First Trimester Use of Artemisinin-Based Combination Therapy and Risk of Low Birth Weight

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Abstract

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Introduction: There is a lack of sufficient evidence on the safety of use of artemisinin-based combination therapies (ACT) among pregnant women, especially during early pregnancy. We determined the association between exposure to ACT during the first trimester of pregnancy and occurrence of low birth weight (LBW), and small for gestational age (SGA) among the offspring of pregnant women.

Methods: We performed a secondary analysis of data from a prospective cohort study of pregnant women recruited at three health and demographic surveillance system (HDSS) sites in the sub-Saharan African countries of Mozambique, Burkina Faso and Kenya. Data from the HDSS allowed earlier pregnancy identification and linkage and tracking of pregnancy outcomes to determine birth weight and gestational age at delivery. Exposure to any antimalarials was ascertained through a combination of data collected from clinic registers, prescription records and self-reported usage by the women.

Results: There was no difference in the pooled prevalence of LBW among children born to pregnant women who were exposed to quinine, ACT and had no exposure to antimalarials, 21.0%, 11.3% and 10.2%, respectively. Children whose mothers had exposure to ACTs during the first trimester had 21% lower occurrence of LBW when compared to children born to mothers exposed to ACTs in second or third trimester, this difference was not statistical significant (95%CI: -7-49%).

Conclusion: ACT exposure during the first trimester was not associated with an increased risk of LBW.

Our findings support the use of ACT for treatment of malaria during the first trimester of pregnancy.

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Dedication

To my parents (Augusto and Isabel) who sacrificed a lot and dedicate their life for my education and wellbeing. To my brothers (Olinda, Ivo, Paulo and Augusto Jr) who understood and taught me what is love and sharing. To my Country, Mozambique, that has been promoting health and education for everyone even under the most adverse circumstances.

Introduction

Artemisinin-based combination therapy (ACT) is the recommended first line treatment of *Plasmodium falciparum* uncomplicated malaria. Yet, there is a lack of sufficient safety evidence of artemisinin derivative products used by pregnant women due to ethical concerns of enrolling pregnant women in randomized controlled trials (RCT). ACTs are not recommended by the World Health Organization (WHO) for use in the first trimester of pregnancy, unless there are no other drugs available, due to concerns based upon animal studies suggesting their potential for embryotoxicity and teratogenicity[1]. Preclinical studies show that artemisinins are embryotoxic and teratogenic in multiple animal species[2-6]. However, in settings where ACTs are the recommended treatment for uncomplicated malaria, it is very likely that a woman will be inadvertently exposed in the early pregnancy due to their high market availability and because the women and health care providers being unaware of a woman's pregnancy status at the time of prescribing an antimalarial [7].

Malaria infection in pregnancy is associated with maternal anemia and intrauterine growth restriction (IUGR), leading to poor pregnancy outcomes such as low birth weight (LBW). Malaria accounts for 14% to 25% of LBW in Sub-Saharan Africa [8,9]. Due to these concerns, multiple measures to prevent malaria in pregnancy are recommended. These measures include the use of long lasting insecticide treated nets (LLITNs), administration of intermittent preventive treatment in pregnancy with sulfadoxine-pyremethamine (IPTp-SP), and malaria case management [10]. The WHO currently recommends the use of artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria in adults, children and in pregnant women in their 2nd or 3rd trimester. Seven days of quinine with clindamycin is recommended for uncomplicated malaria in the first trimester of pregnancy[1]. All these measures have shown an impact on reducing malaria mortality and morbidity including the occurrence of LBW [11].

The WHO defines LBW as a birth weight of live born infant of less than 2500g [12]. LBW is a result of a short gestational period, IUGR or a combination of both processes, and contributes globally to high

neonatal and infant mortality and morbidity [12]. The global prevalence of LBW among live births is 15.5% representing as many as 20 million infants in the world, of which 95% of are from developing countries. Sub-Saharan Africa (SSA) alone accounts for 4 million infants born LBW [13]. A LBW neonate born in SSA is 9 times more likely to die than a normal-weight baby in the first month of life [14,15]. Moreover, infant mortality rates increase exponentially the lower the birth weight [16].

A related measure to LBW that accounts for gestational age is the small-for-gestational age (SGA) measure. SGA is weight less than the 10th percentile of birth weight for the newborn's gestational age [17]. Infants who are growth-restricted experience higher rates of fetal and infant death, birth asphyxia, hypothermia, hypoglycemia, meconium aspiration, and long-term neurological impairment [16].

Given the widespread use of ACT in malaria endemic region, inadvertent exposure in the first trimester of pregnancy is common[18]. Therefore, it is imperative to gather and generate safety data and analysis of ACT exposure in the first trimester.

