KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
KUMASI, GHANA
COLLEGE OF HEALTH SCIENCES
SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF GLOBAL AND INTERNATIONAL HEALTH

EFFICACY, SAFETY AND TOLERABILITY OF DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN PREGNANCY IN GHANA.

BY

JOSEPH OSARFO (MBChB, MPH)

JUNE, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

EFFICACY, SAFETY AND TOLERABILITY OF DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN PREGNANCY IN GHANA.

BY

JOSEPH OSARFO (MBChB, MPH)

A THESIS SUBMITTED TO THE DEPARTMENT OF GLOBAL AND INTERNATIONAL HEALTH, COLLEGE OF HEALTH SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PUBLIC HEALTH

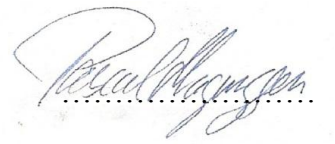
JUNE, 2016

CERTIFICATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Joseph Osarfo (20069581/ PG 4000509)	……	…….
Student name & ID	Signature	Date

Certified by:

D. Harry Tagbor	……	…….
Supervisor (s) name	Signature	Date
Prof. Pascal Magnussen	 ……	…….
Supervisor (s) name	Signature	Date

Certified by:

Dr. Sam Newton	……	…….
Head of Dept. Name	Signature	Date

DEDICATION

I dedicate this work to my parents Mr and Mrs Osarfo, my wife Gifty, my son Nhyira, my sisters Josephine and Joan and all the people who have been a blessing to my life. By allowing God to minister through them, they have been an unending source of strength for me. God bless you all.

ACKNOWLEDGEMENTS

I would like to thank first the Malaria Capacity Development Consortium (MCDC) who sponsored my studies. My heartfelt gratitude goes to my academic supervisors Dr. Harry Kwami Tagbor, Prof. Pascal Magnussen and Prof. Michael Alifrangis for their unbridled patience and extremely valuable guidance. I am very grateful to Dr. Anthony Adusei, Rose Odotei and Emmanuel Nakua of the School of Public Health, KNUST for their continuous encouragement and assistance. A big 'thank you' goes to members of the Drugs Team at the Center for Medical Parasitology, University of Copenhagen for their company during my stay there. Special mention is made of Ulla Abildrup who together with Michael Alifrangis taught and supervised my molecular laboratory analysis. I express profound gratitude to Thaddeus Egondi from Kenya who helped with the initial stages of data analysis and Shirley Bempah who helped with the qualitative aspects of my work. The health management teams of Bosomtwe district and Bekwai municipality, my study areas, deserve commendation for their cooperation and so do the management teams of St. Michael Hospital and Bekwai Government Hospital. I am especially grateful to my colleague medical officers who held the fort during my several travels and the study participants and their families who allowed me into their homes and became friends with me. I am thankful to my colleague MCDC PhD students Victor Asoala, Dr. Alberta Amu Quartey and Dr. Gifty Dufie Antwi for being pillars of strength in difficult times. I also wish to thank Abigail Asigri and Offeibea Asare for running the local MCDC office efficiently. Their contribution to our success will not be forgotten. I am also grateful to the Ato Burndham family and my good friend Mr. Osei for their unending encouragement along the journey. My family has been a lot of support and I thank them immensely.

ABSTRACT

Primarily, the study's original contribution to knowledge is in providing evidence of the safety and efficacy of dihydroartemisinin-piperaquine (DHA-PPQ) use in pregnant women from a clinical trial. DHA-PPQ has potential for use as treatment for uncomplicated malaria in pregnancy. However, there is a paucity of safety data on the use of this drug combination in pregnancy and only two studies have reported its use in pregnancy so far. Additionally, the prevalence of parasite mutations underscoring reported reduced susceptibility to ACTs and SP was assessed to help rationalize drug policy. The diagnostic performance of First Response® malaria rapid diagnostic test (RDT) and perceptions associated with clinical trial participation were assessed in Ghanaian pregnant women to help fill knowledge gaps in these areas.

Second and third trimester pregnant women with asymptomatic parasitaemia were randomized to receive DHA-PPQ and artesunate-amodiaquine (ASAQ) in a non-inferiority trial and followed up on days 1, 2, 3, 7, 14, 28 and 42 after start of study treatment, at delivery and 6 weeks post-partum for adverse events, haematological and parasitological outcomes and neonatal morbidity and mortality data. Mutations at the *Pfcrt*, *Pfmdr1*, *Pfdhfr* and *Pfdhps* genes were investigated using polymerase chain reaction and antigen-antibody reaction-based methods. Results of RDTs at screening were compared to peripheral blood film microscopy used as reference standard. Pregnant women's perceptions of participating in clinical trials were obtained from in-depth interviews with women who participated in the trials and those who did not.

Overall parasitological efficacy for DHA-PPQ was 91.6% (95%CI: 86.7, 95.1) by day 28 and 89.0% (95%CI: 83.6. 93.0) by day 42 in the per protocol population. Efficacy estimates in both arms were comparable.

DHA-PPQ was non-inferior to ASAQ with respect to parasite clearance in both analyses populations. There were no harms associated with the use of DHA-PPQ in the second and third trimesters and it fewer adverse events than ASAQ; 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). Tolerability was high as no participant left the study on account of adverse events experienced. There were no overall changes in white blood cell and differential counts. Close to 90% of parasite isolates from the infected pregnant women were wild type at the *Pfcrt* gene with a similar prevalence of *Pfmdr1* N86 alleles. The *Pfdhfr/Pfdhps* quintuple mutation containing the 540E allele, known to underlie marked SP resistance, was not observed. Participation in clinical trials appeared to be motivated mainly by anticipated health benefits and trust in the research team especially if they are also health workers. Study women were comfortable with blood sampling in their homes but preferred hospital-based sampling better as it supposedly assures blood samples will be used for desired purposes. They conceptualized treatment-emergent adverse events as independent entities that should be separated from drugs.

Dihydroartemisinin-piperaquine is deemed safe and as effective as ASAQ when used to treat uncomplicated malaria during pregnancy. The study results are expected to contribute to evidence that will guide a policy decision on the use of DHA-PPQ use in pregnant women. The predominance of *Pfcrt* K76 suggests the possibility of using chloroquine again in pregnancy but this will have to be in combination with another antimalarial. The predominance of *Pfcrt* K76 and *Pfmdr1* N86 alleles appear to

suggest early declining susceptibility to artemether-lumefantrine, an artemisinin-based combination treatment currently used in pregnancy.

