The safety of artemisinins in first trimester in prospectively followed pregnancies: an observational study

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Abstract

Background Artemisinins, the most effective antimalarials available, are not recommended for falciparum malaria during the first trimester due to safety concerns. Therefore, quinine is used despite poor efficacy. Assessing artemisinin safety requires weighing the risks of malaria and its treatment.

Methods We assessed the effect of first-trimester malaria and artemisinin treatment on miscarriage and major congenital malformations. The effects of falciparum and vivax malaria were studied in antenatal clinics on the Thai-Myanmar border between January 1994 and December 2013. The relationship between artemisinin treatments (artesunate, dihydroartemisinin, or artemether) on miscarriage and malformation was assessed using Cox regression with left-truncation and time-varying exposures.

Findings Of 25485 women, 2558 (10%) had first-trimester malaria. The hazard of miscarriage increased 1·61-fold after an initial first-trimester falciparum episode (95% CI: 1·32, 1·97), 3·24-fold following falciparum recurrence (95% CI: 2·24, 4·68), and 2·44-fold (95% CI: 1·01, 5·88) following recurrent symptomatic vivax. No difference was observed in miscarriage in first-line falciparum treatments with artemisinin (N=183) vs. quinine (N=842) (HR: 0·78; 95% CI: 0·45, 1·34), or risk of major congenital malformations (1·83% [2/109; 95% CI: 0·22, 6·47] and 1·25% [8/641; 95% CI: 0·54, 2·44], respectively).

Interpretation First-trimester falciparum and vivax malaria increases the risk of miscarriage. This observational study of prospectively followed pregnancies found no evidence of an increased risk of miscarriage or major congenital malformations associated with first-line treatment with an artemisinin derivative compared to quinine. Given the low efficacy of quinine and wide availability of highly effective artemisinin combination treatments, it is time to reconsider first-trimester treatment recommendations.

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Panel: Research in context

Evidence before this study

We searched SCOPUS and PubMed for articles published up to October 5 2015 (in any language) addressing the association between first-trimester artemisinin treatment and miscarriage using the search terms: "malaria or plasmodium", "pregnan*", "*artemisinin* OR ACT* OR artesunate OR artemether OR Coartem", "first-trimester OR 'first trimester'", and "miscarriage* OR abortion". We also searched SCOPUS and PubMed for articles published up to October 5 2015 (in any language) addressing the association between first-trimester *Plasmodium* spp. infection and miscarriage using the search terms: "malaria or plasmodium", "pregnan*", "first-trimester OR 'first trimester' OR 'early pregnancy'", and "miscarriage* OR abortion". One study reported on the association between a single first-trimester malaria episode and miscarriage.[1] No randomised-controlled trials of first-trimester artemisinin treatment were identified. No studies have reported on the association between recurrent first-trimester malaria and miscarriage. Nine observational studies of first-trimester artemisinin treatment were identified totalling 935 documented treatments, and a systematic review published in 2007 (webappendix Table 1). These studies found no evidence of an increased risk of miscarriage or major congenital malformations associated with first-trimester artemisinin treatment. Importantly, no published studies examining the association between first-line artemisinin treatment and miscarriage accounted for left truncation, which is necessary when women present at varying gestations due the declining risk of miscarriage as a pregnancy progresses, and few were able to account for confounding by indication and disease severity (webappendix Table 4).

Added value of this study

Assessing the safety of artemisinin derivatives requires weighing the risks of falciparum malaria against those of its treatment. We found that first-trimester falciparum malaria increases the risk of miscarriage, especially after recurrence. However, there was no evidence that first-line treatment with an artemisinin derivative in the first trimester was associated with an increased risk of miscarriage or congenital malformations compared to first-line quinine, which is currently recommended by the World Health Organization. We compared first-line treatment with an artemisinin derivative to first-line quinine in women with first-trimester falciparum malaria in an area of low seasonal transmission, and accounted for confounding by indication and disease severity, thereby separating the effects of infection from the effects of treatment. This study is the first to estimate the association between

recurrent first-trimester malaria and miscarriage, and contributes a further 183 documented first-trimester artemisinin treatments. “Left truncation”, which adjusts for the temporally changing risks of miscarriage and varying gestation at presentation, was also accounted for and is essential to avoid significant bias.[2]

Implications of all the available evidence

Effective treatment of first-trimester falciparum malaria is imperative. Our results add to a growing body of observational evidence that artemisinins, the most effective antimalarials available, are safe in first trimester.

