Improving drug regimens and implementation strategies for malaria prevention in pregnant women in western Kenya
Ouma Okuma, P.

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Chapter 3: A Randomized Controlled Trial of Folate Supplementation When Treating Malaria in Pregnancy with Sulfadoxine-Pyrimethamine


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A Randomized Controlled Trial of Folate Supplementation When Treating Malaria in Pregnancy with Sulfadoxine-Pyrimethamine

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ABSTRACT

Objectives: Sulfadoxine-pyrimethamine (SP) is an antimalarial drug that acts on the folate metabolism of the malaria parasite. We investigated whether folate (FA) supplementation in a high or a low dose affects the efficacy of SP for the treatment of uncomplicated malaria in pregnant women.

Design: This was a randomized, placebo-controlled, double-blind trial.

Setting: The trial was carried out at three hospitals in western Kenya.

Participants: The participants were 488 pregnant women presenting at their first antenatal visit with uncomplicated malaria parasitaemia (density of > 500 parasites/μl), a haemoglobin level higher than 7 g/dl, a gestational age between 17 and 34 weeks, and no history of antimalarial or FA use, or sulfa allergy. A total of 415 women completed the study.

Interventions: All participants received SP and iron supplementation. They were randomized to the following arms: FA 5 mg, FA 0.4 mg, or FA placebo. After 14 days, all participants continued with FA 5 mg daily as per national guidelines. Participants were followed at days 2, 3, 7, 14, 21, and 28 or until treatment failure.

Outcome Measures: The outcomes were SP failure rate and change in haemoglobin at day 14.

Results: The proportion of treatment failure at day 14 was 13.9% (19/137) in the placebo group, 14.5% (20/138) in the FA 0.4 mg arm (adjusted hazard ratio [AHR], 1.07; 98.7% confidence interval [CI], 0.48 to 2.37; p = 0.8), and 27.1% (38/140) in the FA 5 mg arm (AHR, 2.19; 98.7% CI, 1.09 to 4.40; p = 0.005). The haemoglobin levels at day 14 were not different relative to placebo (mean difference for FA 5 mg, 0.17 g/dl; 98.7% CI, –0.19 to 0.52; and for FA 0.4 mg, 0.14 g/dl; 98.7% CI, –0.21 to 0.49).

Conclusions: Concomitant use of 5 mg FA supplementation compromises the efficacy of SP for the treatment of uncomplicated malaria in pregnant women. Countries that use SP for treatment or prevention of malaria in pregnancy need to evaluate their antenatal policy on timing or dose of FA supplementation.
METHODS

Participants

This study was conducted at three government hospitals in western Kenya: Nyanza Provincial General Hospital in the Kisumu District (population 500,000); Bondo District Hospital (district population 300,000), and Siaya District Hospital (district population 480,000). In each site, HIV counselling and testing is provided in the antenatal clinic as part of a program to provide nevirapine to HIV-seropositive pregnant women to reduce vertical transmission of HIV. Malaria transmission is perennial and intense in western Kenya; however, the malaria prevalence among pregnant women in Kisumu is lower than in the rural areas of Bondo and Siaya. Participants were recruited from the daily antenatal clinics in
the participating hospitals, the inclusion and exclusion criteria are summarized in Table 1. The study protocol was approved and reviewed on an annual basis by the institutional review boards of the Kenya Medical Research Institute, and the Centers for Disease Control and Prevention, Atlanta, United States. All participants gave informed consent.