Strengthened targeting of ACT treatment through more dedicated malaria infection testing using tools such as RDTs and monitoring are required. Researchers planning similar studies in pregnant women in the study area need to emphasize transportation to health facilities for blood sampling and potential health benefits.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACT	Artemisinin-combination therapy
AE	Adverse Event
AIDS	Aquired Immune Deficiency Syndrome
AL	Artemether-Lumefantrine
ANC	Antenatal Clinic
ASAQ	Artesunate-Amodiaquine
CRF	Case Report Forms
CQ	Chloroquine
DHA-PPQ	Dihydroartemisinin-piperaquine
EDD	Estimated Delivery Date
EDTA	Ethylenediaminetetraacetic acid
EIR	Entomological Inoculation Rate
GDP	Gross Domestic Product
HIV	Human Immunodeficiency Virus
HPLC	High Pressure Liquid Chromatography
IPTi	Intermittent preventive treatment of malaria in infants
IPTp	Intermittent preventive treatment of malaria in pregnancy
IRS	Indoor Residual Spraying
IST	Intermittent Screening and Treatment of malaria in pregnancy
ITN	Insecticide-treated bednet
LAMP	Loop-mediated isothermal amplification
LBW	Low birth weight
LLIN	Long lasting insecticide-treated bednet
NHIS	National Health Insurance Scheme

NMCP	Nationla Malaria Control Programme
OD	Optical Density
PAM	Pregnancy-associated malaria
PCR	Polymerase Chain Reaction
Pf	*Plasmodium falciparum*
RDT	Rapid Diagnostic Test
RFLP	Restriction Fragment Length Polymorphism
SAE	Severe Adverse Event
SP	Sulphadoxine-Pyrimethamine
SNPs	Single nucleotide polymorphisms
SSOP-ELISA	Sequence Specific Oligonucleotide Probe-Enzyme-Linked Immunosorbent Assay
TMAC	Tetra-methyl ammonium chloride solution
USP	United States Pharmacopoiea
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Pregnant women and children under five years constitute the most vulnerable populations affected by malaria (Snow *et al.,* 2005). Malaria in pregnancy remains a public health challenge in endemic countries and contributes to 2500-10 000 maternal deaths and 100 000-200 000 infant deaths globally every year (Steketee *et al.,* 2001; Guyatt and Snow, 2004; RBM, 2006). Other known adverse outcomes include maternal and newborn anaemia, foetal growth restrictions, low birth weight (LBW) and preterm deliveries, abortions and stillbirths (Shulman *et al.,* 2001; Achidi *et al.,* 2005; Falade *et al.,* 2010; De Beaudrap *et al.,* 2013). These outcomes are preceded by sequestration of *Plasmodium falciparum* in infected red blood cells in the placenta with subsequent inflammatory changes (Menendez *et al.,* 2000; Brabin *et al.,* 2004; Rogerson *et al.,* 2003; Rogerson and Boeuf, 2007).

Close to 55 million pregnancies every year are at risk of pregnancy-associated malaria (PAM) in sub-Saharan Africa where *P.falciparum* is dominant (Snow *et al.,* 2005; Dellicour *et al.,* 2010). Recent modeling studies however report about 12 million pregnant women would actually get malaria infections in the absence of PAM-specific interventions (Walker *et al.,* 2014). Other studies have reported that, at least, a quarter of pregnant women in malaria-endemic regions including the WHO African region have *P.falciparum* infection (Steketee *et al.,* 2001; Desai *et al.,* 2007; Schantz-Dunn and Nour, 2009).

The risk and clinical outcomes of PAM vary by transmission setting. Primigravidae, younger-aged pregnant women and those with human immunodeficiency virus (HIV)

infection tend to have higher risks of infection and more pronounced disease burdens in high and stable transmission areas than multigravidae (ter Kuile *et al.,* 2004; Rogerson *et al.,* 2007; Schantz-Dunn and Nour, 2009). Acquired immunity during latter pregnancies is able to limit the extent of placental sequestration by specific variants of *Plasmodium falciparum* and thus the occurrence of outcomes previously mentioned.

A quarter of cases of severe maternal anaemia (defined as Hb <8.0g/dl), up to 20% of LBW deliveries, 70% of intrauterine growth restrictions and 36% of preterm deliveries are thought to be attributable to malaria in stable transmission areas (Steketee *et al.,* 2001; Desai *et al.,* 2007). Newborns with LBW are more vulnerable to life-threatening conditions and are at increased risk of neonatal/infant mortality. Malaria related LBW has been reported to be associated with a 37.5% fatality rate and accounts for ≥13% of under-five mortality among African children (Murphy and Breman, 2001; Desai *et al.,* 2007). With each successive pregnancy, women acquire pregnancy-specific malaria immunity which lowers their risk of anaemia and LBW babies. *P.falciparum* infection in pregnancy in stable transmission settings is associated with low-grade, often sub-patent parasitaemia and reported to be asymptomatic but a study in Ghana suggested otherwise (Nosten *et al.,* 2004; Tagbor *et al.,* 2008a).

In areas of low and unstable transmission on the other hand, there is little or no acquired immunity. *P.falciparum* infections in pregnancy are typically symptomatic and pregnant women are more susceptible to severe malaria than non-pregnant adults (Shulman *et al.,* 2001; Desai *et al.,* 2007). Sequelae include respiratory distress, hypoglycaemia, coma and maternal/ fetal deaths or wastage (Nosten *et al.,* 1991; Nosten *et al.,* 2004). The parity-dependent protection in multigravidae is less obvious

in low transmission settings and in HIV- positive pregnant women (Nosten *et al.,* 1991; Mount *et al.,* 2004; Espinoza *et al.,* 2005).

Regarding the presence of symptoms or otherwise, asymptomatic parasitaemia is just as important as symptomatic parasitaemia and presents harm for pregnant women and the foetus (Nosten *et al.,* 2007; McGready *et al.,* 2012). It may be argued that asymptomatic malaria infections in pregnancy are even more problematic as the absence of symptoms is likely to divert attention from malaria and eventually avert much-needed treatment.

Studies addressing economic costs of PAM in particular are limited relative to those looking at malaria in general. However, the economic cost of PAM will incorporate various direct and indirect costs to the individual pregnant woman, her household and the health system as a whole (Worrall *et al.,* 2007). These include costs of drugs, service, transportation, preventive interventions, productive time lost, prolonged hospitalization of LBW babies and potential neurological sequelae in the long term.

Various studies in Ghana have shown a steady decline in the prevalence of asymptomatic *P.falciparum* parasitaemia in pregnant women over the last decade or more with reports ranging from 35% in 2000 to 10.1% in 2013 and parasite densities mostly <1000/ul (Glover-Amengo *et al.,* 2005; Tagbor *et al.,* 2006; Bam, 2009; Ofori *et al.,* 2009, Tagbor *et al.,* 2010; Orish *et al.,* 2012; Tay *et al.,* 2013). Similar to previous reports (Rogerson *et al.,* 2007; Schantz-Dunn and Nour, 2009), primigravidae and secundigravidae in Ghana are prone to higher prevalences of infection and LBW compared to multigravidae (Ofori *et al.,* 2009; Tagbor *et al.,* 2010).

There is scarcity of in-country data on the contribution of PAM to the burden of LBW, maternal anaemia and miscarriages. There is also no data on the economic costs of PAM for the country but malaria generally costs Ghana about 1%- 2% of its Gross Domestic Product (GDP) (UNICEF, 2007). The national health insurance scheme (NHIS) currently pays reimbursements of up to 35 Ghana cedis (about US$9.20 at current exchange rates for March 2015) for cases of simple malaria and about 150 Ghana cedis (about US$40) for complicated malaria (NHIS, 2013).

