In vivo response of Plasmodium falciparum to chloroquine in pregnant and non-pregnant women in Siaya District, Kenya*


Chemoprophylaxis using chloroquine (CQ) in suppressive doses has been recommended to protect pregnant women in malarious areas from the adverse effects of malaria during pregnancy. In a malaria-endemic area of western Kenya with CQ-resistant Plasmodium falciparum, we determined the prevalence and density of falciparum infection in gravid and nulligravid women and compared the in-vivo parasite response to CQ using two regimens: 25 mg/kg body weight (CQ25) divided over a period of three days (for high-density parasitaemias) and 5 mg/kg body weight (CQ5) weekly for 4 weeks (for low-density parasitaemias). P. falciparum infections were present in 102 (42%) of 244 pregnant women. A greater proportion of primigravidae were parasitaemic (68%) than nulligravidae (50%, \( P = 0.02 \)) or multigravidae (33%, \( P < 10^{-3} \)). Primigravidae showed a higher geometric mean parasite density. In the CQ25 treatment group, failure to clear parasites by day 7 was more common in primigravidae than nulligravidae (\( P = 0.008 \)) or multigravidae (\( P = 0.15 \)). In the CQ5 treatment group, primigravidae were more likely to show increasing parasite density than nulligravidae or multigravidae.

In this area of Kenya, virtually all women in their first pregnancy are exposed to malaria and are at greatest risk for malaria infection; compared with other women, they show higher parasite densities and are least likely to respond to chloroquine treatment in areas of chloroquine resistance. Malaria control strategies might be targeted to this group of women, but we are pessimistic about the efficacy of weekly CQ5 where there is chloroquine resistance.

In areas with Plasmodium falciparum malaria, the vulnerability of pregnant women and their fetuses to malaria infection could result in stillbirth, abortion, or low-birth-weight infants (1). The World Health Organization has therefore recommended the use of an antimalarial drug in a regular suppressive dose throughout pregnancy for women living in such areas (2). Chloroquine (CQ) is the drug of choice at 5 mg/kg body weight (CQ5) weekly in areas where P. falciparum is sensitive to CQ.

Weekly suppressive doses of chloroquine in schoolchildren have been effective in suppressing peripheral parasitaemia and clinical malaria in areas with CQ-sensitive parasites (3). However, high levels of compliance in chemoprophylaxis programmes for children are difficult to maintain (4), and a community trial of malaria chemoprophylaxis with the WHO-recommended regimen showed a low rate of coverage among pregnant women (5).

CQ-resistant P. falciparum is now established across most of East and Central Africa where in-vivo parasite resistance has been observed in 10–60% of persons treated with CQ at a dose of 10 mg or 25 mg/kg body weight (CQ25) (6, 7). The failure rate of these higher dosage regimens suggests that weekly doses of CQ5 would not effectively maintain the peripheral blood free of parasites and reduce the parasite blood load, particularly in the placenta.

Given a less than optimally effective drug and difficulty in delivering chemoprophylaxis, the strategy of antimalarial intervention in pregnant women in Africa must be assessed carefully. An understanding of drug efficacy in the target popu-
lation is an important prerequisite to developing an effective antimalarial policy for pregnant women. To evaluate the efficacy of chloroquine in pregnant women living in a malaria endemic area of Kenya, we determined the prevalence of *P. falciparum* infection in gravid and nulligravid women in this district, and assessed the in-vivo parasite response to regimens of CQ25 divided over three days in women with high initial parasite densities and CQ5 weekly for 4 weeks in women with low initial parasite densities.

**MATERIALS AND METHODS**

The study was conducted in Siaya District, Nyanza Province, in Western Kenya from 13 June to 16 July 1986. The district is approximately 1000–1400 metres above sea level. *P. falciparum* is endemic and accounts for 70–90% of malaria infections, while *P. malariae* and *P. ovale* are transmitted with lower frequency. Although the area experiences two rainy seasons (March–May and October–November), malaria transmission occurs throughout the year.

**Parasitaemia screening.** All pregnant women attending the prenatal clinic at the District Hospital on 13, 16, and 19 June 1986 were screened for *P. falciparum* infection using a Giemsa-stained thick blood smear. All asexual parasites and leucocytes were counted in adjacent fields by oil-immersion microscopy until ≥300 leucocytes were counted and the asexual parasite density per mm³ of blood was estimated using a value of 8000 leucocytes/mm³ of blood. All nulligravid women 14–17 years of age at one local school were screened in the same way.