Introduction

The relationships between first-trimester malaria, treatment, and miscarriage remain poorly documented because these events often occur before women present to antenatal care.[3,4] A single first-trimester malaria episode is associated with miscarriage,[1] and women with first-trimester malaria who are not adequately treated are at high risk of placental malaria.[5] Since women are usually not protected by preventive interventions until second trimester,[3] early diagnosis and effective treatment of first-trimester malaria is essential to limit its deleterious effects.[6]

Artemisinin derivatives (hereafter referred to as artemisinins) are the most effective antimalarials available. Artemisinin-based combination therapies (ACTs) are recommended by the World Health Organization (WHO) for first-line treatment of falciparum malaria, except during the first trimester of pregnancy.[7] Animal studies have raised concerns about artemisinin safety in first trimester, but data in humans are limited. In animals, artemisinins are embryotoxic and teratogenic because they deplete embryonic erythroblasts, causing miscarriage and congenital malformations (primarily cardiovascular and skeletal).[8] If artemisinins are also embryotoxic or teratogenic in humans, the embryo-sensitive period is predicted to be between 6 and 13 weeks gestation, when erythroblasts are the primary form of circulating red blood cells.[9] Because of these safety concerns, quinine is still recommended for uncomplicated first-trimester falciparum malaria rather than artemisinins, despite being an inferior treatment.[7] Available data on first-trimester artemisinin safety comes from observational studies of inadvertent treatments, which are common but rarely documented.[4] No specific adverse effects have been observed in humans in 935 documented first-trimester artemisinin treatments (webappendix Table 1),[10–19] which although reassuring, has not been sufficient to change treatment recommendations.[7]

The Shoklo Malaria Research Unit (SMRU) screens pregnant women frequently for malaria due to a lack of effective preventative interventions (webappendix Table 2).[20] Prospective data are collected on confirmed *Plasmodium* spp. infections, antimalarial treatment, and pregnancy outcomes, providing an important source of observational evidence on first-trimester artemisinin safety. In this setting a single first-trimester malaria episode (falciparum and vivax) increased the odds of miscarriage, but first-trimester artemisinin treatment was not associated with miscarriage.[1] However, for analytic clarity in this earlier study women with recurrent infections were excluded, which reduced the number of

artemisinin treatments to 44 and overestimated the effect of malaria, since recurrent infection depends on the fetus surviving the initial infection. Here, we extend this seminal study by including women with recurrent malaria, which may be either novel, recrudescent, or relapse in the case of vivax malaria, and three further years of data. Assessing artemisinin safety requires weighing the risks of malaria and its treatment. Therefore, we sought to assess the effect of both first-trimester malaria and artemisinin treatment on miscarriage and major congenital malformations.

Methods

Study area and population

Prospective data on women attending SMRU antenatal clinics has been collected since 1986. The Oxford Tropical Research Ethics Committee granted ethical approval for audits of SMRU clinical records (OXTREC 28-09), and the Tak Province Community Ethics Advisory Board provided local permission (T-CAB-4/1/2015). Data on first-trimester malaria from some of the records included in this analysis have been published previously.[1,20–23]

Procedures

At SMRU antenatal clinics women are encouraged to present early and return weekly throughout their pregnancy for malaria screening, consisting of a finger-prick blood sample that is examined by trained microscopists using Giemsa stained thick and thin blood films (webappendix Table 2).[6] Women are also encouraged to present if they feel unwell, and to deliver at SMRU clinics. The first consultation involves taking obstetric and medical histories, a detailed clinical examination, and gestational age estimation.[24] With each positive screen, information on species, parasitaemia, symptoms, and treatment are recorded. Women are also asked about recent antimalarial treatments at outpatient clinics, and these treatments (usually mefloquine-artesunate (MAS) for *P. falciparum*) are entered retrospectively. Presumptive malaria treatment is not used, and pregnancy termination is unavailable.