**Interventions**

A study nurse or clinical officer randomized participants to FA 5 mg tablets (FA 5 mg arm), FA 0.4 mg tablets (FA 0.4 mg arm), or placebo tablets (FA placebo arm); all were identical in appearance and taste (Laboratory and Allied, Nairobi, Kenya). Participants received a 14-day supply. At day 14, all women received a supply of folic acid 5 mg tablets for 14 days to ensure that pregnant women were not deprived of FA. The first doses of FA or placebo were given together with SP (three tablets of Malodor [Laboratory and Allied]; 1,500 mg of sulfadoxine and 75 mg of pyrimethamine at once) under supervision. Participants were observed for half an hour; if vomiting occurred, the SP dose and FA tablet were repeated. Participants were instructed to take the FA or placebo tablet daily and were asked to bring the tablets at every visit for a tablet count. All participants were supplemented with iron tablets according to the national guidelines (200 mg three times per day). From August 2004 onwards, all participants received insecticide-treated nets (ITNs) as part of the enrolment procedure to reduce the chance of new malaria infections. Participants were instructed to return to the clinic on days 2, 3, 7, 14, 21, and 28, or whenever they felt ill and thought they needed treatment. On follow-up visits, women were questioned about side effects, and signs and symptoms of clinical malaria. The axillary temperature was measured, and blood was obtained for a malaria blood smear; haemoglobin was repeated on days 14 and 28. Women who were ill or had complications that did not allow them to continue participation were referred to the appropriate departments in the hospital and followed until recovery. Women who failed treatment with SP received quinine 600 mg three times per day for seven days. Women who had not cleared parasitaemia after seven days of quinine therapy were treated with mefloquine.

Haemoglobin was measured to the nearest 0.1 g/dl using a portable haemoglobin monitor (HaemoCue, Mission Viejo, California, United States). Peripheral thick and thin blood films were stained with 10% Giemsa, and examined under oil immersion for malaria parasites. A thick film was considered negative if 100 microscopic fields showed no parasites. Malaria parasites and leukocytes were counted in the same fields until 300 leukocytes were counted. Parasite densities were estimated by assuming a count of 8,000 leukocytes/ml of blood. For quality control of the blood smear reading, 10% of the negative samples and 20% of the positive samples at screening, and 20% of all follow-up samples were checked by a different microscopist during the study. HIV testing involved parallel use of two rapid testing methods: Determine HIV-1/2 (Abbott Laboratories, Dainabot, Tokyo, Japan) and Unigold HIV-1/2 (Trinity Biotech, Bray, Ireland), as per Kenya Ministry of Health guidelines for voluntary counselling and testing. Capillus HIV-1/2 (Cambridge Diagnostics, Wicklow, Ireland) was performed on discordant samples. The method of Mount et al. [22] was used to test the urine for sulfa compounds. The sickle cell profile was determined using cellulose acetate electrophoresis (Helena Laboratories, Beaumont, Texas, United States).

**Objectives**

We investigated whether FA supplementation in a high or a low dose affects the efficacy of SP for the treatment of uncomplicated malaria in pregnant women.

**Outcomes**

Outcome measures were the prevalence of SP treatment failure at days 3, 5, 7, 14 (primary outcome), and 28 and change in haemoglobin level comparing day 0 (day of SP treatment) to days 14 and 28. Treatment failures were defined according to the guidelines for an area of low to moderate transmission (Table 2) [23]. The main difference from the protocol for areas of high transmission is that in the moderate transmission protocol an afebrile patient who still had parasitaemia on day 7 post-treatment was classified as a late parasitological failure and given rescue treatment, whereas such patients would not have been classified as parasitological failures in the high-transmission protocol. Because of the adverse consequences that asymptomatic parasitaemia can
### Table 2. Definition of Treatment Failure

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early treatment failure</strong></td>
<td>Development of danger signs or severe malaria* on days 1, 2, or 3 in the presence of parasitaemia</td>
</tr>
<tr>
<td></td>
<td>Parasitaemia on day 2 higher than day 0 count, irrespective of axillary temperature</td>
</tr>
<tr>
<td></td>
<td>Parasitaemia on day 3 with an axillary temperature $\geq 37.5,^\circ C$</td>
</tr>
<tr>
<td><strong>Late clinical failure</strong></td>
<td>Development of danger signs of severe malaria after day 3 in the presence of parasitaemia without previously meeting any criteria of early treatment failure</td>
</tr>
<tr>
<td></td>
<td>Presence of parasitaemia and an axillary temperature $\geq 37.5,^\circ C$ on any day from days 4 to 28 without previously meeting any criteria of early treatment failure</td>
</tr>
<tr>
<td><strong>Late parasitological failure</strong></td>
<td>Presence of parasitaemia on any day from days 7 to 28 and an axillary temperature $&lt; 37.5,^\circ C$ without previously meeting any of the criteria of early treatment failure or late clinical failure</td>
</tr>
<tr>
<td><strong>Treatment failure</strong></td>
<td>Adequate clinical and parasitological response</td>
</tr>
<tr>
<td></td>
<td>Cumulative early, late clinical failure, and parasitological failure</td>
</tr>
<tr>
<td></td>
<td>Absence of early or late treatment failure on day 28</td>
</tr>
</tbody>
</table>

*Definition of treatment failure based on recommendations of the World Health Organization [23].