**In-vivo drug study.** Women screened in the above fashion, who were infected with *P. falciparum* only and had no history of antimalarial drug use in the previous seven days and/or negative results on urine testing for chloroquine using a modified Haskins procedure (8) and who gave informed consent, were enrolled and followed on days 1, 2, 7, 14, 21, and 28. Gravid and nulligravid women with screening parasite densities ≤1500/mm³ were treated with CQ5, weekly, for 4 weeks; women with parasite densities >1500/mm³ of blood were treated with CQ25 divided over three days as 10, 10 and 5 mg/kg. All drug doses were administered by health staff. To avoid risk to women treated with weekly CQ5, these women were counselled to return to the clinic if they experienced any feverish illness or malaria symptoms and they were carefully questioned on each return visit regarding symptoms in the interval since the last visit. Women on the CQ5 regimen who exhibited an increasing parasite density or malaria symptoms were treated with a therapeutic dose of an alternative antimalarial drug (see below).

Information collected at enrolment included name, age, reproductive history, and history of fever and antimalarial drug use in the previous seven days. On each follow-up visit, any fever or antimalarial drug use since the previous visit and pruritus or vomiting following CQ treatment were noted.

At enrolment and on all follow-up visits except on day 1, the following specimens were obtained on all study subjects: Giemsa-stained thick and thin blood smears, and whole blood on filter-paper for chloroquine determination (9). Non-pregnant status of the nulligravidae was confirmed on day 14 of the study by urine tests for human chorionic gonadotropin. One 15-year-old who denied being pregnant was repeatedly positive by urine testing and was included in the gravid group.

In the CQ25 group, resistant infection (RII type or early RI type according to the WHO classification (10)) was defined as the presence of *P. falciparum* parasites in peripheral blood on day 7. Absence of parasites on day 7 but reappearance of parasites on day 14 or 21 was presumed to be due to either RI resistance or early new infection. The CQ5 regimen was evaluated on day 28 after 4 doses of drug; women on this regimen were categorized as follows: aparasitaemic, low-level parasitaemia (<2-fold increase in parasite densities and/or density of <2000/mm³ of blood), or high-level parasitaemia (all others). Women on CQ25 with a resistant infection and women on CQ5 with a high-density parasitaemia were treated with amodiaquine at a dose of 25 mg base per kg body weight (AQ25), divided over three days or with three tablets of pyrimethamine–sulfadoxine (fixed combination). Any woman who was parasitaemic on day 28 was treated with a therapeutic dose of amodiaquine or pyrimethamine–sulfadoxine.

**Statistical testing.** Differences in proportions were tested using χ² and Fisher’s exact test; differences in means were tested using Student’s t-test. Significance was designated at *P* < 0.05.

**RESULTS**

**Malaria parasite screening.** A total of 244 pregnant women were screened and *P. falciparum* was identified on peripheral blood smears in 102 (42%) (Table 1); 68% of 66 primigravidae were parasitaemic compared with 33% of 178 multigravidae (χ² = 24.41, *P* < 10⁻⁶). Fifty percent of 98 nulligravidae

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*Senior Slide or Pregnancy from Roche Laboratories*, Division of Hoffman-La Roche Inc., Nutley, NJ, USA. Use of trade names is for identification only and does not imply endorsement by the U.S. Public Health Service or Department of Health and Human Services.
Table 1. *Plasmodium falciparum* prevalence and parasite density in women in Siaya District, Kenya

<table>
<thead>
<tr>
<th>Pregnancy number</th>
<th>No. of women</th>
<th>No. positive for <em>P. falciparum</em></th>
<th>Geometric mean parasite density (per mm³ of blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>49 (50)*</td>
<td>462</td>
</tr>
<tr>
<td>1</td>
<td>66</td>
<td>45 (68)</td>
<td>1538</td>
</tr>
<tr>
<td>2–3</td>
<td>77</td>
<td>26 (34)</td>
<td>336</td>
</tr>
<tr>
<td>4–5</td>
<td>52</td>
<td>15 (29)</td>
<td>299</td>
</tr>
<tr>
<td>≥6</td>
<td>49</td>
<td>16 (33)</td>
<td>330</td>
</tr>
<tr>
<td>Total pregnant</td>
<td>244</td>
<td>102 (42)</td>
<td>644</td>
</tr>
</tbody>
</table>

* Figures in parentheses are percentages.

were parasitaemic; this was lower than the prevalence in primigravid women (*P* = 0.02) and higher than the prevalence in multigravid women (*P* = 0.003). The geometric mean parasite density (GMPD) was significantly higher in primigravidae than in nulligravid or multigravid women (*P* < 0.0001).