First-trimester non-malaria febrile morbidity was defined as fever (temperature $\geq 37 \cdot 5^{o}C$) not associated with malaria. Malaria is defined as the presence of asexual stages of plasmodia parasites in the peripheral blood, counted per 500 white blood cells or 1000 red blood cells. Hyperparasitaemia was

defined as parasitaemia ≥4%, and severe malaria was defined according to signs of vital-organ dysfunction. Symptomatic malaria was defined as patent parasitaemia and a history of fever (past 48 hours) or temperature ≥37.5°C. Vivax malaria was treated with oral chloroquine. Falciparum malaria was treated with oral quinine in first trimester, or an artemisinin-based treatment in second and third trimester (either artesunate, artemether-lumefantrine, dihydroartemisinin-piperaquine, or mefloquine-artesunate). Mefloquine monotherapy was also given for falciparum malaria until 1996. Clindamycin was added to quinine and artesunate 7-day treatments in 2007 to augment efficacy. According to WHO recommendations, artemisinins were given in first trimester for quinine failures, hyperparasitaemia, severe malaria, or if the fetus is no longer viable.[7] Webappendix Table 3 gives details on treatment regimens and drug manufacturers.

Primary exposures were malaria and first-line artemisinin treatment in first trimester, defined as <14 weeks gestation. The primary outcome was miscarriage, defined as fetal death before 28 weeks gestation because infant respiratory support is unavailable. The ability to determine gestation and fetal viability at SMRU improved after ultrasound was introduced in 2002 (webappendix Table 2).[24,25] The date of miscarriage was recorded consistently as the date of expulsion of the uterine contents, either spontaneously or through surgical intervention, which can occur some time after intra-uterine death. The secondary outcome was major congenital malformations. A surface examination was performed on all newborns by trained staff; a physician verified all malformations, except for some early neonatal deaths. Artemisinin-based treatments were first deployed in the general population in 1994. Therefore, we included women that presented to antenatal care during first trimester with a viable fetus between January 1 1994 and December 31 2013.

Statistical analysis

Cox proportional-hazards models accounting for left truncation (webappendix Table 4) and time-varying exposures were used for all miscarriage analyses, with censoring at the gestation time of miscarriage, gestation time when last seen, or 28 weeks gestation. To assess the association between malaria and miscarriage, women entered the analysis at the gestation time of their first antenatal visit. Multivariable models adjusted for year of first consultation, gravidity, smoking, and first-trimester non-

malaria febrile morbidity. To assess the association between first-line artemisinin treatment and miscarriage (primary analysis), we included women first-trimester falciparum malaria, and compared first-line quinine (including quinine plus clindamycin) to first-line mefloquine monotherapy, artemisinin (all derivatives) following quinine failure, and first-line artemisinin (all derivatives). Women entered the analysis at the gestation time of their first falciparum malaria episode. Treatments administered after determination of fetal non-viability were excluded. Multivariable models adjusted for year of first consultation, disease severity pertaining to the first falciparum malaria episode (asymptomatic, symptomatic, or hyperparasitaemic/severe), and first-trimester non-malaria febrile morbidity. The incidence risk of major congenital malformation was described by first-trimester falciparum malaria and first-line treatment. Malformations were grouped by organ system to increase the likelihood of detecting teratogenic signals.[26] All analyses were performed in Stata Version 13 (StataCorp, College Station, Texas, US).

Role of the funding source

Our funding sources (Wellcome Trust and Bill & Melinda Gates Foundation) had no role in study design, data collection, analysis, or interpretation, writing of the paper, or the decision to submit for publication. The corresponding author had full access to all data and had final responsibility in the decision to submit for publication.