**See Table 1 for danger signs or signs of severe malaria in adults.**

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have for mother and foetus, we decided to use the more conservative protocol.

Malaria was defined as the presence of asexual-stage parasite of any species in thick smears, independent of clinical signs. A parasite density in the highest tercile at enrolment of the total study population was defined as a high parasite density. A young age was defined as under 20 years. Because of the short duration of follow-up and limited sample size, no attempt was made to assess the effect of FA on megaloblastic anaemia.

### Sample Size

We calculated that a sample size of 600 women—200 in each arm—would allow us to detect an increase from 5% to 15% in the parasitological failure rate at day 7 with 80% power and 95% confidence, allowing for a 25% loss to follow-up. However, we did not feel comfortable continuing the trial at an overall treatment failure rate of over 40% at day 28 and an intervention which may contribute to this, because it is recommended that first-line therapy be changed at a 25% failure rate [23,24]. An interim analysis was performed in October 2003 with stopping criteria defined as a difference in the treatment failure rate at day 14 with a p-value of less than 0.01; we used day 14 and not day 7 because we considered day 14 a more appropriate time point for assessing SP resistance. Because this criterion was met, enrolment was stopped with 488 women enrolled. The investigators remained blind until data cleaning, analysis, and quality control of the blood smears were completed.

### Randomization—Sequence Generation

One of the investigators generated a randomization list with a block size of 12 using the statistical program SAS (SAS system for Windows version 8; SAS, Cary, North Carolina, United States).

### Randomization—Allocation Concealment

All FA treatment and placebo tablets were prepared off site and were identical in appearance and taste (Laboratory and Allied). The study arm was allocated by the study staff according to the study arm indicated on the medicine envelope. The envelope was opened by the participant, and the medicine envelopes were put in sealed, opaque envelopes with consecutive numbers according to the randomization list by an investigator.

### Randomization—Implementation

A trained clinical officer or nurse randomized eligible women by assigning them the next envelope in order of enrolment. The envelope was opened by the participant, and the study arm was allocated by the study staff according to the arm indicated on the medicine envelope.

### Blinding

All FA treatment and protocol tablets were prepared off site and were identical in appearance and taste (Laboratory and Allied). All study staff participants were blind to the treatment in each arm.

### Statistical Methods

We analysed the data on an intention-to-treat basis using a pre-established analysis plan. Cumulative treatment failures by follow-up day were compared among treatment arms using the Chi-squared test. We used the Kaplan-Meier curve to examine differences in patterns between treatment arms, and Cox proportional hazards regression analysis to examine the effect of treatment arm on time to treatment failure after we confirmed that the Cox proportional hazard assumption was met. For day 14 post-treatment, we repeated the Cox proportional hazards regression while adjusting for potential confounders; factors examined included site of enrolment, the use of an ITN, ethnicity, education level, socioeconomic status, sickle cell status (carrier versus not a carrier), gravidity, young age, HIV status, and a high parasite density at enrolment. Analysis of covariance was used to assess the effect of treatment arm on haemoglobin level [25]. Factors were removed from models if the p-value was 0.05 or more. The statistical program SAS (SAS system for Windows version 8) was used for all analyses. All tests were two-sided; $p < 0.05$ was considered significant, except for the efficacy between study arms when a $p < 0.013$ was considered significant to adjust for multiple comparisons and the interim analysis (the 58
A $p$-value of 0.05 was subtracted by 0.01 to account for the interim analysis, and the remaining value was divided by 3 to account for the comparisons between the arms; a confidence interval (CI) of 98.7% was used for the efficacy analysis.

RESULTS

Participant Flow

Between November 2003 and November 2005, a total of 4,524 women were screened; 488 met all enrolment criteria, and 415 (85%) women completed the study (Figure 1). The study arms were similar in baseline characteristics (Table 3). Most infections were *Plasmodium falciparum* (98.0%), nine were mixed *P. falciparum*/*P. malariae*, and one was pure *P. malariae*.