The Assessment of Safety of Antimalarial drug use during early Pregnancy (ASAP) study was a multi-country prospective cohort study of pregnant women to evaluate whether ACT exposure in early pregnancy increased the risk for miscarriage, stillbirths, congenital malformations and low birth weight when compared to current therapeutic options [19]. The risk of miscarriage, stillbirths and congenital anomalies have been reported elsewhere [20]. In this analysis we aim to evaluate the association between ACT exposure during pregnancy and LBW and SGA among the offspring of pregnant women.

Objective

The primary objective of this study was to determine the association between exposure to ACT during the first trimester of pregnancy and LBW and the occurrence of SGA by comparing women exposed to ACT versus quinine and to no antimalarials during pregnancy. In addition, this study assessed the risk of LBW associated with ACT exposure during the first trimester versus later gestational ACT exposure.

Methods

Study design

ASAP was a multi-centered prospective cohort study of pregnant women, conducted in three SSA sites associated with the Malaria in Pregnancy Consortium (MiPc). The sites were located in Asembo-Siaya County, Kenya; Nanoro, Burkina Faso; and Manhiça District, Mozambique. In all three sites *Plasmodium falciparum* is the main malaria etiologic agent with high transmission rates. While Nanoro and Manhiça have a clear seasonal transmission pattern increasing during the rainy season, Asembo has year-round transmission[19].

All three ASAP sites have health and demographic surveillance system (HDSS) platforms and are active members of the International Network of field sites with continuous demographic Evaluation of Populations and Their Health (INDEPTH) in developing countries[21-24]. Within their defined communities, HDSS sites ensure recording of all vital status (births, deaths, migration) and other demographic events such pregnancy through full enumeration of residences and its members during twice a year household visits and the use of community key informants[24]. The ASAP cohort study was designed to leverage this platform. Additional recruitment and data collection strategies were employed for the ASAP study to identify pregnancies, antimalarial exposures, determine gestational age at the time of exposure to antimalarials, monitor pregnancy outcomes, and systematically assess infant outcomes[19]. The emphasis was placed on identifying first-trimester pregnancy exposures to antimalarials through identifying and recruiting women as early as possible in pregnancy.

ASAP data collection procedures

Enrollment into the Study

Pregnant women were identified through household visits, community key informants, and at antenatal care visits in a health facility within the HDSS area. Only women who planned to remain in the study area through delivery and who were willing and able to provide informed consent were enrolled in the study. Exclusion criteria common across all study sites were refusal to participate or to be followed up

through the end of pregnancy, or any condition that would interfere with the ability to provide informed consent or provide an accurate medical history. In Mozambique and Burkina Faso, pregnant women were identified within the HDSS through repeated household visits. In addition, field workers visited village reporters on weekly basis to learn about any pregnancies identified in their community. All identified pregnant women were invited to the antenatal care (ANC) and assessed for eligibility. Baseline information was then collected and the data entered into a pregnancy register. In addition, electronic records from outpatient and inpatient visits were recorded through the HDSS platform and linked to the study records to identify possible exposure to ACTs during the first trimester of the pregnancy.

In Kenya, in addition to the above HDSS procedures, recruitment was broadened to include identification of women of childbearing age who were participating in the population-based infectious disease surveillance (PBIDS) in order to detect pregnancies as early as possible through this additional community based strategy.

Data collection procedures

At enrollment, each site collected demographic data, information on possible risk factors and potential confounding factors via a detailed questionnaire administered to enrolled participants at first ANC visit, at antenatal follow-up visits and at the end of pregnancy. Drug exposure ascertainment was based on a combination of retrospective patient self-report, health facility treatment records, inpatient and outpatient visit databases and prospective self-report. For pregnancy outcomes ascertainment, health facility assessments and home base assessment were used. To ensure standardization across sites, nurses and/or midwives were trained on assessment of drug exposure, gestational age assessment using a portable ultrasound scans, and newborn examination. To promote and improve adherence to protocol, community engagement activities were regularly conducted.

Ascertainment of Antimalarial Drug Exposures

The ascertainment of drug exposure included prospective and retrospective self-report and linkage to treatment records at local health facilities, drug prescribing and dispensing clinics. "Possible" ACT exposure was defined as exposure identified in only one data source and "confirmed" ACT exposure as exposures identified in 2+ data sources.

Drug identification – Before study initiation an assessment of antimalarials available in the study areas was conducted and country-specific visual aids and pictorial keys were prepared to assist with recall during the data collection interviews. Study staff reviewed participant's drug packages, tablets and prescriptions when available. They also reviewed and abstracted prescribing information from ANC cards and/or patient hospital files, where available. Data collected included timing of exposure, medication name, dose, and treatment duration.

Self-Reported Drug Exposure – Each study participant was asked about current and previous pregnancy exposure to antimalarials during the baseline interview. This evaluation was conducted post-ascertainment of pregnancy status at first antenatal consultation and repeated at each follow-up antenatal visits. In Kenya, the PBIDS program allowed prospectively collected data on drug exposure and morbidity on a weekly and then bi-weekly basis. The timing of drug exposures was determined by using estimated gestational age [19].

