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Dihydroartemisinin-piperaquine versus artesunate-amodiaquine for treatment of malaria infection in pregnancy in Ghana: an open-label, randomized, non-inferiority trial

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ABSTRACT

Objective: To determine whether dihydroartemisinin-piperaquine (DHA-PPQ) is non-inferior to artesunate-amodiaquine (ASAQ) for treating uncomplicated malaria infection in pregnancy.

Methods: 417 second/ third trimester pregnant women with confirmed asymptomatic *Plasmodium falciparum* parasitaemia were randomized to receive DHA-PPQ or ASAQ over 3 days. Women were followed up on days 1, 2, 3, 7, 14, 28 and 42 after treatment start and at delivery for parasitological, haematological, birth outcomes and at 6-weeks post-partum to ascertain the health status of the babies. Parasitological efficacy (PE) by days 28 and 42 were co-primary outcomes. Analysis was per-protocol (PP) and modified intention-to-treat (ITT). Non-inferiority was declared if the two-sided 95% confidence interval for PE at the endpoints excluded 5% lower efficacy for DHA-PPQ. Secondary outcomes were assessed for superiority.

Results: In PP analysis, PE was 91.6% for DHA-PPQ and 89.3% for ASAQ by day 28 and 89.0% and 86.5% respectively by day 42. DHA-PPQ was non-inferior to ASAQ with respect to uncorrected PE {adjusted difference by day 28 (DHA-PPQ-ASAQ); 3.5% (95%CI: -1.5, 8.5) and day 42: 3.9% (95%CI: -2.7, 10.4)}. ITT analysis gave similar results. PCR to distinguish recrudescence and reinfection was unsuccessful. DHA-PPQ recipients had fewer adverse events of vomiting, dizziness and general weakness compared to ASAQ. Both drugs were well-tolerated and there was no excess of adverse birth outcomes.

Conclusion: DHA-PPQ was non-inferior to ASAQ for treatment of malaria infection during pregnancy. No safety concerns were identified. Our findings contribute to growing evidence that DHA-PPQ is useful for control of malaria in pregnancy.

keywords: Dihydroartemisinin-piperaquine, artesunate-amodiaquine, malaria in pregnancy, treatment, safety, efficacy, tolerability, non-inferiority trial, Ghana

INTRODUCTION

Pregnancy-associated malaria remains a challenge in endemic countries. *Plasmodium falciparum* infections in this population, symptomatic or not, carry an important burden of maternal anaemia and death, pregnancy wastage, preterm delivery and low birth weight (LBW) with increased risk of infant deaths [1-6]. Measures to mitigate these adverse outcomes include sleeping under long-lasting insecticide nets (LLINs), intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) and prompt diagnosis and treatment using artemisinin-based combination therapies (ACTs) [7]. Screening pregnant women intermittently with malaria rapid diagnostic tests (mRDT) and treating positives using ACTs (intermittent screening and treatment, ISTp) has also been studied and showed variable outcomes compared to IPTp-SP [8, 9]. The ISTp approach was suggested to counteract reported declining SP efficacy [10] but the WHO has reiterated the use of SP for IPTp [11].

Dihydroartemisinin-piperaquine (DHA-PPQ) and artemether-lumefantrine (AL) were officially adopted in Ghana in 2010 as first-line alternatives to ASAQ for malaria case management. At the time of planning the study, no specific guidelines existed in relation to the use of DHA-PPQ in pregnancy due to few data on safety and efficacy in this population. Studies, including a systematic review, have however reported good safety and efficacy for DHA-PPQ used as treatment and prevention in children and non-pregnant adults [12-15]. In a recent systematic review including pregnant participants, IPT using DHA-PPQ was associated with 84% reduction in the incidence of malaria parasitaemia compared to placebo [16].

Early reports of DHA-PPQ treatment of PAM showed a day 63 PCR-corrected efficacy of 92.2% in Western Cambodia [17] and lower risks of perinatal death and parasitaemia at delivery in women receiving DHA-PPQ compared to women receiving quinine, chloroquine, or SP in Papua New Guinea [18]. However these trials were not randomized. A multi-country trial of four ACTs for treating malaria in pregnancy (including DHA-PPQ, ASAQ, AL and artesunate-mefloquine) in Ghana, Burkina Faso, Malawi and Zambia reported a day 63 PCR-adjusted cure rate of 99.2% for DHA-PPQ [19].