During 1,671 (99.4%) of the 1,682 routine visits made at or before day 14, the participant reported that she took the FA daily; the tablets were brought at 1,454 of the routine visits (86.4%) and a correct count was established at 1,307 visits (89.9%).

Outcomes and Estimation

From day 3 onwards, women in the FA 5 mg arm were more likely to fail treatment than women in the other arms (Figure 2; log rank test $p < 0.01$ comparing the FA 0.4 mg arm or the FA placebo arm to the FA 5 mg arm). On day 14 the number of treatment failure was 38 out of 140 women (27.1%) in the FA 5 mg arm, 20 out of 138 women (14.5%) in the FA 0.4 mg arm, and 19 out of 137 women (13.9%) in the FA placebo arm (Table 4). In multivariate analysis using Cox proportional hazards regression, compared to FA placebo, treatment failure by day 14 was twice as likely when FA 5 mg was used (hazard ratio [HR], 2.19; 98.7% CI, 1.09 to 4.40; $p = 0.005$), whereas FA 0.4 mg did not affect treatment failure risk (HR, 1.07; 98.7% CI 0.48 to 2.37; $p = 0.8$) (Table 5).

We did not find an effect of treatment arm on haemoglobin levels at day 14 or day 28 among 288 women who completed 28 days of follow-up without treatment failure (Figure 3). Among 386 women who had a haemoglobin available at day 14, the increases in mean haemoglobin in the FA 5 mg and FA 0.4 mg arms were not statistically different compared to the FA placebo arm (0.17 g/dl; 98.7% CI, 0.19 to 0.52 g/dl; $p = 0.3$, and 0.14 g/dl; 98.7% CI, −0.21 to 0.49 g/dl; $p = 0.4$, respectively; adjusted for maternal HIV infection, location of residence, high parasite density infection, and haemoglobin at enrolment).
During the course of the study, 20 participants (4.1%) developed rashes (4, 8, and 8 in the FA 5 mg, FA 0.4 mg, and FA placebo arms, respectively). No severe adverse skin reactions or maternal deaths occurred. Premature delivery was experienced by 14 participants (2.9%) (6, 5, and 3 in the FA 5 mg, FA 0.4 mg, and FA placebo arms, respectively), and eight participants (1.6%) had a stillbirth or early neonatal infant death during the study (3, 2, and 3 in the FA 5 mg, FA 0.4 mg, and FA placebo arms, respectively).

**DISCUSSION**

Interpretation

This study shows that the combined use of SP and daily FA supplementation in a dose of 5 mg compromised the efficacy of SP for the treatment of malaria parasitaemia in pregnant women. A plausible biological mechanism is available. FA is required for DNA synthesis in both humans and protozoa. Malaria parasites can utilize exogenous FA (the salvage pathway) as well as synthesize FA de novo (biosynthesis) [26], though biosynthesis seems to be the preferred method [27]. Antifolates such as SP act on two enzymes important for sequential steps in the biosynthesis of FA for the parasite, dihydropteroate synthase and dihydrofolate reductase, respectively. It has been established that malaria parasites can differ in their ability to use exogenous FA, but the mechanism is unknown [28,29]. If the biosynthesis pathway is compromised, e.g., by sulfadoxine, parasite strains that are able to use exogenous FA can compensate for the lack of FA through the