Pregnant women who reported the use of an anti-malarial drug in the previous 7 days were less likely to be parasitaemic (20% of 40 women) than those who denied recent drug use (46% of 204 women; *P* = 0.003). Among pregnant women with no recent history of antimalarial drug use, the prevalence of parasitaemia was similar in women with a history of fever in the previous seven days (46% of 117 women) and with no recent history of fever (45% of 88 women).

In-vivo parasite resistance studies. Seventy-two gravid and 42 nulligravid women were enrolled and followed for at least 7 days. The distribution by CQ5 and CQ25 treatment group and parasite clearance is shown in Tables 2 and 3.

In the CQ25 treatment group, failure to clear parasites by day 7 was more common in primigravidae (7 of 20) than nulligravidae (0 of 17, *P* = 0.008) or multigravidae (1 of 10, *P* = 0.15). For both gravid and nulligravid women, the clearance of

Table 2. Characteristics and follow-up of gravid and nulligravid women with *Plasmodium falciparum* malaria treated with chloroquine phosphate, 25 mg/kg body weight

<table>
<thead>
<tr>
<th>Pregnancy status</th>
<th>No of women</th>
<th>Mean pregnancy number</th>
<th>Mean age (years)</th>
<th>Initial geometric mean parasite density/mm³</th>
<th>No. of women parasitaemic on follow-up visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>Nulligravid</td>
<td>17</td>
<td>0</td>
<td>14.3</td>
<td>2537</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>Primigravid</td>
<td>20</td>
<td>1</td>
<td>17.9</td>
<td>4828</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Multigravid</td>
<td>10</td>
<td>3.4</td>
<td>24.6</td>
<td>3311</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Total gravid</td>
<td>30</td>
<td>1.8</td>
<td>19.9</td>
<td>4343</td>
<td>8 (27)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are percentages

Table 3. Characteristics and follow-up of gravid and nulligravid women with *Plasmodium falciparum* malaria treated with chloroquine phosphate, 5 mg/kg body weight, weekly

<table>
<thead>
<tr>
<th>Pregnancy status</th>
<th>No. of women</th>
<th>Mean pregnancy number</th>
<th>Mean age (years)</th>
<th>Initial geometric mean parasite density/mm³</th>
<th>No. of women on day-28 follow-up with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Apasitaemia</td>
</tr>
<tr>
<td>Nulligravid</td>
<td>25</td>
<td>0</td>
<td>14.5</td>
<td>232</td>
<td>6 (24)*</td>
</tr>
<tr>
<td>Primigravid</td>
<td>7</td>
<td>1</td>
<td>19.7</td>
<td>849</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Multigravid</td>
<td>26</td>
<td>4.2</td>
<td>24.8</td>
<td>158</td>
<td>13 (50)</td>
</tr>
<tr>
<td>Total gravid</td>
<td>33</td>
<td>3.5</td>
<td>23.8</td>
<td>227</td>
<td>16 (48)*</td>
</tr>
</tbody>
</table>

* Low-density parasitaemia was defined as ≤2-fold increase in day-3 parasite density and were asymptomatic, these women were continued on the CQ 5 mg/kg regimen. High parasitaemia was defined as >2-fold increase in parasitaemia from the day-3 level to a level of >2000/mm³, these women were treated with an alternative drug

* Figures in parentheses are percentages
pruritus among pregnant women did not vary with drug dosage: itching was reported by 22 (73%) out of 30 women on CQ25 and 34 (79%) out of 43 women on CQ5. An antihistamine, chlorpheniramine, was used to counteract the pruritus, but its efficacy was not systematically evaluated.

We followed 23 women who were treated with amodiaquine (AQ25) after failing to clear their parasitaemia on chloroquine regimens; 18 (78%) of these women were aparasitaemic seven days after starting treatment. All five failures were among the 16 primigravid women.