Along with declining prevalence of asymptomatic parasitaemia, maternal deaths attributable to PAM have also decreased from 9.4% (proportion of all maternal deaths) in 2004 to current reported levels of 3.4% (GHS, 2005; Biostatistics Unit, Komfo Anokye Teaching Hospital, 2013; MOH, 2014). The apparent decrease in the burden of PAM in Ghana reflects the declining burden of global malaria morbidity and mortality (O'Meara *et al.,* 2010; WHO, 2012a). Increasing international funding has increased availability and coverage of effective control tools like insecticide-treated nets (ITNs), artemisinin-combination treatments (ACTs), intermittent preventive treatment (IPT) in pregnancy, indoor residual spraying (IRS) and rapid diagnostic tests (RDTs).

Rapid diagnosis and effective case management of uncomplicated PAM with ACTs is a major pillar in malaria control. The ACTs are now the best treatment options for uncomplicated multi-drug resistant *P. falciparum* malaria (White, 2006; WHO, 2006; Nosten and White, 2007) and are deployed for such use in the second and third trimesters of pregnancy based on confidence in their safety during this period of pregnancy (WHO, 2007; WHO 2010a).

The World Health Organization does not recommend ACT use in the first trimester because animal model studies suggest some level of embryotoxicity (Finaurini *et al*, 2010). Rather, quinine in combination with clindamycin is recommended for use in the first trimester

Following policy change from chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) to the ACTs as first line treatment, Ghana initially adopted artesunate-amodiaquine (ASAQ) for treatment of uncomplicated malaria including uncomplicated PAM in the second and third trimesters. The choice of ASAQ was based on a number of factors including reported good efficacy and opportunities for local manufacturing (Koram *et al.,* 2005). National treatment guidelines recommended the use of quinine specifically in the first trimester and also as an alternative antimalarial during the rest of pregnancy. However, implementation of the policy faced challenges of ineffective communication strategies on the part of public health sector managers that failed to deal adequately with widespread complaints of adverse events and safety concerns from the population ((MOH, 2009). The absence of an alternative treatment at this period led to a loss of public confidence in ASAQ.

Subsequently, two other ACTs; artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DHA-PPQ) were included officially as alternative treatments for uncomplicated malaria (MOH, 2008a). To set the appropriate context, DHA-PPQ was recommended by WHO for use in 2010 while AL was already available in the country but could only be accessed from private drug outlets at high costs (up to US$12.00) such that the majority of people could not afford it. While ASAQ and AL are used in pregnancy, DHA-PPQ is not yet indicated for such use due to paucity of safety data in pregnancy.

Targeting ACT treatment by means of parasitological confirmation of parasitaemia is recommended and will help reduce pregnant women's exposure to antimalarials whose safety profiles in pregnancy are uncertain (WHO, 2012c; Fried, 2012). The recommendation has the additional benefit of reducing drug pressure and delaying development of resistant parasite strains. Laboratory confirmation of malaria is routinely done using peripheral blood film microscopy and rapid diagnostic tests (RDT) where the former is not readily available for various reasons. The diagnostic accuracy of malaria RDTs is influenced by parasite densities and transmission settings (Moody, 2012; Ayeh-Kumi *et al.,* 2011).

Resistance to antimalarial drugs used is the single most important threat to malaria control and there have been reports of reduced parasite susceptibility to the artemisinins/ artemisinin-combination treatments in use now (Mukhtar *et al.,* 2007; Noedl *et al.,* 2008; Dondorp *et al.,* 2009; Mutabingwa *et al.,* 2005). In the context of intermittent preventive treatment of malaria in pregnancy, resistance to SP has been reported but the WHO has recently reaffirmed its continuous use (Mbaye *et al.,* 2006; WHO, 2013). In light of these observations, it is important to monitor the efficacy and continuous usefulness of these control tools and studies of prevalence of parasite genetic mutations in the population provide a cheap means to carry out this activity.

1.2 Problem Statement

Dihydroartemisinin-piperaquine has potential for use as treatment for uncomplicated falciparum malaria in pregnancy in the second and third trimesters in endemic countries in addition to ASAQ and AL. It has a good safety and efficacy profile in children and non-pregnant adults (Denis *et al.,* 2002; Hung *et al.,* 2004; Karema *et al.,* 2006; Valecha *et al.,* 2010). However, there is a paucity of safety data on the use of this drug combination in pregnancy and only two published studies have reported its

use in pregnancy so far. One was an uncontrolled pilot study of 50 women (Rijken *et al.,* 2008) in which DHA-PPQ was used to treat recurrent parasitaemia and reported to be safe and efficacious with a polymerase chain reaction (PCR)-adjusted efficacy of 92.2% by day 63. The other was a cross-sectional study of DHA-PPQ as treatment for multi-drug resistant falciparum and vivax malaria (Poespoprodjo *et al.,* 2014) which concluded DHA-PPQ was as safe as other antimalarials compared in the study. In addition to the limitation of data from only two studies, caution is to be applied in the interpretation of these results because of challenges relating to lack of controls, small sample size and the absence of randomization with its attendant biases.

In Ghana and other developing countries where self-medication is commonly practiced (Abuaku *et al.,* 2004; Buabeng *et al.,* 2007; Asante *et al.,* 2010; Hill *et al.,* 2014), it is possible pregnant women may become inadvertently exposed to DHA-PPQ during the early weeks of gestation when they have not realized that they are pregnant. Often times, the local drug shops from which pregnant women obtain medicines for self-medication are manned by attendants who are not trained to guide drug use in pregnancy.

The potential for inadvertent pregnancy exposure to DHA-PPQ is further compounded by the absence of adequately-functioning pharmacovigilance platforms in most developing countries including Ghana (Kennedy *et al.,* 2004; Pirmohamed *et al.,* 2007; Olsson *et al.,* 2010; Kuermmele *et al.,* 2011) such that there are no structures to monitor pregnant women who enter into such situations.

There is thus a need for more rigorous investigation of the safety and efficacy of DHA-PPQ for treatment of uncomplicated falciparum PAM in well-designed and

conducted trial that will increase confidence in the reports of DHA-PPQ safety and efficacy in pregnancy.

Primarily, the study sought to answer the question of whether DHA-PPQ was as safe and efficacious as ASAQ when used to treat uncomplicated malaria in 2nd / 3rd trimester pregnancy in a non-inferiority trial. Parasitological efficacies by days 28 and 42, haematological and biochemistry parameters as well as adverse events were to be assessed. A non-inferiority study design was adopted because a new drug was compared to a standard treatment that is deemed effective. The study hypothesis was that DHA-PPQ is not inferior to ASAQ with respect to parasitological efficacy within a 5% non-inferiority margin. Artesunate-amodiaquine was selected as the active control for this study because it is the firstline treatment for uncomplicated malaria in Ghana and also because it is commonly prescribed for malaria in pregnancy in the study area. The study was conducted in second and third trimester pregnant women because there are still apprehensions about the use of artemisinins in the first trimester of pregnancy despite limited data that suggest otherwise (McGready *et al.,* 2012; Tagbor *et al.*, 2014).

Within the context of antimalarial treatment, the study lends to answering some pertinent secondary questions regarding the diagnostic accuracy of First Response® malaria rapid diagnostic test in pregnant women, prevalence of antimalarial resistance markers *Pfcr*t, *Pfmdr1*, *Pfdhfr*, *Pfdhps* in pregnant women and perceptions of drug trial participation among Ghanaian pregnant women.