The objectives of the current study were to assess the efficacy, safety and tolerability of DHA-PPQ for treatment of asymptomatic *P.falciparum* infection in the second and third trimesters as data

in pregnancy is limited. Understanding how well DHA-PPQ clears existing infections is important when considering this drug as an alternative treatment for malaria in pregnancy and perhaps its potential for IPTp indirectly**.** The control was ASAQ which is standard in Ghana and has high efficacy with PCR-adjusted cure rates of ≥95% in pregnant women [19, 20]. The study design was, therefore, a randomized non-inferiority trial of DHA-PPQ with the co-primary outcomes being parasitological efficacy at days 28 and 42 after starting treatment. In consideration of the potential advantages of DHA-PPQ over ASAQ, including better tolerability, once daily dosing (ASAQ is given in two divided doses in Ghana) and the longer terminal half-life of piperaquine compared to amodiaquine [13, 21], a margin of 5% lower efficacy in DHA-PPQ was considered clinically relevant.

METHODS

Study design

This was an open-label, individually-randomized (1:1), non-inferiority trial with two arms.

Study area and population

The study was conducted from July 2011 to October 2012 in the Bosomtwe and Bekwai areas in the middle forest belt of Ghana, where malaria transmission is moderate to high year round with peaks in the rainy season (March-July and September-November). *P.falciparum* accounts for ≥90% of all malaria, and infection prevalence was up to 50% among children <5 in Bosomtwe district in 2012 [22]. The study population comprised pregnant women of gestational age 15-32 weeks accessing antenatal care (ANC) at St. Michael's Hospital in the Bosomtwe district and the Bekwai Government Hospital. Gestational age and foetal viability at recruitment were determined using ultrasound scan.

Sample Size

A day-28 PCR-corrected parasite clearance of 95.5% was assumed for ASAQ treatment in pregnant women based on a study in Tanzania [21]. At 90% power and a two-sided 5% significance level, 361 pregnant women per study arm would be needed to demonstrate noninferiority of DHA-PPQ to ASAQ. Allowing for a 20% loss to follow up, the sample size was estimated at 452 in each study arm [23].

Participant selection and study procedures

Detailed selection and study procedures are provided in the supplementary appendix. Women were only eligible if they had *P.falciparum* parasitaemia by both RDT (First Response®, Premier Medical Corporation, India) and microscopy. Exclusion criteria included multiple gestation, positive HIV

status, severe renal/cardiac disease or Hb<7.0g/dl. Women were randomized to receive either a co-blistered preparation of ASAQ or co-formulated DHA-PPQ over 3 days. According to standard guidelines, participants received IPTp-SP at study completion if there was at least a month left to term or if they were withdrawn from the study. All women received LLINs, daily iron and folic acid supplementation according to national policy.

Follow-ups were conducted on days 1, 2, 3, 7, 14, 28 and 42 after treatment start for assessment of adherence to treatment and occurrence of adverse and severe adverse events (AEs and SAEs) and blood sampling for haematology, microscopy and filter spot preparation. Women with recurrent infections at days 7, 14, 28 and 42 or any day between days 3 and 42 for unscheduled visits were given Quinine tablets at a dose of 10mg/Kg three times daily for seven days. Assessments were conducted at delivery and 6 weeks post-partum for perinatal outcomes including birth weight, stillbirths, congenital abnormalities and neonatal morbidity and mortality.

For maternal peripheral blood sampling, study workers collected 5ml of venous blood into EDTA tubes labelled with the sampling date, participant's identity number and follow-up day. Labour ward midwives were trained to cut 1cm x 1cm placental blocks from the maternal surface close to the insertion of the cord for preparation of placental smears. They were also trained to draw blood from the umbilical vein from the placental stump of the cord after baby had been separated from the cord. Haematological parameters were analysed using an autoanalyzer (Sysmex KX-21N, Sysmex Corp, Japan). Dried blood spots prepared using Whatmann 1MM filter papers (Whatmann, Maidstone, England) were stored in individual zip-lock bags with silica gel. Thick and thin blood films were prepared for microscopy. A laboratory technologist read all blood films and quantified parasitaemia against 200 white blood cells, assuming a WBC count of 8,000/µl of blood. A thick blood film was declared negative only after 100 high power fields had been examined. A second microscopist read 15% of all slides. Agreement with the study microscopist was 89% with a kappa statistic of 0.6 suggesting moderate agreement. Discrepancies were settled by taking the results from the second microscopist.

