and the number of nonmalaria febrile episodes was analyzed before and after adjustment for measured covariates, globally and for each syndrome.

We then re-ran the analysis without considering the events that occurred during the first 6 months of life to take into account the potential effect of maternal immunoglobulin that could confer a passive immunity to the child. All these analyses were performed with Stata software, version 11.0 (StatCorp).

\textbf{Ethics}

The study protocol was approved by the Ethics Committee of the University of Abomey-Calavi (Faculté des Sciences de la Santé) in Benin and the Consultative Committee of Ethics of Institute of Development Research.

\textbf{RESULTS}

\textbf{Description of the Population}

Among the 4602 consultations that occurred during the follow-up, 711 were follow-up visits scheduled by nurses to evaluate the child’s health following a previous consultation. They were excluded from the analysis. Among the remaining 3891 consultations, fever was recorded in 1212, including 624 with a negative malaria RDT and TBS results. The median follow-up duration was 17.8 months (interquartile range, 17.4–18.1). Placental malaria infection had no significant effect on this duration.

The prevalence of placental malaria infection was 11.2% (62 of 553 infants). Placental malaria infection was significantly associated with a younger maternal age, primiparity, and birth during the rainy season (Table 1). The associations between placental malaria infection and socioeconomic level and village of birth were borderline nonsignificant ($P = .060$ and $P = .057$, respectively).

In addition, 63.1% of the infants had $\geq 1$ nonmalaria fever episode during follow-up. Among them, the median number of nonmalaria fever episodes was 1 (interquartile range, 1–2). The maximum numbers of total, gastrointestinal and respiratory febrile episodes were 6, 3, and 5, respectively. Incidence rates of febrile episodes in the study population are plotted in Figure 2. The incidence rate was higher for children born from a mother with an infected placenta during the entire follow-up.

\textbf{Risk Factors of Nonmalaria Fever Episodes}

Multivariate analyses showed that placental malaria infection was significantly associated with a higher risk of nonmalaria fever ($P = .019$). The village of residence was strongly associated with fever episodes, consistent with the existence of a spatial heterogeneity of risk ($P = .0008$). There was a nonsignificant trend in favor of an association between prematurity and nonmalaria fever ($P = .066$).
The same pattern of association was observed between placental malaria infection and gastrointestinal or respiratory febrile syndrome ($P = .020$ and $P = .006$, respectively). Concerning gastrointestinal syndrome, the only other factor significantly associated with nonmalaria fever was the child’s 5-min Apgar score ($P = .007$). Finally for respiratory febrile episodes, apart from placental malaria infection, prematurity was the only significant association ($P = .023$), despite a nonsignificant trend in favor of an association with sex ($P = .073$) and the child’s 5-min Apgar score ($P = .081$).

As shown in Table 2, the incidence of febrile episodes was 9.03 per 100 person-months in case of placental malaria infection and 6.83 per 100 person-months in the population without placental malaria infection. The infants born to mothers whose placenta was infected by *P. falciparum* presented 1.3-fold more febrile episodes than the others (IRR, 1.3, 95% confidence interval [CI], 1.0–1.7). This association persisted after adjustment (IRR, 1.4, 95% CI, 1.1–1.8). Moreover, when gastrointestinal and respiratory syndromes were considered, the same significant associations were observed (adjusted IRR, 1.6, [95% CI, 1.1–2.5] for gastrointestinal and 1.5 [1.1–2.1] for respiratory syndromes). The goodness of fit of the 3 models, represented in Figure 3, is consistent with the fact that the binomial negative model was well suited to the data.

The same pattern of results was obtained when considering only consultations after the age of 6 months (data not shown).

**DISCUSSION**

Our main result was that placental malaria infection is strongly associated with an increased risk of appearance of nonmalaria fever episodes between birth and 18 months of life. This association remained significant after 6 months of age, suggesting that the effects cannot be explained solely by the immunity potentially induced by maternal immunoglobulins during the first months of life. Furthermore, this association was still significant despite a strong influence of the
Table 2. Incidence Rates of Nonmalaria Febrile Episodes and Incidence Rate Ratios of the Plasmodium falciparum Placental Infection

<table>
<thead>
<tr>
<th>P. falciparum Placental Infection</th>
<th>Yes (n = 62)</th>
<th>No (n = 491)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total febrile episodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of events</td>
<td>85</td>
<td>539</td>
</tr>
<tr>
<td>Incidence/100 person-months&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
<td>9.03 (9.01–9.05)</td>
<td>6.83 (6.83–6.84)</td>
</tr>
<tr>
<td>Crude IRR (95% CI)</td>
<td>1.3 (1.0–1.7)</td>
<td>Reference</td>
</tr>
<tr>
<td>Adjusted IRR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>1.4 (1.1–1.8)</td>
<td>Reference</td>
</tr>
<tr>
<td>Gastrointestinal febrile syndromes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of events</td>
<td>35</td>
<td>162</td>
</tr>
<tr>
<td>Incidence/100 person-months&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
<td>3.72 (3.71–3.73)</td>
<td>2.05 (2.05–2.06)</td>
</tr>
<tr>
<td>Crude IRR (95% CI)</td>
<td>1.8 (1.2–2.6)</td>
<td>Reference</td>
</tr>
<tr>
<td>Adjusted IRR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>1.6 (1.1–2.5)</td>
<td>Reference</td>
</tr>
<tr>
<td>Respiratory febrile syndromes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of events</td>
<td>62</td>
<td>345</td>
</tr>
<tr>
<td>Incidence/100 person-months&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
<td>6.59 (6.57–6.61)</td>
<td>4.37 (4.37–4.38)</td>
</tr>
<tr>
<td>Crude IRR (95% CI)</td>
<td>1.5 (1.1–2.0)</td>
<td>Reference</td>
</tr>
<tr>
<td>Adjusted IRR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>1.5 (1.1–2.1)</td>
<td>Reference</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IRR, incidence rate ratio.