Results

Between January 1994 and December 2013, 55636 pregnant women presented to SMRU clinics, of whom 46% (25485/55636) presented during their first trimester with a viable fetus (Figure 1). Of these, 10% (2257/23118) miscarried, 9% (2367/25485) were lost to follow-up before 28 weeks gestation, and 10% (2558/25485) had first-trimester malaria (Figure 1). Women with first-trimester malaria were more likely to miscarry or be lost to follow-up and tended to present for antenatal care earlier, be younger, be primigravid, and smoke, compared to women without first-trimester malaria (all *p* values <0·0001) (Table 1).

Of the 2558 women with first-trimester malaria, 47% (1207/2558) had falciparum malaria, 60% (1532/2558) had vivax malaria, and 7% (181/2558) had both vivax and falciparum (either separate or

mixed infections). Recurrent first-trimester falciparum malaria occurred in 13% (162/1207) women, and recurrent first-trimester vivax malaria in 9% (139/1532). Most (971/1207; 80%) women with first-trimester falciparum malaria were treated initially with quinine, and 15% (183/1207) were treated initially with artemisinin (i.e. first-line artemisinin treatment) (Table 1). Of the 971 women who received first-line quinine treatment, 13% (129/971) were rescued with artemisinin (usually artesunate monotherapy or artesunate plus clindamycin) following recurrence. Of the 183 first-line artemisinin treatments, 20% (37/183) were for hyperparasitaemia (administered orally) or severe disease (administered parenterally). First-line treatment of first-trimester falciparum malaria occurred at a median of 8·2 gestation weeks (IQR: 5·3 – 11·1). Loss to follow-up was similar between antimalarial treatment groups, except women receiving MAS were less likely to be lost (p = 0·0417) (webappendix Table 6). Rates of falciparum malaria during pregnancy and miscarriage and the frequency of first-line quinine and artemisinin treatments in first trimester over time are shown in Figure 2.

Association between initial and recurrent first-trimester malaria and miscarriage

In 1207 women with first-trimester falciparum malaria, 17% (165/983) miscarried and 19% (224/1207) were lost to follow-up, compared to 9% (1963/20978) and 9% (1949/22927) in women with no first-trimester malaria, respectively. In multivariable analyses, the hazard of miscarriage increased 1·61-fold (95% CI: 1·32, 1·97; p <0·0001) with an initial first-trimester falciparum malaria episode, and 3·24-fold (95% CI: 2·24, 4·68; p <0·0001) with recurrent first-trimester falciparum malaria (Figure 3). This association was stronger in women with symptomatic falciparum malaria than in women with asymptomatic falciparum malaria (Figure 2). A single first-trimester hyperparasitaemic or severe falciparum malaria increased the hazard of miscarriage 4·21-fold (95% CI: 2·43, 7·29; p <0·0001) (Figure 3). An initial first-trimester vivax malaria episode, either asymptomatic or symptomatic, increased the hazard of miscarriage slightly (Figure 3). Recurrent symptomatic first-trimester vivax malaria increased the hazard of miscarriage 2·44-fold (95% CI: 1·01, 5·88; p = 0·0473) (Figure 3).

Association between first-line treatment with an artemisinin derivative and miscarriage

Of 1207 women with first-trimester falciparum malaria, 1179 had a known first-line treatment and a viable fetus at the time of first-line treatment (Figure 1). Most (842/1179; 71%) received first-line quinine (including quinine plus clindamycin), 11% (129/1179) received first-line quinine followed by

artemisinin (artemisinin rescue), 2% (25/1179) received first-line mefloquine monotherapy, and 16% (183/1179) received first-line artemisinin. First-line artemisinin treatment was not associated with miscarriage when compared to women that received first-line quinine only (HR: 0·78; 95% CI: 0·45, 1·34); p = 0·3645) (Figure 4). Five women received two first-trimester artemisinin treatments; one miscarried and four delivered.

Since animal studies suggest a theoretical embryo-sensitive window in humans of 6 to 13 weeks gestation, we also estimated the association between first-line artemisinin treatment and miscarriage before, during, and after this window.[9] First-line artemisinin treatments before the embryo-sensitive window were associated with a non-significant decrease in the hazard of miscarriage (HR: 0·54; 95% CI: 0·25, 1·15; p = 0·1108), while treatments during the embryo-sensitive window were not associated with a changed hazard of miscarriage (HR: 1·15; 95% CI: 0·46, 2·87; p = 0·7602) (Figure 4).