Parasite DNA was extracted using the Chelex method with some modifications [24, 25] and used for PCR genotyping to differentiate reinfection from recrudescence using procedures described earlier [26].

Data Management and Statistical Methods

Data were double-entered and analysed using Stata 12 (Stata Corp, College Station, Texas, USA) after consistency checks. Parasitological efficacy by day 28 and 42 was defined as absence of parasitaemia and without prior rescue medication at any point. Secondary outcomes included changes in

maternal Hb, total white blood cell, differential and platelet counts at days 14, 28 and 42 over baseline, proportions of AEs and SAEs, birth weight, proportions of adverse pregnancy outcomes (spontaneous abortions, preterm deliveries, stillbirths and neonatal mortalities) and proportions of maternal peripheral, placental and cord blood parasitaemia.

Per protocol and "modified intention-to-treat" analyses of the primary outcome were undertaken. The per protocol population comprised women who took ≥ 2 days course of assigned treatments, had ≥ 4 follow-up visits with no major protocol violation with a parasitological outcome at days 28 and 42 days. The "modified intention-to-treat" group included all randomized women who took the first dose of treatment with a parasitological outcome.

For analysis of the co-primary outcomes, treatment differences between study arms (adjusted and unadjusted) and the two-sided 95% confidence intervals around them were calculated using binomial regression with identity link. Non-inferiority was inferred when the lower limit of the confidence interval was above the pre-defined margin of -5%.

Ethics

The Committee on Human Research and Publication Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana approved the study protocol. A data safety and monitoring board (DSMB) monitored the study. The study was registered with ClinicalTrials.gov (NCT01231113).

RESULTS

A total of 3464 women in second and third trimester were screened for peripheral *P.falciparum* parasitaemia between July 2011 and October 2012 using mRDT and microscopy. Of these, 588 (17%) were mRDT positive. As shown in the trial profile (Figure 1), 171 were excluded and the remaining 417 women were recruited. Of the participants, 91.6% (382/417) were followed up on day 28 and 89.0% (371/417) on day 42. Only 56.8% (237/417) and 42.2% (176/417) were available for assessments at delivery and at 6 week post-partum respectively.

Background characteristics of participants were comparable between the two arms and are shown in Table 1. Nulliparous and primiparous women comprised over 50% (221/416). About 60% (239/403) reported owning treated nets but only 40% (137/340) slept under them the night before the survey. Close to two-thirds (252/386) of the women were anaemic (Hb < 11g/dl). The DHA-PPQ arm had more women within the lowest Hb category (14.5% vs 7.8%; p=0.04). Baseline parasite density was <500/µl in 84.4% (342/405) of the women.

Parasitological efficacy of DHA-PPQ versus ASAQ

Differentiation of recrudescent from new infections by PCR genotyping was not considered reliable as the process yielded very few positives at the defined end points. This was most likely due to poor sensitivity at low parasite densities. Consequently, only PCR-uncorrected estimates of parasitological efficacy are reported. In the per-protocol analysis, parasitological efficacy by day 28 was 91.6% (95%CI: 86.7, 95.1) in the DHA-PPQ arm and 89.3% (95%CI: 83.8, 93.5) in the ASAQ arm and 89.0% (95%CI: 83.6, 93.0) and 86.5% (95%CI: 80.6, 91.2%) by day 42 (Table 2). The lower boundaries of the 95% confidence interval around the difference excludes the pre-specified non-inferiority margin of minus 5%. Unadjusted differences (DHA-PPQ efficacy-ASAQ efficacy) were 2.3 (95%CI: -3.8, 8.3) and 2.5 (95%CI: -4.3, 9.1) by days 28 and 42 respectively. Similarly, adjusted differences were 3.5 (95%CI: -1.5, 8.5) and 3.9 (95%CI: -2.7, 10.4) respectively.

Similar results were observed in the modified intention-to-treat population except for the day 42 crude risk difference where non-inferiority could not be excluded as the lower boundary of the 2-sided 95% confidence interval overlapped the minus 5% non-inferiority margin (Table 2). None of the baseline variables; age, gestational age, gravidity, parasite density and Hb concentration was associated with parasitological outcome by days 28 and 42 (data not shown).