**DISCUSSION**

As chemotherapeutic interventions against malaria during pregnancy have increased in complexity owing to the spread of chloroquine-resistant *P. falciparum* across sub-Saharan Africa, the possibility of focusing the strategy on a subgroup of pregnant women is of immediate concern. Although other investigators have shown that young women of low parity appear to be at increased risk for both malaria infection (1, 11–14) and delivery of low-birth-weight infants (1, 11–15), the relative importance of focusing on young women as opposed to women of low parity has not been clear. In our study area of Western Kenya, with a 68% point prevalence of malaria infection in primigravidae, we assume that virtually all women are exposed to malaria at some time during each pregnancy. The observed higher prevalence of parasitaemia and higher parasite densities in primigravidae compared with younger nulligravidae and older multigravidae suggests that in her first malaria-exposed pregnancy a woman is more susceptible to malaria infection, regardless of her age.

Exposure to malaria parasites during a woman's first pregnancy may convey some degree of protection against infection in subsequent pregnancies; this is supported by studies of *Plasmodium berghei* infections in pregnant mice (16). The immunological memory may be associated with cellular response(s) at the placental–uterine interface (11) leading to an anamnestic response in subsequent pregnancies. Although humoral immune mechanisms may differ between the pregnant and non-pregnant state, it is unlikely that a pregnancy-related alteration in the quantity or quality of malarial antibody production would explain the difference we observed in responses between the primigravidae and the multigravidae (17). In areas with lower rates of *P. falciparum* transmission, some women may not be exposed to malaria during their first pregnancy and would be susceptible in the second pregnancy. This

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**Fig. 1.** Mean blood chloroquine levels at follow-up visits in gravid and nulligravid women treated with chloroquine 25 mg/kg body weight or 5 mg/kg body weight weekly.

Parasites was unrelated to initial parasite density. Among all women who were aparasitaemic on day 7, primigravidae were more likely to have reappearance of parasites on day 14 and 21 than nulligravidae or multigravidae.

In the CQ5 treatment group (Table 3), gravid women were more likely to clear their parasitaemia by day 28 than nulligravid women. Primigravidae (3 of 7) were more likely to show an increased parasite density, however, and to require a treatment dose than nulligravidae (5 of 25, P=0.22) and multigravidae (3 of 26, P=0.09).

Whole-blood CQ levels were similar within treatment groups on a given follow-up day for nulligravid, primigravid and multigravid women (Fig. 1) and did not differ significantly between women exhibiting and not exhibiting resistance on day 7. Although vomiting was reported in the interval between CQ administration and the follow-up visit by 11 (15%) of 72 pregnant women, vomiting within 4 hours of drug administration was reported by only 2 women, and their whole-blood CQ levels indicated adequate absorption of the drug.

Pruritus associated with CQ administration was reported at least once during the study by 56 (77%) gravid women but by only 8 (19%) nulligravid women (P<10^{-8}). The prevalence of reported
might explain the observed gradient between the first, second, and all subsequent pregnancies for parasite prevalence and mean parasite densities in women living in other African countries with lower rates of transmission (1, 18).

The proportion of primigravid women who failed to clear their parasitaemia following treatment doses of chloroquine (CQ25) was significantly higher than that of nulligravid and multigravid women. This poor response is similar to that observed in 5–9-year-old children living in this area of Kenya (A. D. Brandling-Bennett, unpublished findings, 1985). Similarly, primigravid women with low initial parasite densities (≤1500 parasites/mm$^3$ of blood), after four weeks of CQ5, were more likely to show increasing parasite densities and require therapeutic doses of the drug. Although the parasite density was higher in primigravidae than nulligravidae and multigravidae, the difference in clearance rates could not be attributed to higher initial parasite density. Although clearance of *P. falciparum* in children in areas of CQ resistance appears to be age related (D. Heymann, personal communication from Malawi, 1986), age could not explain our observed differences in clearance rates in women, since both younger nulligravidae and older multigravidae showed higher rates of clearance than the primigravidae. In areas with CQ-resistant *P. falciparum*, the physiological mechanisms which render the primigravida more susceptible to developing a patent malaria infection may also contribute to a decreased ability to clear the infection following treatment.