Health Facility Records Drug Exposures – Record linkages techniques were used to match pregnant women as recorded in the health facility log-books to the morbidity surveillance database and outpatient department record. This was conducted to ascertain drugs dispensed at the health facilities located within the study catchment areas.

Gestational Age Assessment and Classification

Ascertainment of gestational age is critical for correct exposure classification. Multiple methods were used, including date of last menstrual period, Ballard Score, fundal height and ultrasound as explained

elsewhere[19]. Study staff were trained on newborn examination, Ballard Score assessment, fundal height measurements, and use of ultrasound scans to date the pregnancy. Quality control of these procedures consisted on immediate feedback during training, supervising visits and assessment of a sample of ultrasound scans by a consulting radiologist at University of Washington who provided feedback to the midwives. Fundal height was assessed at every antenatal visit. On the first antenatal visit ultrasound scans were performed whenever possible.

For this analysis we define embryo-sensitive period (ESP) as gestational age between 6 and 12 weeks post last menstrual period, which is equivalent to between 4 and 10 weeks post-conception.

Pregnancy Outcomes Assessment

The ASAP protocol included several pregnancy outcomes of interest namely miscarriages, fetal deaths/stillbirths, low birthweight, small for gestational age, and live births. The present analysis is restricted to birthweight and gestational age. Women were encouraged to deliver at closest health facility where systems were in place to identify and link records. Also, deliveries occurring outside the health facility where actively identified by close monitoring lists of probable delivery and home-based visits or by notification from village recorders whereby a study staff team assessed at home cases as soon as possible. In addition, traditional birth attendants (TBAs) were instructed to alert study staff of the occurrence of any pregnancy outcome, including miscarriage, stillbirth or live birth.

Inclusion criteria for the analysis

For purposes of this study of LBW and SGA we included live-born infants of women enrolled in the ASAP study who had her exposure status ascertained during pregnancy and birth weight collected within the first 10 days of life of her offspring.

Outcomes

The primary outcome for the analysis was low birthweight (LBW), defined as weight at birth below 2500g regardless gestational age [12]. As a secondary outcome, we evaluated small for gestational age

(SGA) defined by weight at birth below 10th percentile of weight for the gestational age according to international reference curves [17,25]. Weight at birth was measured in grams.

Study groups

We identified exposure groups based upon the type and timing of antimalarial drug exposure. These were: a) not-exposed to any antimalarial with or without exposed IPTp-SP; b) exposed to ACT during the first trimester of pregnancy (early ACT exposure); c) exposed to ACT during the second or third trimester of pregnancy (later ACT exposure); and d) exposed to another antimalarial with or without IPTp-SP in first trimester.

For evaluating the association of early pregnancy exposure to ACT and LBW or SGA, the early ACT exposure group was compared to the *non-ACT exposed* group. A secondary comparison was early ACT exposure compared to exposure to an ACT during the second or third trimester.

Potential covariates

Covariates considered for adjustment in the analyses were level of maternal education, mother's age at recruitment, marital status, gravidity, HIV serological status and study site.

Data sources

Each site submitted its patient-level study data along with database documentation. The datasets were merged and used for this analysis.

Bias and control measures

Confounding

Clinical malaria is a potential confounding for the association between ACT exposure and LBW. Pregnant women who present with clinical malaria tend to receive an antimalarial other than IPTp-SP. Also, malaria is associated with LBW occurrence. To address this bias, for the main comparison (association of ACT exposure and LBW or secondary outcomes) we first compared ACT exposed women to women without other antimalarial other than IPTp-SP, then we compare ACT exposed to non-ACT antimalarial

exposed women. With the same concern for the second comparison (association of earlier ACT exposure and LBW or secondary outcomes) we restricted the comparison to women who received an antimalarial other than IPTp-SP.

We adjusted for the mother's age and gravidity. No other potential covariate was used in the multivariate analyses because of sparse data. For example, no exposure to quinine during pregnancy was observed in Asembo. The between-sites comparison of the baseline data showed important heterogeneity. Thus a multilevel approach was chosen to address such heterogeneity.