Of note, a high proportion of women who received first-line MAS miscarried (15/71, 21%), and most miscarriages in the artemisinin treatment group (15/23, 63%) were in women who received MAS specifically. Further, one third of women who received MAS during the embryo-sensitive window miscarried (8/24, 33%). However, this may be explained by the circumstances of this treatment; women received MAS from outpatient clinics (rather than antenatal clinics) where they presented because of illness before they became aware of their pregnancy. Additionally, MAS treatments at outpatient clinics were given at earlier gestations than for the other treatments when women are at greater risk of miscarriage (MAS: 3·8 weeks [IQR: 1·9 – 7·4]; quinine: 10·1 [7·5 – 11·8]; other artemisinins: 12·4 [9·0, 13·3]) (webappendix Table 10).

Major congenital malformations

20628 women presented for antenatal care between 1994 and 2013, of whom 0·85% (175/20628; 95% CI: 0·73, 0·98) had newborns with a major congenital malformation (Figure 1). The incidence of malformation in women with no first-trimester malaria was 0·84% (158/18803; 95% CI: 0·71, 0·98) (Table 2). Malformation incidence was similar in women with first-trimester vivax malaria (0·59%; 95% CI: 0·22, 1·27). In women with uncomplicated first-trimester falciparum malaria, malformation incidence was slightly higher (1·29% [10/773]; 95% CI: 0·62, 2·37), but did not differ from women

who received first-line quinine (8/641; 1·25%; 95% CI: 0·54, 2·44) and women who received first-line artemisinin (2/109 [microphthalmia; imperforate anus]; 1·83%; 95% CI: 0·22, 6·47) (p = 0·7551) (Table 2). Two other newborns of mothers who had hyperparasitaemic or severe first-trimester falciparum malaria had a malformation [syndactyly; cleft lip and palate] (9·09% [2/22]; 95% CI: 1·12, 29·16); both were of mothers who received first-line artemisinin, but only eight women received first-line quinine (Table 2). No newborns of mothers who received artemisinin in first trimester had skeletal or cardiovascular malformations as reported in animal studies.

Discussion

First-trimester falciparum malaria increases the risk of miscarriage, especially after recurrence, but this large prospective observational study found no evidence that first-line treatment with an artemisinin derivative was associated with an increased risk of miscarriage or congenital malformations. Assessing the safety of artemisinin treatment during pregnancy requires weighing the risks of falciparum malaria against those of its treatment. This is the first study to estimate the effects of initial and recurrent first-trimester malaria, their symptomatology, and treatment on miscarriage.

Legitimate ethical concerns regarding randomised-controlled trials of first-trimester artemisinin treatment have meant that only observational studies have been conducted to date, and these have not adjusted for confounding by indication and disease severity in assessing risks and benefits.[27] A major strength of this study is that it was possible to adjust for these important confounders by comparing to nearly one thousand women who received quinine treatment (webappendix Table 4). “Left truncation”, which adjusts for the temporally changing risks of miscarriage and varying gestation at presentation, was also accounted for as this is essential to avoid significant bias.[28] Nevertheless, this study still has limitations common to observational designs. Data were collected over a long period of time, relatively few first-trimester artemisinin treatments were documented, toxicities other than miscarriage and major malformations detectable at birth from surface examination were not captured, and all artemisinin derivatives were analysed together. Several associations of considerable magnitude had wide confidence intervals that crossed null, and we cannot rule out potential confounding effects of time and unmeasured variables, or residual confounding by disease severity. Furthermore, women with first-

trimester malaria were more likely to be lost, raising the possibility of informative right censoring, but this would underestimate the effect of malaria (webappendix Table 4).