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**Table 3. Characteristics of Study Population at Enrolment, Overall and by Treatment Arm**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall, % (n = 488)</th>
<th>FA 5 mg, % (n = 161)</th>
<th>FA 0.4 mg, % (n = 165)</th>
<th>FA Placebo, % (n = 162)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 20 y</td>
<td>50.8</td>
<td>47.8</td>
<td>52.1</td>
<td>52.5</td>
</tr>
<tr>
<td>Gravidaity</td>
<td>52.5</td>
<td>52.8</td>
<td>58.2</td>
<td>46.3</td>
</tr>
<tr>
<td>Trimester of pregnancy</td>
<td>69.1</td>
<td>68.9</td>
<td>71.5</td>
<td>66.7</td>
</tr>
<tr>
<td>Enrolment site</td>
<td>Kisumu</td>
<td>41.6</td>
<td>41.6</td>
<td>39.4</td>
</tr>
<tr>
<td>Bondo</td>
<td>24.0</td>
<td>26.1</td>
<td>24.9</td>
<td>21.0</td>
</tr>
<tr>
<td>Siaya</td>
<td>34.4</td>
<td>32.3</td>
<td>35.8</td>
<td>35.2</td>
</tr>
<tr>
<td>Ethnic group</td>
<td>Luo</td>
<td>91.4</td>
<td>93.2</td>
<td>94.6</td>
</tr>
<tr>
<td>Marriage status</td>
<td>Married</td>
<td>63.9</td>
<td>62.7</td>
<td>61.2</td>
</tr>
<tr>
<td>Education level</td>
<td>None or incomplete primary</td>
<td>45.7</td>
<td>45.3</td>
<td>41.8</td>
</tr>
<tr>
<td>Indicator of socioeconomic statusa</td>
<td>Second complete</td>
<td>8.2</td>
<td>6.8</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>House walls of mud</td>
<td>48.6</td>
<td>50.3</td>
<td>46.1</td>
</tr>
<tr>
<td></td>
<td>Possession of bicycle</td>
<td>83.4</td>
<td>82.6</td>
<td>82.4</td>
</tr>
<tr>
<td></td>
<td>Possession of ITNb</td>
<td>15.0</td>
<td>13.0</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Received an ITN</td>
<td>62.7</td>
<td>63.4</td>
<td>61.8</td>
</tr>
<tr>
<td>HIV status</td>
<td>Positivec</td>
<td>34.1</td>
<td>38.5</td>
<td>28.7</td>
</tr>
<tr>
<td>Sickle cell status</td>
<td>Carrier</td>
<td>20.3</td>
<td>21.1</td>
<td>21.2</td>
</tr>
<tr>
<td>Anemia</td>
<td>Any anemia</td>
<td>86.1</td>
<td>87.0</td>
<td>89.1</td>
</tr>
<tr>
<td>Moderate anemia</td>
<td>Any anemia</td>
<td>13.5</td>
<td>14.3</td>
<td>13.9</td>
</tr>
<tr>
<td>Fever</td>
<td>Documented feverd</td>
<td>3.7</td>
<td>4.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Fever past week</td>
<td>Fever past week</td>
<td>58.6</td>
<td>55.9</td>
<td>56.4</td>
</tr>
<tr>
<td>Parasite density</td>
<td>High</td>
<td>33.4</td>
<td>35.4</td>
<td>30.3</td>
</tr>
<tr>
<td>GMPD</td>
<td>Parasites/l (95% CI)</td>
<td>3.231 (2.879 to 3.626)</td>
<td>3.178 (2.608 to 3.872)</td>
<td>3.149 (2.581 to 3.840)</td>
</tr>
</tbody>
</table>

aThe possession of a bicycle was used as an indicator of high/medium socioeconomic status. A house with mud walls in contrast to a house of bricks, walls of mud with cement, or other materials was used as an indicator of low socioeconomic status.
bPossession of ITN, two missing; HIV status, indeterminate for one woman.
cAny anemia: haemoglobin below 11 g/dl; moderate anemia: haemoglobin below 8 g/dl.
dDocumented fever: An axillary temperature of 37.5 °C or higher.

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**Figure 2. Cumulative Treatment Survival Rates by Intervention Arm among Parasitaemic Pregnant Women Treated with SP and FA**

Participants received the FA intervention up to 14 days past SP treatment; after day 14 every participant received FA 5 mg in accordance with the National Guidelines in Kenya.

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**Figure 3. Log rank test results for cumulative survival of participants treated with different intervention arms**

FA = Folate
FA Placebo
FA 0.4 mg
FA 5 mg

Log rank tests:
FA placebo vs. FA 0.4 mg: P = 0.8
FA 0.4 mg vs. FA 5 mg: P = 0.0007
FA placebo vs. FA 5 mg: P = 0.0014

**Adverse Events**

During the course of the study, 20 participants (4.1%) developed rashes (4, 8, and 8 in the FA 5 mg, FA 0.4 mg, and FA placebo arms, respectively). No severe adverse skin reactions or maternal deaths occurred. Premature delivery was experienced by 14 participants (2.9%) (6, 5, and 3 in the FA 5 mg, FA 0.4 mg, and FA placebo arms, respectively), and eight participants (1.6%) had a stillbirth or early neonatal infant death during the study (3, 2, and 3 in the FA 5 mg, FA 0.4 mg, and FA placebo arms, respectively).
biosynthesis pathway by increasing the flux through the FA salvage pathway [27,28]. However, pyrimethamine may interfere with the utilization of exogenous FA in a competitive way, an action that is thought to be independent of pyrimethamine’s inhibition of dihydrofolate reductase [29,30]. The success and duration of the effect of pyrimethamine may be dependent on the FA levels; large amounts of FA (such as 5 mg daily), but not low doses, may overwhelm pyrimethamine’s ability to block the salvage pathway [30].