Measurement error

Ideally birth weights should be collected on the first day of life, due to the newborn's physiologic changes. A newborn loses almost 6.4% of its weight within the first 48 hours of life[26]. We use a linear regression to obtain the missing weight at birth from the weight collected within first 10 days as previously suggested in the literature [27,28]. The estimated equation has weight as dependent variable and as predictors dummies to indicate each day of life within the first week of life and continuous counts of days between 8th and 10th day of life; dummies for study sites and gestational age at birth as the equation represents

$$weight = \beta_0 + \beta_1 \cdot day_1 + \beta_2 \cdot day_2 + \beta_3 \cdot day_3 + \beta_4 \cdot day_4 + \beta_5 \cdot day_5 + \beta_6 \cdot day_6 + \beta_7 \cdot daycontinuos_{7-9} + \beta_{site2} \cdot site2 + \beta_{site3} \cdot site3 + \beta_{gestage} \cdot age\_gest$$

The estimated $\beta_0 + \beta_{gestage} \cdot$age_gest is the mean weight on day zero. We add this value the residuals in order to preserve the variability on the weight at birth. It is to notice that the first day is included in the model as day zero. The described procedure, assumes that all children are comparable to each other and that there is not error measurement of weight on the date that has been collected. The gestational age might not be accurate and there might be inter-site variance as well.

Statistical methods

Baseline characteristics of different comparison groups

Counts and proportions were employed for categorical variables, continuous arithmetic means, standard deviations, and quartiles. Baseline data were compared by site to assess heterogeneity among sites and by antimalarial exposure to evaluate imbalance of covariates. One-way ANOVA is used to compare means and Fisher's exact chi-squared are used to compare the distribution of frequencies.

Prevalence of low birth weight and of small for gestational age analysis

The prevalence of LBW was computed among groups of comparison. As the measure of association we used prevalence-ratio (PR). The PR is computed through two steps. First a random intercepts logistic regression is fitted; and secondly the fitted proportions are used to compute the prevalence ratio as explained elsewhere[29]. Briefly the fitted models have the form:

$$\text{logit}(LBW\_prevalence) = \beta_s + \beta_1 \cdot exposure_{ACT} + \beta_1 \cdot exposure_{QNN} + \beta_j \cdot covariates + \varepsilon$$

$$\beta_s = \beta_0 + \eta_s$$

The dependent variable is the dummy indicating low birth weight (or small for gestational age)[1]; the predictors of interest are included as the dummies for ACT and QNN exposure; other covariates may also be included in the model. The subscripts on the intercept is to indicate different intercepts on different sites due to some specific site influence η (from a normal distribution); ε represents some random error due to measurement error. The same regression equations are used to compute unadjusted and adjusted pooled prevalences. We also report the 95% confidence intervals (95%CI) of the estimates.

Mean weight and mean gestational age at birth

As measure of association we use mean difference. We use a mixed linear model with random intercepts defined by the site. This regression has the form:

[1] These are 2 different regressions.

$$birthweight = \beta_s + \beta_1 \cdot exposure_{ACT} + \beta_1 \cdot exposure_{QNN} + \beta_j \cdot covariates + \varepsilon$$

$$\beta_s = \beta_0 + \eta_s$$

The dependent variable is birthweight; the predictors of interest are included as the dummies for ACT and QNN exposure; other covariates may also be included in the model. The subscripts on the intercept is to indicate different intercepts on different sites due to some specific site influence η (from a normal distribution); ε represents some random error due to measurement error (from a normal distribution). The effects are the β's. The same regression equations are used to compute unadjusted and adjusted pooled means and its 95%CI.

Software

All analysis was performed on Stata 14 (StataCorp. 2015. Stata: Release 14. Statistical Software. College Station, TX: StataCorp LP).

Ethical Approval

All study sites and the University of Washington had institutional ethical review approvals and the study sites obtained informed consent from all participants.

Results

From the total of 2930 women recruited into the main ASAP study, 381 women were not included in the analysis due to lack of baseline information and 338 women were excluded due to lack of a recorded birth weight. Thus 2134 (72.8%) of pregnancies that ended in a live born infant are included in the analysis (Figure 1 and Table 1). There were 1872 non-exposed women, 236 exposed to ACT and 26 to quinine. A total of 204 cases of LBW were initially counted. However, post-correction due to the effect of first days of life on birthweight, resulted in an extra 32 cases identified and 1 case became normal weight at birth resulting in a total of 235 cases of LBW included in the analysis.

Baseline characteristics

There were important inter-site differences among study participants. Although there were some statistically significant differences between study sites, age at recruitment was similar with mean of 25.7 (SD 6.61) years and ranging from 12 to 49 years (Table 2). There were different levels of illiteracy ranging from 92.1% in Nanoro, 45.1% in Asembo, and 14.5% Manhiça. Almost (98.7%) all women in Nanoro were married or lived in married union whereas in Asembo and Manhiça these values were 78.3% and 63.9%, respectively. A total of 21.9% of women were pregnant for the first time. Women were recruited at an average of 20.8 (SD 8.38) weeks of gestation. Asembo had a considerable (35.4%) percentage of women recruited in the first trimester of pregnancy compared to less than 10% in Nanoro and Manhiça. No woman in Nanoro was reported to be HIV positive whereas in Asembo and Manhiça 21.6% and 24.3% of women were HIV positive, respectively.