The First Response® RDT, a histidine-rich protein 2-based (HRP-2) rapid diagnostic test, was the first type of RDT brought in by the Ghanaian ministry of health / national malaria control programme and is widely used. Studies have reported

sensitivity of 15.3%-100% and specificity up to 90% for HRP-2 RDTs in pregnant women (Maholtra *et al.,* 2005; Kattenberg *et al.,* 2011; Minja *et al.,* 2012; Matangila *et al.,* 2014). Very high sensitivity and specificity have been reported in children as well (Baiden *et al*., 2012; Osei-kwakye *et al*., 2013) but there are no reports of their diagnostic performance in Ghanaian pregnant women to justify use in this population. Such evaluation is important in light of relatively lower parasite densities found in pregnant women compared to children in areas of perennial and high malaria transmission.

Similarly, current prevalence data of parasite genetic mutations underlying antimalarial resistance or reduced parasite susceptibility to currently used antimalarials is lacking in pregnant women. Extrapolations to pregnant women have been made based on such data from other populations especially children. However, prevalence of molecular markers of resistance may differ in pregnant women for reasons of better immunity and reduced drug pressure compared to children.

Pregnant women's participation in biomedical research is pertinent to generating relevant data directly from them instead of having to make extrapolations from other populations groups. Their perception of clinical trial participation was assessed to help fill knowledge gaps regarding this issue in African pregnant women as most available literature relates to North American and European pregnant women. This aspect of the study was also included to help increase the depth of work to be done at a time when there were challenges with recruiting an adequate sample size.

1.3 Study Rationale

Primarily, the study is expected to generate data directly from pregnant women on the parasitological efficacy and safety of DHA-PPQ treatment of uncomplicated PAM and contribute to knowledge regarding the safety of ACT use in pregnancy. Dihydroartemisinin-piperaquine has potential for use in treating uncomplicated PAM with a number of advantages over ASAQ. Dihydroartemisinin-piperaquine has a more convenient once-daily dosing compared to the twice-daily dosing regimen for ASAQ. It also has the advantage of a longer post-treatment prophylaxis and time for haematological recovery due to the longer half-life of piperaquine. The data generated is expected to contribute significantly to evidence that will guide a policy decision on the use of DHA-PPQ use in pregnancy.

Secondly, the study provides prevalence data of molecular markers known or associated with reduced parasite susceptibility to ACTs and SP in pregnant women in a perennial transmission area. This will enable early detection of changing antimalarial drug efficacy and serve as baseline for further studies in this regard. Additionally, the study provides the first assessment of the sensitivity, specificity and predictive values of the First Response® RDT specifically in pregnant women and will help justify its continuous use in this population or otherwise.

Lastly, the study also provides insights into pregnant women's acceptability of DHA-PPQ and ASAQ, perceptions of clinical trials and their impact on participation therein. This information will help fill knowledge gaps in understanding the mindset of pregnant women regarding participation and/or willingness to participate in drug trials. It is expected this will in turn enhance their participation and especially retention once recruited.

1.4 Study Objectives

1.4.1 General objective

The main aim of the study was to assess the safety, efficacy and tolerability of DHA-PPQ compared to ASAQ for treatment of uncomplicated *P.falciparum* malaria in second and third trimester pregnancy.

1.4.2 Specific objectives

1.4.2.1 Primary Specific Objective

1. To assess the efficacy of DHA-PPQ in the treatment of asymptomatic *P.falciparum* infection in pregnancy.

1.4.2.2 Secondary Specific Objectives

2. To assess the safety of DHA-PPQ in pregnancy compared to ASAQ.
3. To assess the prevalence of single nucleotide polymorphisms in the *Pfcrt*, *Pfmdr1*, *Pfhdfr* and *Pfdhps* genes in *P.falciparum* isolates in Ghanaian pregnant women.
4. To assess diagnostic accuracy of the First Response® HRP-2 malaria rapid diagnostic test compared to light microscopy.
5. To explore experiences accrued during participation in a trial and the social constructs thereof among pregnant women in Ghana.
6. To identify factors informing willingness to participate in drug trials among pregnant women in Ghana.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter entails a review of previous research and current developments relating to the objectives of the study and further clarifies gaps in knowledge that are to be addressed in the present study. It presents background information to the adverse outcomes of PAM and the relevant context for understanding the perceptions of pregnant women regarding participation in clinical trials.

2.2 Epidemiology and Pathogenesis of pregnancy-associated malaria

Dellicour *et al* (2010) estimate that about 54.7 million pregnant women, 30 million of which were in Africa, were potentially at risk of PAM in areas of stable *P.falciparum* transmission in 2007. More recent modelled estimates suggest 12 million pregnant women would have been exposed to PAM and over 90% would have developed placental malaria in the absence of specific interventions to control PAM (Walker *et al.*, 2014).

Pregnancy-associated malaria outcomes and risks depend on transmission settings, parity and the extent of acquired immunity at the time of pregnancy. Primigravidae, younger-aged and HIV-infected pregnant women tend to have higher risks of infection compared to multigravidae in high and stable transmission areas (ter Kuile *et al.*, 2004; Schantz-Dunn and Nour, 2009). The apparent parity-dependent protection is less in low transmission settings and in those with HIV comorbidity (Espinoza *et al.*, 2005)

Girls and teenage women living in intense transmission areas acquire IgG mediated *P.falciparum* sub-species specific immunity to malaria as they grow up. The antibodies are directed against variant specific antigens expressed on the surfaces of infected red blood cells (Hviid, 2005). The non-sterilizing characteristic of this immunity arises as parasite strains expressing novel surface antigens will not be combated by existing antibodies and thus be able to establish an infection. During the first pregnancy, this immunity is compromised or down-regulated and the young women revert to a state of increased susceptibility to *P. falciparum* infection (Brabin, 1983; Rogerson and Boeuf 2007). Down-regulation of immunity was first believed to be part of a general maternal immuno-modulation mechanism that functions to protect the foetus and placenta from immunological rejection (Smith, 1996) but this explanation failed to account for the observed greater susceptibility in primigravidae compared to multigravidae.

It has been shown that the greater susceptibility in primigravidae is related to the fact that the placenta offers an "immune-independent" site where a sub-population of *P.falciparum*-infected red blood cells expressing distinct surface antigen/adhesion molecules called VAR2CSA bind to the intervillous spaces of the placental via chondroitin sulphate A (Fried and Duffy., 1996; Salanti *et al.,* 2003; Rowe and Kyes, 2004; Viebig *et al.,* 2005). Sequestration of infected erythrocytes in the placenta is an adaptive mechanism to protect the parasites from being cleared in the spleen.

The placenta is described as an "immune-independent" site because in first time pregnancies there are initially no antibodies to the VAR2CSA antigens. Over subsequent pregnancies, immunity is developed to this sub-population of parasites through acquisition of anti-VAR2CSA antibodies which inhibit adhesion of infected red blood cells to placental chondroitin sulphate A (Fried and Duffy, 1996). Research

efforts are currently directed at identifying the conserved regions of the large VAR2CSA antigen to serve as an immunogen in the quest for a vaccine against PAM (Fried *et al.,* 2013).