Secondary outcomes

Between treatment start and day 28, the ASAQ group showed an increase of 0.7g/dl (10.1 g/dl vs. 10.8g.dl; p<0.0001) in Hb while the DHA-PPQ arm had an increase of 0.6g/dl (10.0g/dl vs. 10.6g/dl; p=0.0001). Similar increases were observed between day 0 and day 42; (10.1g/dl vs. 11.2g/dl; p<0.0001) for ASAQ and (10.0g/dl vs. 10.8g/dl; p<0.001) for DHA-PPQ groups. There was no difference in mean Hb between study arms at day 28 (10.8g/dl in ASAQ arm vs. 10.6 g/dl in DHA-PPQ arm; p=0.25) (Table 1 in supplementary appendix). The ASAQ group, however, had a higher mean Hb at day 42 (11.2g/dl vs. 10.8g/dl; p=0.01). Neutrophil and lymphocyte counts showed significant variation over baseline at days 14, 28 and 42 in each arm (Tables 2 and 3 in supplementary appendix). There were however no differences between study arms regarding differential and platelet counts (Table 1 in supplementary appendix).

There were no differences in mean maternal Hb levels at delivery (Table 3). Birth weight records were obtained for less than half (162/417) of study women. Mean birth weight was 2.95Kg (95%CI: 2.86, 3.04) in the ASAQ arm and 2.94 kg (95%CI: 2.82, 3.06) in the DHA-PPQ arm. Overall, LBW was observed in 9.3% (15/162) of babies and was higher in the DHA-PPQ arm [13.2% (12/91) vs. 4.2% (3/72); p=0.05]. A significantly lower prevalence of peripheral parasitaemia was observed in the DHA-PPQ group at delivery (37.1% vs. 21.7%; p=0.01) but there were no differences in the preva-

lence of cord and placental parasitaemia.

Generally, the drugs were well tolerated. Adverse events were mild and included general weakness, vomiting, nausea, dizziness, abdominal pains and anorexia. These peaked on day 3 (Table 4) but were mostly resolved by day 7 (Table 5). Women in the ASAQ arm reported more AEs; anorexia (12.0% vs. 22.3%; p=0.007), vomiting (19.5% vs. 29.4%; p=0.02), dizziness (14.5% vs. 26.6%; p=0.003) and general weakness (38.5% vs. 62.5%; p<0.0001). Adverse events were recorded on day 14 but there were no differences between study arms (Table 4 in supplementary appendix). Adverse events reported were similar to self-reported complaints present within the month preceding enrolment (Table 5 in supplementary appendix).

No maternal mortality was recorded. There were eight cases of SAEs due to hospitalizations; 4 in each arm. The DHA-PPQ arm recorded a case each of severe anaemia (Hb 3.8g/dl), pregnancy induced hypertension (PIH), antepartum haemorrhage (placenta abruptio) and severe diarrhoea. In the ASAQ arm, there were three cases of severe diarrhoea and a suspected appendicitis. Furthermore, two cases each of neonatal deaths and stillbirths were recorded in the DHA-PPQ arm while one case of stillbirth was observed in the ASAQ arm. Three cases of polydactyly and a case of gum swelling were observed in the DHA-PPQ arm but no congenital anomaly was recorded for the ASAQ arm.

DISCUSSION

The study found that DHA-PPQ was non-inferior to ASAQ with respect to uncorrected parasitological efficacy and appeared to be better tolerated. No safety concerns were identified. These findings concur with previous studies where DHA-PPQ was found non-inferior to other ACTs such as artesunate-mefloquine and artemether-lumefantrine and reported to be comparable to ASAQ [19, 27-29].

The day 42 PCR-uncorrected and unadjusted efficacy of ASAQ was slightly less than 90% in both per protocol and modified intention-to-treat populations and is comparable to PCR-uncorrected cure rates observed in the PREGACT study which reported a day 63 PCR-uncorrected cure rate of 82.3% but a corrected cure rate over 95% [19]. Similarly, the day 28 uncorrected unadjusted efficacy for ASAQ in the PP population compares favourably with the PCR-uncorrected estimate for the same time point in Tanzanian pregnant women [20]. The day 42 PCR-uncorrected unadjusted DHA-PPQ efficacy in the PP is also comparable to the uncorrected estimates for day 63 in Ghana, Malawi and Zambia in the PREGACT study [19]. If PCR genotyping had been successful, much higher efficacy estimates may have been observed as majority of day 42 positives would likely have been new infections and the two antimalarials may have been good alternatives in terms of efficacy. The finding of DHA-PPQ non-inferiority to ASAQ should be interpreted with caution in light of some study limita-

tions discussed below.