No quinine exposures during pregnancy were identified in Asembo (Table 3). Important differences on distribution of exposure groups were present (p-value=0.031). The age at recruitment was similar among different exposure groups. Women exposed to ACT or quinine had 5 weeks lower mean gestation age at recruitment compared to the non-exposed group, with 44.5% of those exposed to ACT

identified in first trimester of pregnancy. Also, there tended to be more women who were HIV positive among those exposed to quinine in the first trimester.

Mean weight at birth

The mean weight at birth in grams was 2871.1, 3093.9 and 3080.2 in Nanoro (Burkina Faso), Manhiça (Mozambique) and Asembo (Kenya), respectively. These mean values are below the standard defined weight as 3500g. Although non-statistically significant, infants born to women exposed to quinine during the first trimester weighed 108.9 g (95%CI: -89.1 – 306.9g) less than infants not exposed to quinine. Similarly, babies exposed (confirmed or possible) to quinine during first trimester of pregnancy weighted 29.3g (95% CI: -100.2 – 41.6g) more than non-exposed to no antimalarial (Table 4).

Prevalence of LBW

The pooled prevalence of LBW was 21.0%, 11.3% and 10.2% among confirmed quinine, artemisinin and antimalarials non-exposure, respectively. These prevalence values were not statistically different by exposure group and did not change materially after adjustment for age at recruitment, gravidity and HIV status (Table 5). Moreover, first trimester exposure to ACTs had a non-significant 21% (95%CI: -7 – 49%) relatively less occurrence of LBW when compared to exposure to ACTs in the second or third trimester.

Prevalence of SGA

We found no association between first trimester exposure to ACT and occurrence of SGA (Table 6). The quinine confirmed exposed group during first trimester of pregnancy had non-significant 22% (95%CI: -74 – 119%) relatively higher occurrence of SGA than non-exposed and non-significant 32% (95%CI: -86 – 149%) relatively higher compared to the artemisinin group. These associations become stronger but still not statistical significance when restricting the exposure to the embryo sensitive period.

Discussion

We leveraged the use of the HDSS platform to determine the occurrence of LBW and SGA among women exposed to artemisinin therapy or quinine during pregnancy. Results from this analysis indicate no evidence of an increased risk of LBW (PR: 1.01; 95%CI: 0.45 – 1.57) or SGA (PR: 0.93; 95%CI: 0.50 – 1.36) among infants born to women with confirmed first trimester exposure to artemisinin treatment compared to babies born to women unexposed to antimalarials. Quinine exposure during the embryo-sensitive period was associated with a non-statistically significant level of prevalence of LBW (PR: 1.89; 95%CI: 0.47 – 3.13) when compared to unexposed pregnancies.

The prevalence of LBW in Manhiça and Kenya (6.1% and 11.0% respectively) in our study was lower than what national community surveys (14.1% and 7.6% respectively) have reported [30,31]. However, these two sites are relatively underrepresented within the national sample frames. Additionally, many interventions contributing to reduction of LBW have been strengthened since these national surveys were conducted, such as increase of antenatal coverages that detects earlier pregnancies and malaria prevention, e.g., high coverage IPTp and intra-domiciliary bed net use programs. Thus our findings may be a reflection of the contribution of such interventions.

The prevalence of SGA found in this study varied between 15.3% among infants born to women non-exposed to antimalarials during pregnancy to 23.9% among infants born to women exposed to quinine during pregnancy. These estimates are consistent with recent estimates of SGA based on 22 birth cohort studies from the SGA-Preterm Birth working group[32].

More than half of the women had their first antenatal visit during the second trimester of the pregnancy. This finding is very similar to DHS reports[30,31]. However, Asembo is an exception given that at least a third of the recruited women had the first antenatal visit in first trimester. This could have played

a role on earlier use of IPTp-SP which could have reduced the occurrence of malaria and thus less need of quinine or an ACT at this site.

HDSS procedures mandate frequent visits to a household. This increases the likelihood of detecting early pregnancies and facilitates follow-up of pregnancy outcomes. Nevertheless, cultural barriers[7] still pose challenges to the field workers to identify not yet visible pregnancies as almost half of the pregnancies were detected during second semester.

Limitations

We included in the analysis only pregnancies ending as singleton live-birth and with birth weight collected within 10 days of life. This could lead to bias because the small gestation for age (the outcome) is associated with less probability to survival. Thus the inclusion in the data analysis is conditioning on the outcome. However, we do not expect this to be an important source of bias because the neonatal mortality rate in these sites is small (less than 30 per 1000 live-births).