The purpose of regular antimalarial chemoprophylaxis during pregnancy is to clear or prevent placental parasite infections, since it is the placental infection that is associated with delivery of low-birthweight infants (1). Peripheral parasitaemia does not necessarily mean that there is a placental parasite infection (1), and we cannot be sure that clearance of peripheral parasites will be accompanied by the clearance of placental infection. However, it is certain that persistence of parasites in the peripheral blood bodies ill for placental parasite clearance.

For areas in sub-Saharan Africa, where there is a great need to introduce effective malaria interventions, we are pessimistic about the efficacy of chemoprophylaxis in pregnant women using weekly chloroquine 5 mg/kg. We believe that malaria control strategies could be focused on primigravid women or those in their first malaria-exposed pregnancies, but this is the very group that is least likely to respond to a chemotherapeutic intervention. As chloroquine-resistant parasites spread, a better understanding of the problem is needed leading to further chemotherapeutic options for pregnant women. In areas with moderate chloroquine resistance, it may be worthwhile to assess the effect of intermittent full-dose treatment with chloroquine rather than continual prophylaxis. Likewise, further understanding of the immunological mechanisms responsible for the increased susceptibility during the first malaria-exposed pregnancy is needed: with this knowledge, malaria prophylaxis could be provided alone or in conjunction with tetanus toxoid in a prenatal clinic. This would offer an attractive addition or alternative to chemoprophylaxis.

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**RÉSUMÉ**

**RÉPONSE IN VIVO DE *PLASMODIUM FALCIPARUM* À LA CHLOROQUINE CHEZ DES FEMMES (ENCEINTES OU NON) DU DISTRICT DE SIAYA, KENYA**

On a recommandé une chimio prophylaxie à la chloroquine (CQ) à des doses suppressives pour protéger les femmes enceintes des régions impaludées contre les effets indésirables du paludisme pendant la grossesse. Au Kenya occidental, dans une région d’endémie du paludisme où *Plasmodium falciparum* chloroquinorésistant est installé, nous avons
déterminé la prévalence et la densité des infestations à *falciparum* chez les femmes enceintes ou non, et nous avons comparé la réponse *in vivo* des plasmodies à la chloroquine administrée à deux posologies différentes: 25 mg/kg de poids corporel (CQ25) sur une période de 3 jours (pour les parasites à forte densité parasitaire), et 5 mg/kg de poids corporel (CQ5) par semaine pendant 4 semaines (pour les parasites à faible densité). *P. falciparum* a été retrouvé dans les foetts sanguins de 10% (42%) femmes enceintes sur les 244 examinées. Une plus grande proportion de primigestes (68%) que de nulligestes (50%, \( P = 0.02 \)) ou de multigestes (33%, \( P < 10^{-4} \)) étaient parasitées. Les primigestes présentaient également une moyenne géométrique de la densité parasitaire sensiblement plus élevée que les nulligestes ou les multigestes.

Chez les 30 femmes enceintes et 17 femmes non enceintes du groupe traité par la CQ25, la non-élimination des parasites au 7e jour s’est rencontrée plus couramment chez les primigestes que chez les nulligestes (\( P = 0.008 \)) ou les multigestes (\( P = 0.15 \)). Chez les 33 femmes enceintes et 25 femmes non enceintes du groupe traité par la CQ5, les primigestes (43%) étaient plus susceptibles de présenter une remontée de la densité parasitaire que les nulligestes (20%) ou les multigestes (12%).vingt-trois femmes ont été traitées par l’amidoquine, à raison de 25 mg/kg de poids corporel. Après l’échec de l’éléménation de la parasitémie, 18 d’entre elles (78%) présentaient une parasitémie nulle 7 jours après le traitement. Les cinq échecs ont tous été observés chez des primigestes (au nombre de 16).

La forte prévalence de la parasitémie chez les primigestes laisse à penser que, dans cette région du Kenya, toutes les femmes sont exposées au paludisme au cours de leur première grossesse. Nos résultats portent à croire que les femmes présentent, au cours de leur première grossesse en région d’endémie, un risque maximal d’infestation, avec des densités parasitaires élevées, et qu’elles sont moins aptes à répondre au traitement à la chloroquine dans les régions de choroquinorésistance. Les stratégies de lutte antipaludique pourraient être axées sur ces femmes mais nous sommes pessimistes quant à l’efficacité du traitement par la chloroquine à raison de 5 mg/kg de poids corporel par semaine, dans les régions de chloroquinorésistance.

**REFERENCES**