It is likely that FA supplementation affects other antifolate antimalarial combinations as well, such as chlorproguanil-dapsone, dapsone-pyrimethamine, and cotrimoxazole. Cotrimoxazole will increasingly be used as a prophylactic drug among HIV-positive pregnant women. Further study into the effect of concomitant FA supplementation in malarial areas is needed.

We did not collect blood at enrolment and follow-up visits to be able to differentiate between recrudescent and new malaria infections. After day 14, all groups switched to FA 5 mg, so we were not able to assess the extent to which FA contributes to SP treatment failure after 14 days. Several studies indicate that FA supplements do not predispose to increased risk of malaria acquisition [31,32], and thus we hypothesized that the difference between treatment arms observed in this study after day 14 is mainly caused by recrudescence.

**Overall Evidence**

Our results are supported by studies among symptomatic, nonpregnant persons in areas of different malaria endemicity. A randomized, placebo-controlled study in Gambia reported approximately twice as common SP treatment failures among children with symptomatic malaria supplemented with a high dose of FA (5 mg daily for children <15 kg, 7.5 mg daily for children 15–20 kg, and 10 mg daily for children >20 kg) compared to the FA placebo group in an area with low seasonal malaria transmission [19]. In a low-to-moderate malaria transmission area in Kenya, a randomized, open-label study among symptomatic participants (all ages) showed a comparable cumulative survival curve when assessing the interaction of SP and FA (5 mg daily) [20]. Dzinjalamala et al. [21] recently noted significantly higher mean FA levels at enrolment among children with a treatment failure to SP for symptomatic malaria (28 day

### Table 4: Cumulative Treatment Failures and Relative Risk Reduction by Follow-Up Day among Participants Who Completed the Study

<table>
<thead>
<tr>
<th>Days Post-SP Treatment</th>
<th>FA 5 mg, n (%)</th>
<th>FA 0.4 mg, n (%)</th>
<th>FA Placebo, n (%)</th>
<th>Total, n (%)</th>
<th>Relative Risk Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3</td>
<td>14 (10)</td>
<td>5 (10)</td>
<td>10 (35)</td>
<td>41</td>
<td>0.94 (0.69 to 1.25)</td>
</tr>
<tr>
<td>Day 7</td>
<td>21 (15)</td>
<td>10 (23)</td>
<td>9 (35)</td>
<td>41</td>
<td>0.96 (0.69 to 1.35)</td>
</tr>
<tr>
<td>Day 14</td>
<td>38 (27)</td>
<td>20 (45)</td>
<td>19 (35)</td>
<td>41</td>
<td>0.96 (0.69 to 1.35)</td>
</tr>
<tr>
<td>Day 28</td>
<td>78 (55)</td>
<td>49 (55)</td>
<td>51 (37)</td>
<td>41</td>
<td>0.96 (0.69 to 1.35)</td>
</tr>
</tbody>
</table>

### Table 5. The Effect of Daily FA Dose on Time to Treatment Failure of SP among Pregnant Women by Follow-Up Time Point

<table>
<thead>
<tr>
<th>Day</th>
<th>Ratio Type</th>
<th>HRs (98.7% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3</td>
<td>Unadjusted</td>
<td>FA Placebo, 0.4 mg, 1.07 (0.58 to 1.98)</td>
</tr>
<tr>
<td>Day 7</td>
<td>Unadjusted</td>
<td>FA Placebo, 0.4 mg, 1.07 (0.58 to 1.98)</td>
</tr>
<tr>
<td>Day 14</td>
<td>Unadjusted</td>
<td>FA Placebo, 0.4 mg, 1.07 (0.58 to 1.98)</td>
</tr>
<tr>
<td>Day 28</td>
<td>Unadjusted</td>
<td>FA Placebo, 0.4 mg, 1.07 (0.58 to 1.98)</td>
</tr>
</tbody>
</table>