Compared to other study sites, Asembo (Kenya) had higher proportion of missing information of weight at birth variable, representing 12% of all pregnancies recruited (figure 1). The vast majority of these missing birth weights are among babies born at home, at which no evaluation on birth day was possible. We used an imputation technique to address this problem. The employed imputation assumes that all babies in one site are similar regardless of potential unmeasured biological differences (gender, mother anthropometrics). This may have contributed to lower prevalence of LBW because babies who died due to conditions linked to LBW didn't get their weights recorded and thus they do not contribute on the imputation. However, we do not expect this bias to have contributed much on the overall direction of the association.

In Nanoro all women were HIV negative according to the database documentation. The HIV prevalence in Nanoro is very small and our findings are likely to be correct. However, we do not rule out the possibility of poor documentation of HIV status.

Conclusions

We found no evidence of an increased risk of LGW or SGA among infants born to women with confirmed first trimester exposure to an ACT. Our findings add to the support for use of ACT for uncomplicated *Plasmodium falciparum* malaria during the first trimester of pregnancy. The existence of the HDSS platform greatly facilitated active surveillance pregnancy pharmacovigilance.

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Tables

Table 1 – Detailed data processing steps from the total pregnancy recorded to final sample included in the analysis, ASAP cohort, 2015

		Asembo	Manhiça	Nanoro	Total
	Total pregnancy records	**1453**	**763**	**714**	**2930**
		↓	↓	↓	↓
		↓	↓	↓	↓
Step 1	**Removed because of no baseline data**	**314**	**49**	**18**	**381**
	Out migration				
	Withdrawl or refused follow-up				
	Maternal death				
	Detected at outcome				
	Entered after 28 weeks				
	No GA information				
	Preganancy end date error				
	No follow-up				
		↓	↓	↓	↓
		↓	↓	↓	↓
Step 2	**Removed due to**	**144**	**40**	**28**	**212**
	Twins	14	0	14	28
	Weight at birth missing	81	40	14	135
	More than 1 pregnancy follow up	49	0	0	49
		↓	↓	↓	↓
		↓	↓	↓	↓
Step 3	**Removed because of no weight collected after 10th day of life**	**202**	**0**	**1**	**203**
		↓	↓	↓	↓
		↓	↓	↓	↓
Step 4	**Records included in the Analysis**	**793**	**674**	**667**	**2134**
	LBW cases	87	41	107	235

LBW – low birth weight; GA – gestational age

Table 2 - Baseline characteristics of pregnancies included for low birth weight analysis per study site, ASAP cohort, 2015

	Asembo		Manhiça		Nanoro		Total		p-value
Characteristic	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
N participants	793		674		667		2134		
Age at recruitment (years)									
Range	15 - 48		12.4 - 41.9		15 - 49		12.4 - 49.0		
Mean (SD)	25.8 (6.65)		24.2 (6.24)		27.0 (6.63)		25.7 (6.61)		< 0.001
Education									< 0.001
Never went to school or primary incomplete	358	45.1	98	14.5	614	92.1	1070	50.1	
Primary completed	374	47.2	331	49.1	52	7.8	757	35.5	
Secondary completed	61	7.7	245	36.4	1	0.1	307	14.4	
Marital Status									< 0.001
Married or living together	621	78.3	431	63.9	658	98.7	1710	80.1	
Not married	172	21.7	243	36.1	9	1.3	424	19.9	
Gravidity									< 0.001
Primigravida	167	21.1	185	27.4	116	17.4	468	21.9	
1-3 pregnancies	382	48.2	396	58.8	313	46.9	1091	51.1	
4 or more pregnancies	244	30.8	93	13.8	238	35.7	575	26.9	
Gestational age at recruitment (weeks), mean (SD)	18.5 (10.54)		21.2 (5.65)		23.2 (6.94)		20.8 (8.38)		< 0.001
Gestational age in categories									< 0.001
First trimester	281	35.4	29	4.3	45	6.7	355	16.6	
Second timester	263	33.2	418	62.0	294	44.1	975	45.7	
Third trimester	249	31.4	227	33.7	328	49.2	804	37.7	
HIV status									< 0.001
Positive	171	21.6	164	24.3	0	0.0	335	15.7	
Negative	583	73.5	447	66.3	648	97.2	1678	78.6	
No information	39	4.9	63	9.3	19	2.8	121	5.7	

SD – standard deviation; Except for the means all p-values are computed through Fisher's exact chi-squared test. For the means are computed from one-way ANOVA.