Less than half of the estimated sample size was recruited; largely because of a lower-than-expected prevalence of parasitaemia among ANC attendants. This may be related to a nationwide distribution of LLINs at the initial stages of the study although only 40% of women reported sleeping under them the night before enrolment. Furthermore, there was a period of 3 months when no women were recruited due to protests against health finance reforms piloted in the Ashanti region at the time. Coupled with loss to follow up, there were implications for reduced study power, diminished treatment differences and a bias towards non-inferiority. However, with the sample size achieved, our study indicates that efficacy of DHA-PPQ is comparable to that of ASAQ, excluding an important difference and the point estimate was in favour of DHA-PPQ. This is consistent with other studies [17, 19] indicating the combination has high efficacy at clearing malaria infection. The longer period of post-treatment prophylaxis for DHA-PPQ compared to ASAQ (by extension, more reinfections in ASAQ) may explain why the uncorrected efficacy was slightly higher. Losses to follow-up for birth outcomes were high but those who were followed until delivery were comparable to those enrolled at baseline and there were no systematic differences in those followed until delivery between study arms (see supplementary appendix).

The longer period of post-treatment prophylaxis for DHA-PPQ compared to ASAQ (by extension, more reinfections in ASAQ) may explain why the uncorrected efficacy was slightly higher. It is possible treatment efficacy may be more similar if reinfections had been corrected for. Based on its high efficacy at clearing malaria infection and longer post-treatment prophylaxis, DHA-PPQ has potential for use as IPTp. Recent studies in East Africa showed IPTp-DHA-PPQ had lower prevalence of histologically confirmed placental malaria, parasitaemia at delivery and incidence of malaria compared to IPTp-SP [9, 30].

It is unclear why PCR-genotyping to differentiate recrudescent from new infections was unsuccessful and may have arisen from a number of causes. Silica gels in individual zip-lock bags probably should have been changed more often than was done considering the relatively long storage duration of some of the filter papers (≥10 months) at possibly high temperatures/ humidity until actual DNA extraction for analysis at the msp1, msp2 and glurp genes. These may have contributed to DNA degradation and obstructed the expected high sensitivity of the standard PCR and subsequently reduced PCR sensitivity. Nonetheless, every *P.falciparum* infection in pregnant women may have important consequences for the health of baby and mother and the PCR-uncorrected estimates reported still hold relevance.

The relatively low agreement of 89% between the microscopy readers is acknowledged as a limitation. The standard is to have each slide double-read with a third read where there are dis-

agreements. However, the impact thereof is mitigated by roughly even distribution of false positive and false negative slides (52% vs 48%) such that the effects of a misclassification bias was minimized.

The occurrence of a higher mean Hb at day 42 in the ASAQ arm compared to the DHA-PPQ arm contrasts with reports of better haematological recovery associated with DHA-PPQ [13, 22, 31]. This may be a chance finding and may be a follow-on from the lower Hb in more women at baseline in the DHA-PPQ group despite the integrity of the randomization process. Contrary to reports of decreased white blood cell counts with DHA-PPQ [32, 33], the present study did not observe this. Reduced neutrophil counts over baseline (Table 1 in supplementary appendix) may be adverse events associated with artemisinin derivatives [34] while the observed increased lymphocyte count is consistent with pregnancy itself [35]. It is possible that the lack of differences between study arms may be due to inadequate power on account of the low numbers assessed for some of the haematological indicators.

The higher prevalence of LBW in the DHA-PPQ arm is thought to be a chance finding and not attributable to DHA-PPQ. Available evidence from studies using DHA-PPQ for treatment or prevention of malaria in pregnancy [17, 19, 9, 30] do not suggest an association between DHA-PPQ use and excess of LBW. Nutritional status was not assessed but the women had similar background characteristics. The common AEs observed were comparable to those reported for both study drugs in previous studies and were fewer in the DHA-PPQ arm [12, 16, 19, 30]. No participant left the study or stopped the treatment on account of adverse events, suggesting good tolerability of both drugs especially DHA-PPQ on account of better side-effect profile. Adverse events reported at day 14 were deemed to be more likely pregnancy-related than a result of study treatment.