Placental sequestration underlines the pathogenesis of PAM. Malaria parasites in the intervillous spaces induce chemoattractant secretions from the syncytiotrophoblast which then attract maternal mononuclear cells (Rogerson and Boeuf, 2007; Lucchi *et al.,* 2008). Both mononuclear cells and syncytiotrophoblast release chemokines which serve to attract additional monocytes. Accumulated monocytes, macrophages, neutrophils and lymphocytes distort the balance of type-1 and type-2 T-helper cells needed for optimal development of the foetus. There is a shift towards type-1 T-helper cell dominance leading to production of pro-inflammatory cytokines including interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), and interleukins of the variety IL-1, 8 and 10 (Suguitan *et al.,* 2003; Boyou-Akotet *et al.,* 2004).

The inflammatory processes cause thickening of the syncytiotrophoblast basement membrane, increased syncytial knotting and necrosis (Galbraith *et al.,* 1980; Walter *et al.,* 1982; Yamada *et al.,* 1989; Ismail *et al.,* 2000) and compromise the placental function of nutrient transfer to the foetus. These processes underlie the adverse outcomes of PAM previously described (Menendez *et al,* 2000; Schulman *et al.,* 2001; Rogerson *et al.,* 2003; Tako *et al.,* 2005). TNF-α is reported to suppress erythropoiesis and induce oxidative stress on red blood cell membranes (Suguitan *et al.,* 2003) and may explain the adverse outcome of maternal anaemia. Both IFN-γ and TNF-α mediate spontaneous abortions by stimulating uterine contractions (Suguitan *et al.,* 2003; Okoko *et al.,* 2003)

Placental insufficiency has also been suggested to occur through a potential decrease in the functional mass of the placenta consequent to impaired placental vascular development following placental malaria-associated dysregulation of angiopoietins and vascular endothelial growth factor (VEGF) (Muehlenbachs *et al.,* 2006; Silver *et al.,* 2010). This dysregulation may eventually lead to a reduction in placental transport and secretory functions, foetal growth restriction and finally LBW (Umbers *et al.,* 2011).

Placental insufficiency could also be mediated through a compromised integrity of the syncytiotrophoblast that arises as a result of inflammation-mediated processes and make it more permeable to unwanted substances from mother to foetus (Scifres and Nelson, 2009). The consequence of placental malaria infection is most inimical to fetal growth in the third trimester as nutrient demands are highest during this period but infection in early pregnancy could also hinder trophoblastic invasion and impair placental vascular development leading to placental insufficiency.

Though the adverse outcomes of PAM are essentially attributed to *P.falciparum* infection, there is evidence to show *P.vivax* also contributes to LBW, preterm delivery and maternal anaemia (Nosten *et al.,* 1991; Allen *et al.,* 1998; Nosten *et al.,* 1999; Singh *et al.,* 1999; Poespoprodjo *et al.,* 2008; Rijken *et al.,* 2012; Chotivanich *et al.,* 2012). However, there may be some differences in the severity and epidemiology of *P.vivax* PAM outcomes as the extent of parasite accumulation in the placenta is limited relative to *P.falciparum* (Poespoprodjo *et al.,* 2008). Furthermore, multigravidae rather than primigravid and secundigravid women bear the brunt of *P.vivax* PAM possibly because the development of antibodies with successive pregnancies noted for *P.falciparum* does not play any role in *P.vivax* infections in pregnancy (Poespoprodjo *et al.,* 2008; ter Kuile and Rogerson, 2008).

From the foregoing, it is important to devote resources to avoid or reduce the extent of placental infections through the recommended control tools of IPTp-SP, treatment with ACTs and ITN use.

2.3 Diagnosis of pregnancy-associated malaria with rapid diagnostic tests

Parasitological confirmation of malaria prior to prescribing antimalarials is now recommended (WHO, 2012c) following the introduction of ACTs which are more costly than previously-used CQ and SP. It is important treatment of malaria is targeted to those really in need of it to reduce drug pressure and avert development of resistant parasite strains. Adherence to the recommendation will help to reduce unnecessary exposure of pregnant women to new antimalarials whose safety profiles in pregnancy are uncertain (Fried, 2012) and also allows for better comparison of malaria-related data from various endemic countries.

Laboratory diagnosis of malaria has been done routinely with blood film microscopy that can be very sensitive with detection levels as low as 5-10 parasites/µl in expert hands (Moody, 2002). However, factors such as high procurement and maintenance costs, lack of expertise and shortage of required reagents have challenged availability of microscopy in resource-poor settings in malaria endemic areas. Other methods have included PCR and loop-mediated isothermal amplification (LAMP) but these are expensive, restricted to the research arena mostly or are yet to be adapted for routine or field use (Moody, 2002; Abdul-Ghani *et al.*, 2012).

Rapid diagnostic tests (RDTs) offer a relatively cheaper alternative that does not require extensive training or equipment for diagnosing PAM at point of care in resource-poor areas. Rapid diagnostic tests diagnose malaria by detecting parasite

antigens (histidine-rich protein2 (HRP-2), lactate dehydrogenase (LDH) and aldolase) in blood by antibody-antigen reactions.

It utilizes capture and detection loci representing test and control lines respectively on a nitrocellulose membrane. Capture antibodies bind parasite antigens in a blood sample in an immobile phase while detection antibodies are conjugated to an indicator in a mobile phase. The antibody-indicator complex moves on to further bind the parasite antigen previously captured by immobile phase antibodies and produces a visible line if the antigen in question is present (Murray *et al.,* 2008).

The threshold of detection for most brands of RDTs is 200 parasites/ul and the recommended sensitivity (ability to correctly detect those who have malaria) and specificity (ability to correctly identify those who do not have malaria) are 95% and 90% respectively for all malaria parasite species (WHO, 2010b; WHO/FIND/CDC, 2013). Many of the RDTs target HRP-2 which is expressed solely by P. *falciparum* in all its blood stages including gametocytes (Shiff *et al.,* 1993; Hayward *et al.,* 2000). Rapid diagnostic tests based on HRP-2 are reported to be more sensitive and heat stable than RDTs based on the other parasite antigens but persistence of HRP-2 in the blood stream for up to 35 days after an infection is cleared presents difficulty in interpreting test results, especially in areas of intense transmission (Moody, 2002; Karbwang *et al.,* 1996; Humar *et al.,* 1997; Kattenberg *et al.,* 2012).

Against PCR as a reference standard, HRP-2 tests in pregnant women showed sensitivity of 15.3%-100% and specificity up to 90% while microscopy showed sensitivity ranging 60%-70% and specificity >90% (Maholtra *et al.,* 2005; Minja *et al.,* 2012; Matangila *et al.,* 2014).

A systematic review of RDT performance in pregnant and parturient women (Kattenberg *et al.,* 2011) reported HRP-2 RDT sensitivity compared to peripheral and placental smears using placental histology as a reference standard. Histidine-rich protein 2 based tests showed overall sensitivity of 94% and specificity of 81% while peripheral microscopy showed sensitivity ranging from 21% to 100% and placental blood smear showed overall sensitivity of only 54% and specificity of 97%. Compared to peripheral film microscopy, HRP-2 RDTs showed high sensitivity of over 90% compared to LDH tests that showed a wide ranged sensitivity of 15-96% sensitivity

The diagnostic performance of HRP-2 RDTs does not appear to depend to a significant extent on the method used as a gold standard from the performance indicators presented above but evaluating RDT performance in pregnant women using peripheral blood microscopy as the gold standard has been questioned (Kattenberg *et al.,* 2011; Conroy *et al.,* 2012). This is because of placental sequestration leading to absent or undetectable parasite levels in peripheral blood that will be missed on microscopy. Placental histology and PCR have been suggested as more appropriate standards.