We found no evidence that first-line treatment with an artemisinin derivative increased the rate of miscarriage compared to first-line treatment with quinine. There was a higher risk of miscarriage in women who received an artemisinin derivative during the putative embryo-sensitive window, but this may be explained at least in part by the administration of mefloquine-artesunate at earlier gestations to symptomatic women in the routine outpatient clinics compared to the active surveillance of antenatal clinics. In rats, embryotoxicity of artesunate was attenuated when co-administered with mefloquine.[29] Primates, including humans, might be less sensitive to the effects of artemisinins because of differences in placentation and the visceral yolk sac, which could result in different levels of embryonic exposure to artemisinins.[8,30] Additionally, a three-day artemisinin regimen means that the exposure period is relatively short in humans because organogenesis is three days in rats but three months in humans.[8,30] Therefore, artemisinin-induced depletion of embryonic erythroblasts severe enough to cause miscarriage in rats might not translate to humans, but may still cause congenital malformations.

We cannot draw firm conclusions on the possible effects of first-trimester artemisinin treatment on congenital malformation because of relatively small numbers of treatments and cases. Furthermore, the incidence of major congenital malformations is most likely an underestimation since only those detectable at birth from surface examination and heart auscultation were recorded routinely, and major malformations (particularly cardiovascular) are often not detected or confirmed until later in life. Only four newborns whose mother received first-line artemisinin treatment during first trimester had a major congenital malformation, and the organ systems involved were inconsistent with the types of malformations induced by artemisinins in animal studies.

These results have important implications for malaria treatment and control policies, and future studies of artemisinin safety. Recurrent first-trimester vivax malaria is associated with miscarriage, yet radical cure is not possible during pregnancy with currently available drugs. First-trimester falciparum malaria is strongly associated with miscarriage, especially after recurrence. We found no evidence of harm associated with first-line artemisinin treatment of first-trimester falciparum malaria. Quinine is

comparatively poorly tolerated and associated with a shorter time to recurrence than artemisinin in pregnant women.[31] Furthermore, we found that women who received artemisinins following quinine failure were more likely to miscarry than those who received first-line artemisinin treatment. Early and effective treatment is imperative, especially since current preventive measures do not adequately cover early pregnancy.[3] Artemisinins are the most effective antimalarials available and have been recommended as first-line treatment in the general population by the WHO since 2006. Yet, artemisinin safety in first trimester is still uncertain. This paper contributes a further 183 well-documented first-trimester artemisinin treatments, and adds to a growing body of observational evidence supporting the use of artemisinins in the first trimester of pregnancy.[1,12,13,15–19,23,32] Given the wide availability of ACTs, their excellent tolerability and efficacy, the likely reduced future availability of quinine, and the rarity of congenital malformations, now may be the time to endorse the use of artemisinin derivatives for the treatment of first-trimester falciparum malaria, accompanied by robust pharmacovigilance.

Tables and Figures

Figure 1. Study profile. Included women were enrolled earlier in pregnancy (p <0·001), slightly younger (p <0·001), less likely to be primigravid (p <0·001), more likely to have a history of miscarriage (p <0·001), and had higher haematocrit levels at their first consultation (p <0·001) compared to excluded women (webappendix Table 6).

Table 1. Cohort demographics, N = 25485

Variable	No first-trimester malaria (22927)	First-trimester malaria (2558)
Miscarried[1]	1963 (9)	294 (14)
Lost to follow-up before 28 weeks gestation[2]	1949 (9)	418 (16)
Gestation at first consultation, weeks	9·0 {7·2, 11·3}, 0 – 14·0	8·4 {6·6, 10·6}, 0.1 – 14·0
Maternal age, years	26 {21 – 31}, 13 – 51	23 {19 – 30}, 13 – 46
13 – 20 years	5443 (24)	909 (36)
21 – 25 years	5959 (26)	628 (25)
26 – 30 years	5709 (25)	482 (19)
31+ years	5816 (25)	539 (21)
Primigravid	5628 (25)	821 (32)
Current smoker	5362 (26)	764 (35)
History of miscarriage	6200 (27)	758 (30)
Haematocrit (first consultation), %	36 {33 – 38}, 9 – 52	34 {31 – 37}, 13 – 48
Severe anaemia (haematocrit <20%)	9 (0)	13 (1)
Non-malaria febrile morbidity in 1st trimester	310 (1)	38 (1)
Number of antenatal malaria screens	23 {14 – 28}, 1 – 40	22 {15 – 28}, 1 – 38
Estimated gestational age from ultrasound	16714 (73)	1648 (64)
Details of initial first-trimester malaria		
Symptoms		
Asymptomatic	NA	919 (36)
Symptomatic	NA	1639 (64)
First-line treatment of first-trimester falciparum malaria		
Quinine	NA	971 (81)
Mefloquine monotherapy	NA	25 (2)