There was no excess of SAEs in the DHA-PPQ arm and only severe diarrhoea was thought to be plausibly linked to the study treatments, especially ASAQ [36, 37], in terms of time between exposure and occurrence. The case of placental abruption, though occurring within 3 days after start of treatment, is not likely to be attributable to DHA-PPQ as there is no known plausible mechanism by which DHA-PPQ may cause it. The occurrence of polydactyly in the DHA-PPQ arm is similar to that reported among Ghanaian pregnant women who received AQ for treatment [38]. It is deemed to be within background occurrence and not related to DHA-PPQ. A pilot study of DHA-PPQ in pregnant women [17] reported one case each of Patau's syndrome (trisomy 13) and umbilical hernia but not polydactyly. Congenital malformations were reported for all four ACTs including DHA-PPQ with a prevalence range of 0.8%-2.0% in the PREGACT Study [19].

Similarly, the other SAEs observed were not considered to be major deviations from background occurrences of pregnancy complications. In the first quarter of 2014, 3 cases of antepartum haemorrhage and 18 cases of hypertensive diseases in pregnancy were managed at St. Michael's hospital

(unpublished data).

Conclusion

The results of this study add to a growing evidence base that DHA-PPQ is effective and safe for treatment of malaria in pregnancy. The better tolerability and single daily dosage regimen for DHA-PPQ is expected to augur well for better adherence. Future research is needed regarding long term effects on developmental milestones in babies born to mothers who received DHA-PPQ treatment and to evaluate rarer adverse events including possible LBW. Furthermore, if DHA-PPQ were to be considered for IPTp, more data on effectiveness of the 3-day regimen under routine operational conditions as well as acceptability studies would be required.

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Table 1: Baseline demographic and clinical characteristics of study women

variable	ASAQ (%) (N=205)	DHA-PPQ (%) (N=212)	p-value	Total (%)
Education (n=415)			0.91	
None	24 (11.8)	20 (9.5)		44 (10.6)
Primary	29 (14.2)	32 (15.2)		61 (14.7)
[a]Junior High School	129 (63.2)	135 (64.0)		264 (63.6)
[b]Senior High School	19 (9.3)	19 (9.0)		38 (9.2)
Tertiary	3 (1.5)	5 (2.4)		8 (1.9)
Age (n=417)			0.05	
15-19	40 (19.5)	29 (13.7)		69 (16.5)
20-24	56 (27.3)	77 (36.3)		133 (31.9)
25-29	56 (27.3)	67 (31.6)		123 (29.5)
≥30	53 (25.9)	39 (18.4))		92 (22.1)
Gestational Age (n=405)			0.61	
<24 weeks	111 (56.4)	112 (53.9)		223 (55.1)
≥24 weeks	86 (43.7)	96 (46.2)		182 (44.9)
Mean gestational age	22.3(95%CI; 21.6, 22.9)	22.7(95%CI; 22.1, 23.4)	0.33	
Parity (n=416)			0.12	
Nulliparous	54 (26.3)	74 (35.1)		128 (30.8)
Primiparous	46 (22.4)	47 (22.1)		93 (22.3)
Multiparous	105 (51.2)	90 (42.7)		195 (46.9)
ITN Ownership (n=403)			0.84	
Yes	117 (58.8)	122 (59.8)		239 (59.3)
No	82 (41.2)	82 (40.2)		164 (40.7)
Slept in ITN last night (n=340)			0.19	
Yes	61 (36.8)	76 (43.7)		137 (40.3)
No	105 (63.3)	98 (56.3)		203 (59.7)
Parasite Density (n=405)			0.99	
<500/µl	169 (84.5)	173 (84.5)		342 (84.4)
≥500/µl	31 (15.5)	32 (15.5)		63 (15.6)
Geometric Mean Parasite Density	238 (95%CI;199, 285)	236 (95%CI; 193, 289)	0.28	
[c]Hb (n=386)			0.04	
7-8.9	15 (7.8)	28 (14.5)		43 (11.1)
9-10.9	115 (59.6)	94 (48.7)		209 (54.1)
≥11	63 (32.6)	71 (36.8)		134 (34.7)
Mean Haemoglobin	10.1 (95%CI; 9.9, 10.2)	10.0 (95%CI; 9.8, 10.2)	0.44	

[a] Junior High School refers to 3 years while [b]Senior High School refers to 6 years of secondary school education post primary school.
[c]Haemoglobin in g/dl.