Table 3 - Baseline characteristics by exposure level (no exposure, possible or confirmed), ASAP cohort, 2015

			No exposure		Possible or confirmed exposure					Confirmed exposure					Confirmed exposure				
					Artemisinins		Quinine		p-value	Artemisinins		Quinine		p-value	Artemisinins		Quinine		p-value
Characteristic	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**		**N**	**%**	**N**	**%**		**N**	**%**	**N**	**%**	
N participants	2134	100.0	1872	100.0	236	100.0	26	100.0		103	100.0	26	100.0		133	100.0	0		
Site									0.031					< 0.001					< 0.001
Nanoro	667	31.3	608	32.5	37	15.7	22	84.6		37	35.9	22	84.6		0	0.0	0		
Manhiça	674	31.6	648	34.6	22	9.3	4	15.4		19	18.4	4	15.4		3	2.3	0		
Asembo	793	37.2	616	32.9	177	75.0	0	0.0		47	45.6	0	0.0		130	97.7	0		
Age at recruitment (years)																			
Range	12.4 - 49.0		12.4 - 49.0		15.0 - 46.0		17 - 36			15.0 - 45.0		17 - 36			15.0 - 46.0		-		
Mean (SD)	25.7 (6.61)		25.6 (6.58)		26.1 (6.91)		24.7 (6.05)		0.468	25.7 (6.71)		24.7 (6.05)		0.708	26.3 (7.07)		-		0.288
Education									0.031					0.380					0.013
Never went to school or primary incomplete	1070	50.1	943	50.4	109	46.2	18	69.2		50	48.5	18	69.2		59	44.4	0		
Primary completed	757	35.5	650	34.7	101	42.8	6	23.1		39	37.9	6	23.1		62	46.6	0		
Secondary completed	307	14.4	279	14.9	26	11.0	2	7.7		14	13.6	2	7.7		12	9.0	0		
Marital Status									0.035					0.037					0.355
Married or living together	1710	80.1	1498	80.0	186	78.8	26	100.0		84	81.6	26	100.0		102	76.7	0		
Not married	424	19.9	374	20.0	50	21.2	0	0.0		19	18.4	0	0.0		31	23.3	0		
Gravidity									0.428					0.719					0.351
Primigravida	468	21.9	406	21.7	54	22.9	8	30.8		24	23.3	8	30.8		30	22.6	0		
1-3 pregnancies	1091	51.1	970	51.8	110	46.6	11	42.3		49	47.6	11	42.3		61	45.9	0		
4 or more pregnancies	575	26.9	496	26.5	72	30.5	7	26.9		30	29.1	7	26.9		42	31.6	0		

Gestational age at recruitment (weeks), mean (SD)	20.8 (8.38)		21.5 (8.00)		16.0 (9.87)		16.8 (5.56)		< 0.001	16.6 (9.24)		16.8 (5.56)		< 0.001	17.1 (10.24)		-	< 0.001
Gestational age in categories									< 0.001					< 0.001				< 0.001
First trimester	355	16.6	245	13.1	105	44.5	5	19.2		50	48.5	5	19.2		55	41.4	0	
Second trimester	975	45.7	878	46.9	78	33.1	19	73.1		37	35.9	19	73.1		41	30.8	0	
Third trimester	804	37.7	749	40.0	53	22.5	2	7.7		16	15.5	2	7.7		37	27.8	0	
HIV status									0.279					0.446				0.067
Positive	1678	78.6	1,478	79.0	177	75.0	23	88.5		81	78.6	23	88.5		96	72.2	0	
Negative	335	15.7	289	15.4	45	19.1	1	3.8		14	13.6	1	3.8		31	23.3	0	
No information	121	5.7	105	5.6	14	5.9	2	7.7		8	7.8	2	7.7		6	4.5	0	

Table 4 - Mean birthweight in grams, by exposure status and study site, ASAP cohort, 2015

	Pooled Mean Weight at Birth		Pooled mean difference Unadjusted		Mean difference	
Characteristic		**95% CI**		**95% CI**		**95% CI**
Site						
Nanoro (Burquina Faso)	2871.1	2838.2 - 2904.0				
Manhiça (Mozambique)	3093.9	3061.6 - 3126.1				
Asembo (Kenya)	3080.2	3036.9 - 3123.6				
Confirmed or possible Exposure						
No exposure	3015.4	2991.8 - 3038.9	0.0	-	0.0	
Artemesinin	3062.0	2995.7 - 3128.3	46.6	-23.8 - 116.9	29.3	-41.6 - 100.2
Quinine	2902.6	2702.9 - 3102.4	-112.7	-313.9 - 88.4	-108.9	-306.9 - 89.1
Confirmed Exposure						
No exposure	3015.4	2991.8 - 3038.9	0.0	-	0.0	
Artemesinin confirmed	3014.0	2912.8 - 3115.2	-1.4	-105.3 - 102.5	-20.1	-124.3 - 84.1
Quinine confirmed	2902.6	2701.3 - 3104.0	-112.7	-315.5 - 90.0	-107.9	-307.8 - 92.0
Confirmed ESP Exposure						
No exposure	3015.4	2991.8 - 3038.9	0.0	-	0.0	
Artemesinin confirmed	3043.7	2924.0 - 3163.5	28.4	-93.8 - 150.5	23.0	-98.7 - 144.6
Quinine confirmed	3002.1	2744.5 - 3259.7	-13.3	-272.0 - 245.4	-39.4	-294.5 - 215.7
Confirmed earlier versus later ACT Exposure						
Earlier	3043.7	2940.3 - 3147.2	105.6	-89.3 - 300.6	152.7	-43.0 - 348.4
Later	2938.1	2772.9 - 3103.4	0.0	-	1.00	-

All weights from 2134 newborns are included in the analysis post imputation.