**Figure 3. Haemoglobin Levels by Intervention Arm at Different SP Treatment Time Points**

Haemoglobin levels (mean and 98.7% CI) are shown by type of FA intervention at enrolment, 14 days, and 28 days post-treatment with SP for malaria among 287 pregnant women who completed 28 days of follow-up without treatment failure. Mean haemoglobin was obtained by analysis of covariance and was adjusted for HIV, site of residence (rural versus urban), and high parasite density. On days 14 and 28 the haemoglobin was adjusted for haemoglobin at enrolment as well. Participants received the intervention up to 14 days past SP treatment; after day 14 every participant received FA 5 mg in accordance with the National Guidelines in Kenya.

*Significant HRs.
*Adjusted for young age and high-density parasitaemia.

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follow-up compared to children with an adequate parasitological and clinical response in Malawi.

**Generalizability**

SP is recommended for the treatment and prevention of malaria in pregnancy. Although our results are based on the treatment of uncomplicated malaria in pregnant women, they will have implications for the use of SP as IPTp as well. Many countries have introduced IPTp with SP [5]. We cannot assess from our study the effect of FA 5 mg on the preventive action of SP on malaria, but FA 5 mg will affect the treatment action of SP when malaria parasitaemia is present. Depending on the endemicity of malaria in an area, it can be expected that 1%–50% of pregnant women may carry malaria parasitaemia, without noticing it; particularly in the placenta [1,3]. Given the present results, countries using IPTp should consider evaluating their FA recommendations in the antenatal clinic to optimize SP efficacy. Options to consider include using low-dose (0.4 mg) FA tablets daily or suspending FA 5 mg for 14 days after SP treatment, which would disrupt an important routine of daily intake of FA for the prevention of anaemia. The first option may be preferable; our data show no difference in efficacy of SP between 0.4 mg FA daily and withholding FA 5 mg for 14 days. Effects of regimens on haemoglobin levels were similar. However, this study was not designed to assess the optimal FA dose to prevent FA deficiency and adverse events such as megaloblastic anaemia in the presence of malaria parasitaemia. International guidelines recommend folic acid doses of 0.4 or 0.6 mg daily during pregnancy [13–15]. Although these international recommendations for FA supplementation are based on studies conducted in developed countries, the few studies in sub-Saharan Africa assessing FA deficiency among pregnant women suggest that such deficiency is relatively uncommon, ranging from 3%–10%; an exception was Togo (68% FA deficiency among pregnant women) [33–38]. A dose of 1 mg of FA daily in combination with malaria prophylaxis was sufficient to abolish FA deficiency among primigravidae in Zaria, Nigeria [39]. Given the international recommendations, the relatively low prevalence of FA deficiency in pregnancy, and the compromised efficacy of SP for malaria treatment when FA 5 mg is used, we believe it is reasonable to recommend FA 0.4 mg daily for pregnant women in malarious areas in sub-Saharan Africa.

Resistance to SP was high in the study area. However, at present, no safe and efficacious alternative drug is available for the treatment and prevention of malaria in pregnancy. Kenya has moved now to artemisinin-based combination therapy for children and quinine as first-line therapy for malaria parasitaemia. International guidelines recommend a 48-hour interval between FA and SP treatment when FA 5 mg is used, we believe it is reasonable to recommend FA 0.4 mg daily for pregnant women in malarious areas in sub-Saharan Africa.

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**Author Contributions**

MFP, FOTK, JGA, LS, and AMeVe designed the study. PO, JGA, and AMeVe analyzed the data. PO and AMeVe enrolled patients. PO, MEP, MfH, FOTK, KO, JGA, PAK, RWS, LS, and AMeVe wrote the paper. PO supervised field work and coordinated all field activities. MfH provided supervision to the study team in the field, backstopping and providing support to PO, who was primarily responsible for the team on the ground in Kenya, and provided input into the interpretation of data. KO carried out and supervised the laboratory procedures. RWS provided initial input into the design of the study along with the lead author and other authors and provided input on drafts of the manuscript as it was developed.

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