Table 2: Parasitological efficacy by study arms in the two analyses populations

PER PROTOCOL POPULATION

	Treatment group						Crude difference	95% CI	Adjusted difference	95% CI
	ASAQ efficacy			DHA-PPQ efficacy						
	N	n	%	N	n	%				
Day 28	178	159	89.3	190	174	91.6	2.3	(-3.8, 8.3)	3.5	(-1.5, 8.5)
Day 42	178	154	86.5	190	169	89.0	2.5	(-4.3, 9.1)	3.9	(-2.7, 10.4)

MODIFIED INTENTION-TO-TREAT POPULATION

	Treatment group						Crude difference	95% CI	Adjusted difference	95% CI
	ASAQ efficacy			DHA-PPQ efficacy						
	N	n	%	N	n	%				
Day 28	184	164	89.1	199	180	90.4	1.3	(-4.8, 7.4)	3.3	(-2.3, 8.9)
Day 42	180	156	86.7	191	167	87.4	0.7	(-6.1, 7.6)	2.1	(-2.7, 8.9)

N is total number assessed at end point. n is number without malaria infection and % represents efficacy. 'Adjusted' refers to differences in parasitological efficacy controlling for age, gestational age, Hb, gravidity and parasite density at enrolment. Differences are obtained as efficacy in DHA-PPQ group minus efficacy in ASAQ group.

Table 3: Comparison of delivery and 6-week post-partum assessments between study arms

	ASAQ	DHA-PPQ	p-value
Maternal Hb at delivery {*N, mean (95%)}	74,12.0 (11.5, 12.4)	87,11.5 (11.2, 11.9)	0.10
&Peri. parasitaemia (N)	97	120	0.012
Yes	36 (37.1%)	26 (21.7%)	
No	61 (62.9%)	94 (78.3%)	
$Plac. parasitaemia(N)	90	116	0.43
Yes	19 (21.1%)	30 (25.9%)	
No	71 (78.9%)	86 (74.1%)	
Cord parasitaemia(N)	85	105	0.90
Yes	26 (30.6%)	33 (31.4%)	
No	59 (69.4%)	72 (68.6%)	
Gestation (N)	96	122	0.92
≥37 weeks n (%)	91 (94.8%)	116 (95.1%)	
<37 weeks n (%)	5 (5.2%)	6 (4.9%)	
Congenital abnormality	0	4 (3 cases of polydactyly and 1 case of gum swelling)	
Stillbirths	1	2	
Miscarriage	1	1	
Intrauterine death	1	0	
Neonatal deaths	0	2	
Neonatal Jaundice	2	2	

*N is number assessed for outcome &Peripheral parasitaemia $Placental parasitaemia

Table 4: Frequency of adverse events on day 3 after start of study treatment

Adverse events	ASAQ (N=184)	DHA-PPQ (N=200)	p-value
Anorexia *[n(%)]	41 (22.3)	24 (12.0)	**0.007**
[&]Nausea	5 (2.6)	0 (0.0)	**0.02**
Vomiting	54 (29.4)	39 (19.5)	**0.02**
Abdominal pain	53 (28.8)	48 (24.0)	0.29
Diarrhoea	21 (11.4)	26 (13.0)	0.64
Dizziness	49 (26.6)	29 (14.5)	**0.003**
Sleeplessness	31 (16.9)	21 (10.5)	0.07
Nightmares	30 (16.3)	20 (10.0)	0.07
Visual disturbance	10 (5.4)	10 (5.0)	0.85
Tinnitus	16 (8.7)	11 (5.5)	0.22
General weakness	115 (62.5)	77 (38.5)	**<0.0001**
Itching	34 (18.5)	28 (14.0)	0.23

[*]Adverse events presented as no. reporting AE and percentage. Occurrences of AEs were not mutually exclusive as individuals mentioned more than one. [&]for nausea (ASAQ=194, DHA-PPQ=209).

Table 5: Frequency of adverse events on day 7 after start of study treatment

Adverse events	ASAQ (N=182)	DHA-PPQ (N=187)	p-value
Anorexia *[n(%)]	17 (9.3)	11 (5.9)	0.21
Nausea	0	0	
Vomiting	24 (13.2)	15 (8.0)	0.11
Abdominal pain	37 (20.3)	35 (18.7)	0.70
Diarrhoea	11 (6.0)	11 (6.0)	0.96
Dizziness	25 (13.7)	15 (8.0)	0.08
Sleeplessness	36 (19.8)	21 (11.2)	**0.02**
Nightmares	20 (11.0)	8 (4.3)	**0.02**
Visual disturbance	3 (1.7)	0	0.08
Tinnitus	16 (8.8)	10 (5.4)	0.20
General weakness	67 (36.8)	48 (25.7)	**0.02**
Itching	20 (11.0)	17 (9.1)	0.54

* Adverse events presented as no. reporting AE and percentage. Occurrences of AEs were not mutually exclusive as individuals mentioned more than one.