<sup>a</sup> Incidence rates globally estimated on the sample.

<sup>b</sup> IRR from binomial negative regression model adjusted for age of the mother at delivery, parity, parents’ schooling, socioeconomic level, sex, village of birth, season of birth, prematurity, 5-minute Apgar score, and nutritional status.

Village of residence in multivariate analyses, also suggesting a spatial variation of childhood fever morbidity in the area previously reported in Malawi and Nigeria [22–24]. There was no significant interaction between gravidity and placental infection on the occurrence of nonmalaria fever (P = 0.64). When we considered fever episodes as related to gastrointestinal or respiratory clinical signs, similar associations were obtained with placental malaria infection. Interestingly, among the other risk factors evaluated in this study, prematurity and Apgar score, both related to the child’s potential frailty, presented a significant association with nonmalaria fever episodes.

Our protocol was particularly well suited to detecting nonmalaria fevers in the study population during the entire follow-up period. Indeed, not only was the recruitment quasi-exhaustive within the study area and during the inclusion phase, but fever episodes were also actively screened once a week by means of a health agent network, and mothers were invited to bring their infants to the health center for free attendance in case of suspected fever. Furthermore, every day, our team checked that the children in whom a fever had been diagnosed the day before consulted at the dispensary. If not, our team went to the family to evaluate the health status of the child and to perform RDT and TBS for malaria diagnosis. This protocol, different from the majority of programs focusing on the determinants of fever, leads us to believe that the vast majority of fevers were detected.

The protocols classically used are house-to-house surveys, based on interviews with parents, or community surveys in healthcare structures, both likely to generate bias [25, 26], with the objective of supplying information to health policy makers [27]. We found no influence of socioeconomic and educational factors with the occurrence of nonmalaria fever, consistent with most studies in developing countries [28–31]. This could reflect the narrow gap in the socioeconomic categories in the households of the study areas. A recent study showed that the primary reason given for not visiting the hospital when ill was the distance to the facility from home [32, 33]. We did not find this association, which could be the result of the frequent visits the team made to children’s homes. This very close follow-up together with the fact that all care was free could be responsible for a possible overestimation of the number of consultations. Nevertheless, since neither the women nor the health teams knew whether or not the placenta was infected, the extent of the potential overestimation of consultations was probably similar in the 2 groups (ie, those with and those without placental malaria infection).

Because the study had been designed primarily for malaria risk factor analysis, otorhinolaryngological and bronchopulmonary infections could not be differentiated with sufficient accuracy, since for instance, respiratory rate was not collected and thoracic radiography was not available [34]. Likewise, both gastrointestinal and respiratory syndromes could simultaneously occur at a given consultation. The definition of the syndromes was therefore unspecific and the 3 outcomes were not independent. However, taking this shortcoming into account, we are fully convinced that the broader definition of “nonmalaria fever” (ie, axillary temperature >37.5°C with negative results of malaria RDT and TBS), the main outcome of the study, can be supported by our data. For this reason, we consider that the main objective has been met and that placental malaria infection is positively associated with the occurrence of nonmalaria fever during the first months of life.

The role of differential exposure to infectious diseases cannot be entirely removed. However, as shown by our multivariate analysis, the village of residence and placental infection were associated with the risk of nonmalaria fever. This result is consistent with the existence of both a potential spatial variation of exposure and the role of placental infection.

To our knowledge, this is the first time that such an association has been clearly shown. Inversely, the association between placental malaria infection and a higher susceptibility...
to malaria has been described several times, including in this population [7–10]. It has been conjectured that placental infection may alter infants’ immunological response and be responsible for an immune tolerance phenomenon [11]. In the present study, we suggest that immune tolerance could be responsible for the development of a tolerogenic environment, involving a number of immune effectors [12–14] that could interact with the development of immunity during the first years of life. Such interactions can concern not only in the malaria-specific immune response but also in general immunity. For example, it has been shown that placental malaria infection was positively associated with a higher risk of congenital cytomegalovirus infection in West Africa [18]. Our results strongly support this hypothesis and need to be confirmed, because the same pattern of results could be explained by differences in individual susceptibility to infections independent of placental malaria, which could be mediated by host genetic polymorphism, as already shown for malaria [35].

**CONCLUSION**

These results suggest an association between *P. falciparum* placental infection and nonmalaria infectious morbidity in the first 18 months of life. It could be related to immune tolerance, leading to impaired immune development that is not specific to malaria infections in infants born to mothers with infected placentas.

**Notes**

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**Potential conflicts of interest.** All authors: No reported conflicts.

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