Artemisinin derivative	NA	183 (15)
Mefloquine-artesunate	NA	71 (6)
Artemether-lumefantrine	NA	10 (1)
Artesunate plus clindamycin	NA	50 (4)
Artesunate monotherapy	NA	49 (4)
Dihydroartemisinin-piperaquine	NA	3 (0)
Other/unknown	NA	8 (1)
Demised before administration	NA	20 (2)

Numbers are median {IQR}, range or frequency (%). Missing: gravidity 10; smoking status 2853; history of miscarriage 9; haematocrit 969; and miscarriage 2367 (i.e. lost to follow up before 28 weeks gestation). Continuous variables were compared between groups using the Student's *t*-test or Mann-Whitney *U* test for normal and skewed distributions, respectively. Categorical variables were compared using the Chi-squared test. [1]In women followed until 28 weeks; women who miscarried presented for antenatal care 1·4 weeks earlier (p <0·0001), since early attendance increases the chances that a miscarriage will be detected. [2]Women lost to follow-up were slightly younger (p <0·0001), and were more likely to be primigravid (p <0·0001) and have first-trimester malaria (p <0·0001) (webappendix Table 7).

Figure 2. Frequency of first-line quinine and artemisinin treatments in first trimester and rates of falciparum malaria during pregnancy and miscarriage over time. The increase in the rate of malaria in 1998 was due to the establishment of SMRU antenatal clinics in migrant communities. Abbreviations: Q = quinine; Q+C = quinine plus clindamycin; ART = artemisinin.

Figure 3. The association between initial and recurrent first-trimester malaria and miscarriage. Numbers are adjusted hazard ratios (HR) (95% confidence interval) or N (%). Del. = delivered. Misc. = miscarried. Hyper = hyperparasitaemic. Cox models include women lost to follow-up before 28 weeks (until gestation time last seen), but percentage calculations for delivered/miscarried do not. Models for falciparum malaria (1 – 4) include women that may have also had first-trimester vivax, malariae, or ovale malaria; see webappendix Table 11 for associations in women with only first-trimester falciparum malaria. Models for vivax malaria (5 – 6) exclude women that also had first-trimester falciparum malaria. Models 2 and 5 exclude women with symptomatic malaria. Models 3 and 6 exclude women with asymptomatic infections. Model 4 excludes women with uncomplicated infections. Models were adjusted for year (by stratification due to non-proportional hazards (p <0.001)), gravidity, current smoking status, and non-malaria fever in first trimester. Age and previous miscarriage were omitted from multivariable models due to collinearity with gravidity. Adjusted results for gravidity, current smoking status, and fever in first trimester are presented from Model 1. 146 women had recurrent first-trimester falciparum malaria (136 had two and 10 had three). 13 women had recurrent (two) asymptomatic first-trimester falciparum malaria. 81 women had recurrent symptomatic first-trimester falciparum malaria (75 had two, and six had three). 17 women had recurrent (two) asymptomatic first-trimester vivax malaria, and none miscarried. 38 women had recurrent (two) symptomatic first-trimester vivax malaria. See webappendix Table 12 for a table version of this figure, including univariable associations.