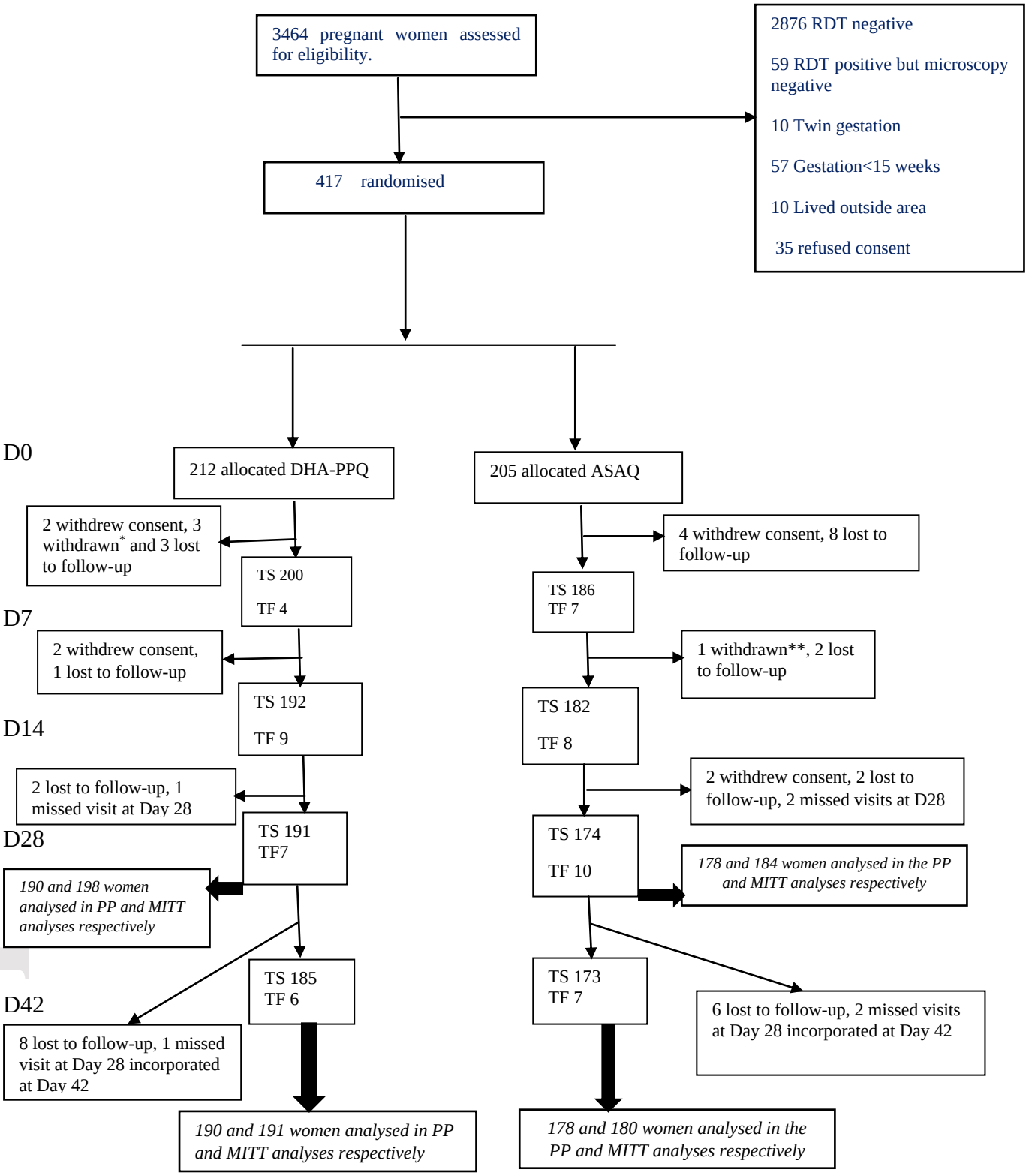

Fig 1: Trial Profile

*1 each withdrawn for persistent vomiting after a repeat initial dose of DHA-PPQ, onset of pregnancy-induced hypertension (PIH) and occurrence of antepartum haemorrhage (placental abruption). **Withdrawn for protocol violation on the part of recruiters (it was realized this was a case of twin pregnancy). TS is treatment success and TF is treatment failure. PP is per protocol and MITT is modified intention to treat. Line arrows show participant flow and block arrows show numbers analysed