Sensitivity of HRP-2 RDTs varies in different transmission settings with varying parasite densities (Moody, 2002). Sequence variation in HRP2 also explains variations in performance of RDTs based on histidine-rich protein 2 (Lee *et al.,* 2006; Wurtz *et al.,* 2013). Furthermore, some parasite strains may have deletions of the gene expressing HRP-2 and will thus provide false negative test results with HRP-2 based RDTs (Houze *et al.,* 2011; Maltha *et al.,* 2012; Koita *et al.,* 2012; Kumar *et al.,* 2013; Wurtz *et al.,* 2013). Differential expression levels of the gene coding for HRP2 in different parasite isolates is also suspected to underlie variability in test performance

(Baker *et al.*, 2011). Other factors that affect RDT performance include assay degradation by sub-optimal storage as a result of humid conditions as well as sub-optimal technique of performing and interpreting RDT results (McMorrow *et al.*, 2008).

A review of literature showed two studies that reported on the diagnostic performance of LDH-based RDTS in pregnant women in Ghana but none on the performance of HRP2 RDTs in this population. Tagbor *et al* (2008b) reported a high sensitivity and specificity; 96.4% and 85.5% for an LDH-based test compared to peripheral microscopy in pregnant women in a perennial and high transmission zone of Ghana. Another study also using an LDH-based test in Northern Ghana, characterized by seasonal transmission, reported a rather low sensitivity of 50.5% and specificity of 87.7% (Ayeh-Kumi *et al.*, 2011) and concluded the RDT in question may not be a useful replacement for microscopy. The low sensitivity was presumably due to the low parasite density observed in the study area; a finding also made by Tagbor *et al* (2008b) when they reported sensitivity of 57% in a sub-analysis of parasite density <50/µl.

One study was found that reported the diagnostic performance of the First Response® HRP2 RDT in a symptomatic population that included some pregnant women (Acheampong *et al.*, 2011). However, the study did not report an analysis of diagnostic performance indicators for the sub-population of pregnant women. Rapid diagnostic tests based on HRP2, either alone or in combination with other parasite antigens, are used in Ghana but there has not been an evaluation of its sensitivity and specificity in pregnancy.

2.4 Control of pregnancy-associated malaria

The WHO recommends a three-pronged control strategy for PAM (WHO, 2012a);

i) intermittent preventive treatment using sulfadoxine-pyrimethamine (IPTp-SP) in high transmission zones

ii) use of insecticide-treated bednets (ITN)

iii) prompt diagnosis and treatment with efficacious antimalaria drugs

2.4.1 Intermittent Preventive Treatment of Malaria in Pregnancy with Sulphadoxine-pyrimethamine

Intermittent preventive treatment of malaria in pregnancy originally entails administering two or three therapeutic doses of sulphadoxine-pyrimethamine (IPTp-SP) at least a month apart to pregnant women beginning from 16 weeks of gestation (WHO, 2006). The policy of IPTp-SP for pregnant women has been shown to be efficacious for prevention of maternal anaemia and LBW (Parise *et al.,* 1998; Verhoeff *et al.,* 1998; Shulman et al., 1999; Rogerson *et al.,* 2000) and is currently implemented in 37 countries in sub-Saharan Africa (Kayentao *et al.,* 2013). Thirty-one of these countries give only 2 doses of SP while the other six, Ghana included, give 3 doses. In HIV-positive pregnant women, more doses of SP may be given. The justification for IPTp lies in the observation that PAM is often asymptomatic and missed on peripheral blood microscopy.

Despite concerns over increasing SP resistance and the continuous usefulness of IPTp-SP as a control strategy (Newman *et al.,* 2003; Mbaye *et al.,* 2006), the policy has been recently revised to reflect 3 or more doses of SP as this regimen has been

associated with higher birth weights and lower risks of LBW in a pooled analysis (WHO, 2012b; WHO, 2013; Kayentao *et al.,* 2013).

The WHO now recommends IPTp-SP for all pregnant women at each antenatal clinic visit even up to the time of delivery (WHO, 2013). In its revision in 2012, the WHO Malaria Policy Advisory Committee stated that IPTp-SP remains effective and that it is not associated with harm even in areas with high prevalence of *P. falciparum* parasites with quintuple dihydrofolate reductase/ dihydropteroate synthetase (*Pfdhfr/Pfdhps*) gene mutations.

However, a recent study in Tanzania has reported lower median birth weights for pregnant women who carry the *Pfdhfr/Pfdhps* sextuple mutation including A581G and this equates to over 40% of the population studied (Minja *et al.,* 2013).

Harrington *et al* (2009) also reported IPTp-SP is linked to an increased parasite density and placental inflammation at delivery and no evidence of improvement in overall pregnancy outcomes. The finding of increased parasite density with IPTp-SP was not corroborated in the study by Minja *et al* (2013).

In a moderate transmission zone of Ghana, Tagbor et al (2010) reported a day 28 PCR- corrected parasitological failure of 5.6% among pregnant women taking single dose SP. Moreover, IPTp-SP both significantly reduced clinical malaria/ maternal anaemia and proved non-inferior on LBW/ maternal anaemia to intermittent screening and treatment (IST) of pregnant women with ASAQ or SP (Tagbor *et al.,* 2010: Wilson *et al.,* 2011). These studies indicate Ghana still retains significant levels of SP effectiveness among pregnant women.

The study comparing intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) to intermittent screening and treatment with ACTs (IST) (Tagbor *et al.,* 2010) did not include placental histology for assessment of acute and chronic placental malaria but this strategy appears to have potential in replacing IPTp-SP in areas with defined seasonal transmission to reduce indiscriminate use of SP when parasite levels are low or absent. Another argument for considering IST is the apparent lack of protection in the first trimester as IPTp-SP begins from the second trimester. First trimester parasitaemia will likely be asymptomatic, especially in high transmission settings and reportedly carries an increased risk of miscarriage (McGready *et al,* 2012). Intermittent screening and treatment may well fill this gap of protection in the first trimester, especially in first pregnancies but this will have to be juxtaposed against first trimester ACT use.

A recent report, however, questions the usefulness of IST (Desai et al., 2015) in that intermittent screening and treatment of malaria in 2^{nd}/ 3^{rd} trimester pregnancy with dihydroartemisinin-piperaquine in Western Kenya showed poorer outcomes of a higher incidence of malaria infection during pregnancy, clinical malaria and a higher prevalence of malaria infection at delivery compared to IPTp-SP and intermittent preventive treatment with dihydroartemisinin-piperaquine. It is unlikely IST will be adopted anytime soon as an alternative approach to tackling malaria in pregnancy.

In 2011,about 83% of pregnant women in Ghana took at least one dose of IPTp-SP during their last pregnancy while 65% took at least 2 doses (MICS, 2011), an improvement from the 44% taking at least 2 doses reported in the 2008 demographic and health survey (GDHS, 2008).

Of 1136 pregnant women delivering at St.Michael's Hospital in the Ashanti region of Ghana between January and June 2011, 11% did not access IPTp-SP at all, 9% took only one dose, 21% took two doses while 59% took all three doses of IPTp-SP (St Michael's Hospital, Accessed in 2013).These data shows there are problems with coverage and uptake.