Figure 4. The association between first-line treatment of first-trimester falciparum malaria and miscarriage, N = 1179. Numbers are adjusted hazard ratios (HR) (95% confidence interval) or N (%). Models are adjusted for severity of the first falciparum malaria episode (asymptomatic, symptomatic or hyperparasitaemic/severe), non-malaria febrile morbidity in first trimester, and year of first consultation. See webappendix Table 13 for a table version of this figure, including univariable associations. *Categorisations are based on treatment of the first falciparum malaria episode (i.e. first-line treatment), except for "ART rescue after Q failure" which refers to artemisinin-based treatment in first trimester following failure of first-line treatment with quinine or quinine plus clindamycin; Q = quinine; C = clindamycin; MFQ = mefloquine monotherapy; ART = artemisinin-based treatments. Hazard ratios are presented for treatments occurring before (<6 weeks gestation) and during (≥6 and <13 weeks gestation) the embryo-sensitive window. No miscarriages occurred in women who received artemisinin treatment after the embryo-sensitive window (≥13 and <14 weeks gestation). We performed a sub-group analysis excluding women with asymptomatic malaria (N = 919); the association between artemisinin treatment and miscarriage changed by <5% (webappendix Table 14). We performed a sub-group analysis in women attending prior to 2007 whose gestational age was

estimated from ultrasound biometry, since the accuracy of gestational age estimates influences the accuracy of the gestation time of antimalarial treatment, and quinine plus clindamycin succeeded quinine monotherapy in 2007 (N = 469); the direction of associations were the same but of greater magnitude (webappendix Table 15).

Table 2. Major congenital malformations by first-line treatment of first-trimester falciparum malaria and organ system

	No malaria, N = 18803	**Uncomplicated falciparum malaria, N = 773[1]**		**Hyperparasitaemic/severe falciparum malaria, N = 31**	
		Quinine, N = 641	**Artemisinin, N = 109**	**Quinine, N = 8**	**Artemisinin, N = 22**
TOTAL	**158 (0·84%)**	**8 (1·25%)**	**2 (1·83%)**	**0 (0%)**	**2 (9·09%)**
Organ system[2]					
Multiple	26 (16·5)	1 (12·5)	0 (0)	0 (0)	0 (0)
Syndromic	4 (2·5)	0 (0)	0 (0)	0 (0)	0 (0)
CNS	33 (20·9)	1 (12·5)	0 (0)	0 (0)	0 (0)
		Anencephaly (10·3)			
Ears/eyes/face/neck	24 (15·2)	0 (0)	1 (50)	0 (0)	0 (0)
			Microphthalmia (8·2)		
Circulatory	13 (8·2)	1 (12·5)	0 (0)	0 (0)	0 (0)
		Heart defect (7·3)			
Respiratory	2 (1·3)	0 (0)	0 (0)	0 (0)	0 (0)
Digestive	59 (37·3)	2 (25)	1 (50)	0 (0)	1 (50)
		Cleft lip and palate (9·7)	Imperforate anus (6·3)		Cleft lip and palate (13·9)
		Cleft lip and palate (8·8)			
Genital	19 (12·0)	0 (0)	0 (0)	0 (0)	0 (0)
Renal	6 (3·8)	0 (0)	0 (0)	0 (0)	0 (0)
Musculoskeletal	42 (26·6)	4 (50)	0 (0)	0 (0)	1 (50)
		Polydactyly (12·0)			Syndactyly (12·5)
		Polydactyly (12·3)			
		Syndactyly and talipes (11·0)			
		Syndactyly (8·9)			
Skin	4 (2·5)	0 (0)	0 (0)	0 (0)	0 (0)
Other	1 (0·6)	0 (0)	0 (0)	0 (0)	0 (0)

Numbers are N (%). [1]23 women received first-line mefloquine or had unknown first-line treatment (zero malformations). [2]Descriptions of malformations are listed under incidence for each organ system, with gestational time of first-line treatment in parentheses.

Contributors

KAM, JAS, RM, FJIF and FN developed the analytical plan. RM, JW, MKP, MP, OM, MJR and PJ gathered the data. KAM analysed the data. KAM, JAS, RM, FJIF, NJW and FN interpreted the data. KAM drafted the manuscript. All authors read and critically revised the draft manuscript, and approved the final manuscript. All authors agreed to be accountable for all aspects of the work.

Declaration of interests

We have no conflicts of interest to declare.

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