Table 5 - LBW prevalence and prevalence rate ratio, per exposure level, ASAP cohort, 2015

	Counts		Pooled Prevalence (%) Unadjusted		Pooled Prevalence (%) Adjusted		Prevalence Rate-Ratio Unadjusted		Prevalence Rate Ratio Adjusted	
Characteristic	**N**	**cases**	**%**	**95% CI**	**%**	**95% CI**	**PRR**	**95% CI**	**PRR**	**95% CI**
Confirmed Exposure										
No exposure	1872	201	10.2	5.7 - 14.7	10.3	5.2 - 15.3	1.00		1.00	
Artemesinin confirmed	103	13	11.2	3.7 - 18.6	10.4	4.1 - 16.6	1.09	0.50 - 1.68	1.01	0.45 - 1.57
Quinine confirmed	26	7	21.0	4.4 - 37.6	18.5	4.7 - 32.3	2.05	0.59 - 3.51	1.80	0.47 - 3.13
Confirmed ESP Exposure										
No exposure	1872	201	10.2	5.7 - 14.7	10.3	5.2 - 15.3	1.00		1.00	
Artemesinin confirmed	74	9	11.3	2.8 - 19.7	10.0	5.4 - 14.7	1.10	0.40 - 1.80	0.98	0.34 - 1.62
Quinine confirmed	16	4	20.6	0.2 - 40.9	19.5	3.1 - 38.7	2.01	0.15 - 3.87	1.89	0.12 - 3.67
Confirmed earlier versus later ACT Exposure										
Earlier (First trimester)	74	9	12.2	4.7 - 19.6	11.8	11.4 - 12.2	0.88	0.29 - 2.64	0.79	0.27 - 2.35
Later (Second or third trimester)	29	4	13.8	1.2 - 26.3	14.9	10.1 - 19.7	1.00	-	1.00	-

The unadjusted pooled prevalences are fitted values from a random-intercepts logistic regression with low birth weight as the dependent variable and site as predictor. For the adjusted pooled prevalence age of the mother, gestational age and gravidity are added on the regression. The prevalence rate-ratio are division of the fitted prevalence on the random intercept at the median.

ESP – Embryo Sensitive Period

Table 6 – Small for gestation age prevalence, per exposure level, ASAP cohort, 2015

	Counts		Pooled Prevalence (%) Unadjusted		Pooled Prevalence (%) Adjusted		Prevalence Rate-Ratio Unadjusted		Prevalence Rate Ratio Adjusted	
Characteristic	**N**	**cases**	**%**	**95% CI**	**%**	**95% CI**	**PRR**	**95% CI**	**PRR**	**95% CI**
Confirmed Exposure										
No exposure	1821	307	15.3	8.0 - 27.4	14.8	7.4 - 27.7	1.00		1.00	
Artemesinin confirmed	100	19	14.7	6.6 - 30.0	13.9	6.0 - 29.1	0.96	0.53 - 1.39	0.93	0.50 - 1.36
Quinine confirmed	26	6	20.7	7.4 - 46.0	18.2	6.1 - 43.2	1.36	0.34 - 2.37	1.22	0.26 - 2.19
Confirmed ESP Exposure										
No exposure	1821	307	15.3	8.0 - 27.4	14.8	7.4 - 27.7	1.00		1.00	
Artemesinin confirmed	71	12	13.7	5.7 - 29.4	12.3	4.9 - 27.7	0.90	0.40 - 1.39	0.83	0.35 - 1.30
Quinine confirmed	16	4	23.9	7.4 - 55.1	23.0	6.8 - 55.0	1.56	0.17 - 2.95	1.54	0.13 - 2.96
Confirmed earlier versus later ACT Exposure										
Earlier (First trimester)	71	12	10.8	2.5 - 36.0	7.3	1.4 - 30.1	0.64	0.24- 1.76	0.46	0.14 - 1.47
Later (Second or third trimester)	29	7	16.7	3.5 - 52.6	16.0	3.0 - 53.5	1.00	-	1.00	-

The unadjusted pooled prevalences are fitted values from a random-intercepts logistic regression with low birth weight as the dependent variable and site as predictor. For the adjusted pooled prevalence age of the mother, gestational age and gravidity are added on the regression. The prevalence rate-ratio are division of the fitted prevalence on the random intercept at the median.

ESP – Embryo Sensitive Period

Figures

Figure 1 - Flow chart of the recruited participants in ASAP cohort utilized in this analysis, 2015