2.4.2 Insecticide-treated nets

Reports on the effectiveness of ITNs in controlling malaria-related anaemia in pregnant women and LBW have yielded mixed results. Some studies showed little or no beneficial effects (Browne *et al.*, 2001; Schulman *et al.,* 1998) while two studies in Gambia and Thailand showed a reduction in maternal anaemia (D'Alessandro *et al.,* 1996; Dolan et al., 1993). Insecticide-treated net use among pregnant women in Western Kenya was only protective among gravida 1-4, showing a 47% reduction in incidence of severe malarial anaemia and 28% decrease in the prevalence of LBW but not beneficial in gravidity ≥5 (ter Kuile *et al.,* 2003). A systematic review of ITNs in pregnancy showed 23% reduction in LBW, 33% decrease in miscarriages/stillbirths and 23% reduction in placental parasitaemia in all gravidae (Gamble *et al.,* 2007).

Overall, ITNs are effective against PAM but requires consistent and correct use. However, despite evidence of increased access, there are still gaps between ownership and utilization (Ahmed and Zerihun, 2010; Gerstl *et al.,* 2010; Ndjinga and Minakawa, 2010). In a study assessing coverage and use of ITNs in the Nkoranza South district of Ghana, only 47% of pregnant women owned ITNs or any other net and of these only 73% had used the ITN the night before the survey (Okyere, 2011).

Use of ITNs is influenced by factors such as knowledge of a causal link between mosquitoes and malaria, knowledge of symptoms of malaria, reservations about using

insecticides, cost of nets, doubts about ITN effectiveness, high temperatures and lack of places to hang nets (Binka and Adongo, 1997; Minja *et al.*, 2001; Mbonye *et al.*, 2006; Belay and Deressa., 2008). According to the Ghana Demographic and Health Survey of 2008, only 20% of pregnant women slept under a long-lasting ITN the night before the survey. This has significantly improved to about 60% as reported in a survey conducted by the University Of Ghana School Of Public Health (unpublished). This substantial increase followed a national campaign supported by UNICEF that distributed close to 11 million long-lasting ITNs in 2011.

2.5 Antimalarial Drug Use in Pregnancy

Following withdrawal of CQ and SP, the ACTs are now the drugs of choice for uncomplicated multi-drug resistant *P.falciparum* malaria (Nosten and White, 2007; White 2006; WHO, 2006). Artemisinin-combination therapy is widely deployed for treatment of uncomplicated malaria in the second and third trimesters of pregnancy in malaria endemic areas based on confidence in the safety of ACT exposure during this period of pregnancy (WHO, 2007; WHO 2010a). In Ghana, the ACTs used in pregnancy are ASAQ and AL while quinine is used in the first trimester.

2.5.1 Artemisinin-combination treatment

The ACTs are a combination of two antimalarials with different mechanisms and duration of action. Specifically, artemisinins combined with longer-acting partner drugs like AQ, lumefantrine, mefloquine, piperaquine and SP are now approved by WHO (WHO, 2013). Combining short-acting artemisinins with other longer-acting antimalarials of varying mechanisms of action will theoretically protect both partners in the combination but more importantly the artemisinin component from parasite resistance development. The probability of a parasite developing resistance

simultaneously to both partner drugs is reduced to about one in a billion in such drug combinations (White, 1999).

The artemisinin component rapidly reduces the parasite burden while the longer-acting partner slowly clears the remaining parasites over 1-2 weeks. The ACTs only need to be taken for three days which will enhance compliance (White, 1999). The longer-acting partner drug must, in theory, persist sufficiently long at concentrations lethal to remaining parasites in the bloodstream. Beyond this point, it is possible that drug concentrations fall to levels that are sub-lethal and thus sub-therapeutic to newly infecting parasites and concerns have been raised over this essentially "monotherapy" situation as it will increase selection pressure and spread of resistant strains.

2.5.2 Mode of action of artemisinin-combination treatment

The mechanism of action of ACTs combines the different mechanisms of the drugs in the combination. This sub-section will be restricted to ASAQ and DHA-PPQ as these are the ACTs of relevance to the present study.

Alterations to the chemical structure of artemisinin gave rise to its derivatives artesunate, arthemeter and dihydroartemisinin (DHA). Dihydroartemisinin is the active metabolite of the artemisinin derivatives and has short half-life of one hour in vivo (Newton *et al.,* 2002; Hien *et al.,* 2004a). Haeme-activated cleavage of an endoperoxide bridge in its structure produces free oxygen radicals (Karunajeewa *et al.,* 2004).

These oxygen radicals irreversibly damage parasite membrane systems and possibly destroy specific transport proteins in the process. Dihydroartemisinin is also known to disrupt parasite mitochondrial electron transport systems and inhibits sarco-

endoplasmic reticulum calcium adenosine triphosphatase (; Eckstein-Ludwig *et al.,* 2003; Karunajeewa *et al.,* 2004).

The artemisinins have a broad range of activity and rapidly kill early asexual ring stages, trophozoites, schizonts and immature gametocytes of all species of Plasmodia causing human malaria (Dondorp *et al.,* 2010). It has the ability to reduce parasite density by up to 10 000 parasites per asexual cycle (White, 1999).

Based on structural similarity, AQ and PPQ are suspected to have a similar mechanism of action as CQ and kills asexual stage parasites by inhibiting detoxification of haeme or parasite-degraded haemoglobin products (Goldberg *et al.,* 1990; Davis *et al.,* 2005). Haeme accumulates and damages parasite cell membranes. Piperaquine is highly bound to plasma proteins, has a large mean volume of distribution, is slowly absorbed and thus has a long elimination half-life of 2-5 weeks which makes it a suitable partner drug in ACT (Hung *et al.,* 2004; Tarning *et al.,* 2005; Myint *et al.,* 2007; Tarning *et al.,* 2008). Considered a pro-drug, amodiaquine is metabolized to the active metabolite desethylamodiaquine by the cytochrome P450 enzyme system (Li *et al,* 2002) and has a terminal elimination half-life of 1-3 weeks (Krishna and White, 1996).

2.5.3 Artemisinin use in pregnancy

Artemisinin use in pregnancy is restricted to the second and third trimesters due to concerns of embryotoxicity and teratogenicity in the first trimester (WHO, 2010). These concerns are based on animal models showing that exposure of rat embryo to artemisinins at a time period corresponding to 4-10 weeks gestation in humans was associated with resorption of foetal red blood cells in the yolk sac with consequent abortions or developmental abnormalities (Longo *et al.,* 2006; Ward *et al.,* 2007).

Dihydroartemisinin has been shown to inhibit proliferation and cause delayed differentiation of primitive human erythroid cell lines but a review of first trimester pregnancy exposures to artemisinins in south-east Asia showed the drug did not appear to unduly interfere with pregnancy (Finaurini *et al.,* 2010; McGready *et al.,* 2012). This observation is possibly because doses of artemisinins that cause embryotoxicity and teratogenicity in rats were substantially higher than those used in humans (Chen *et al.,* 1984; Clark *et al.,* 2004)

Li and Weina (2010) have further suggested that the discrepancy in embryotoxicity to artemisinins between rats and humans lies in the differential duration of production of erythroblasts. They stated that erythroblasts are produced over only one day in rats such that a single exposure to the drug can result in a high proportion of cell deaths. In contrast the process is believed to take much longer in humans, about 12 days, and it is thought unlikely that three days of exposure to ACTs will evoke the degree of toxic effects seen in rats. Lastly, oral administration of ACTs in pregnant women is likely to result in lower peak concentrations and shorter exposure time to artemisinins in which case embryotoxicity will be unlikely. It is possible the in-vivo milieu presents peculiar dynamics that favour a safety margin in humans when early pregnancies are exposed to artemisinins and there is the need for further investigation in this area.

It is important to note that *P.falciparum* tolerance to artemisinins, manifesting as delayed parasite clearance times, has emerged in western Cambodia and spread to neighbouring countries in the region (Noedl *et al.,* 2008; Dondorp *et al.,* 2009; Dondorp *et al.,* 2010; Meshnick, 2012; Plowe, 2014). Ariey *et al* (2014) have recently reported a strong correlation between mutations in the K13-propeller gene on chromosome 13 and artemisinin resistance *in vitro* and *in vivo*. Single nucleotide

polymorphisms (SNPs) in this gene now constitute the most useful markers for artemisinin resistance/ tolerance.

Prior to the report by Ariey *et al* (2014), various explanations had been attempted for the phenomenon of artemisinin tolerance. Miotto *et al* (2013) hypothesized that mass administration of CQ and pyrimethamine in Cambodia some decades ago initially produced parasite variants resistant to most antimalarials aside the artemisinins and that sexual recombination over time has produced sub-populations of *P.falciparum* that are now resistant to the artemisinins. Subsequently, on the basis of genomic profiling, *P.falciparum* parasites from Cambodia had been categorized into sensitive and resistant sub-populations (Plowe, 2014). Other studies had also associated the observed tolerance/ resistance with SNPs in the *P.falciparum* multidrug resistance gene (*Pfmdr1*) and mutation of the gene encoding sarco-endoplasmic reticulum adenosine triphosphate (Sisowath *et al.,* 2009; Jambou *et al.,* 2005).

2.5.4 Amodiaquine use in pregnancy

Only four published studies were found that assessed AQ, ASAQ and AQ-SP use in pregnancy. A high parasitological efficacy rate of 95% by day 28 is reported for ASAQ used to treat malaria in pregnancy in Tanzania and Ghana (Mutabingwa *et al.,* 2009; Dr. Harry Tagbor, personal communication, 2010). Artesunate-amodiaquine was associated with only a marginal excess of complaints of general weakness but not any detectable increase in adverse birth outcomes. A similar exposure of pregnant women to AQ and AQ-SP for treatment and IPTp was associated with a high prevalence of mild adverse events but not severe adverse events of stillbirth, neonatal deaths and congenital abnormalities (Tagbor *et al.,* 2006; Clerk *et al.,* 2008).

Efficacy and occurrence of mild adverse events with ASAQ were similar to reports in children 6-59 months old and in a cohort of adults in whom more than half of reported adverse events were traced to ASAQ (Koram *et al.,* 2005; Agyei *et al.,* 2008; Faye *et al.,* 2010; INESS, 2011). None of the reviewed studies reported any link between AQ use, alone or combined, with bone marrow suppression, agranulocytosis or hepatitis contrary to reports in non-immune traveller using it for chemoprophylaxis (Neftel *et al.,* 1986; Hatton *et al.,* 1986; Phillips-Howard and West., 1990).

2.6 Safety and Efficacy of Dihydroartemisinin-piperaquine in children and non-pregnant adults

Piperaquine, the longer-acting partner drug in DHA-PPQ, was used extensively in China for treatment and mass prophylaxis for close to 16 years until resistance developed in the late 1980s (Changxiong *et al.,* 1989). Limited human toxicological data for PPQ from early pre-clinical and clinical studies indicated that for doses up to 60mg/kg over 2-3 days, the most common AEs were a decreased white cell count, elevated serum albumin, elevated serum alanine aminotransferase (ALT), low serum glucose and transient mild prolongation of the rate-corrected electrocardiographic QT interval and these may not have been clinically significant (Davies *et al.,* 2005; Myint *et al.,* 2007; Ahmed *et al.,* 2008).

Dihydroartemisinin-Piperaquine has been evaluated in large clinical trials involving children and non-pregnant adults in Southeast Asia, China, India, Rwanda, Burkina Faso, Uganda and Kenya (Denis *et al.,* 2002; Ashley *et al.,* 2004; Hien *et al.,* 2004b; Ashley *et al.,* 2005;Giao *et al.,* 2004; Hung *et al.,* 2004; Karema *et al.,* 2006; Mayxay *et al.,* 2006; Smithius *et al.,* 2006; Zongo *et al.,* 2007; Kamya *et al.,* 2007; Valecha *et al.,* 2010; Ogutu et al., 2014) and found to be safe and highly efficacious with cure rates in excess of 95% by days 28 and 63.

Only one study in Rwanda (Karema *et al.*, 2006) reported a cure rate under 90% among a number of trials in Asia and Africa included in an individual patient data analysis.

A recent review (Zani *et al.,* 2014) showed DHA-PPQ is superior to AL and as effective as artesunate-mefloquine (ASMQ) in preventing new infections over 28 days in Africa and Asia respectively. However, while DHA-PPQ maintained its superiority over AL over 63 days in Africa, both DHA-PPQ and ASMQ did not show any consistent benefit in preventing new infections in Asia. The longer terminal half-life of PPQ compared to 3-5 days for lumefantrine (Ezzet *et al.,* 1998; Ezzet *et al.,* 2000) and the more likely stable and perennial malaria transmission patterns found in Africa may account for the observed advantage of DHA-PPQ over AL. Mefloquine has a long terminal half-life of about 3 weeks (Karbwang and White, 1990), comparable to that of PPQ, and is thus expected to have a similar post-treatment prophylactic effect. Studies comparing DHA-PPQ and ASAQ were limited and not emphasized in the review.

Adverse events (AEs) reported with DHA-PPQ treatment include vomiting, diarrhoea, sleep disturbances, nightmares, dizziness, palpitations, abdominal pains, headaches, hypersensitivity reaction and visual disturbances (Myint *et al.,* 2007; Trung *et al.,* 2009; Zwang *et al.,* 2009). The risk of AEs is higher with ASAQ and ASMQ but comparable to AL (Karema *et al.,* 2006; Zongo *et al.,* 2007; Zwang *et al.,* 2009). Haemolysis in study participants with glucose-6-phosphate dehydrogenase defect has been noted (Mayxay *et al.,* 2006; Katrak *et al.,* 2009).

2.7 Dihydroartemisinin-piperaquine use in pregnancy

Two published reports from Southeast Asia describe DHA-PPQ use in pregnant women. Poespoprodjo *et al* (2014) reported DHA-PPQ has been used to treat both uncomplicated and complicated malaria in pregnancy in second and third trimester pregnancies in Papua, Indonesia (an unstable transmission area) since 2006 following development of resistance to CQ and SP. Their study reported over 60% (5/8) and about 3% (1/38) prevalence of miscarriage for first-time pregnancies exposed to DHA-PPQ and oral quinine respectively. The authors acknowledge a possible bias in reporting that the frequent abortions in those who received DHA-PPQ may have been due to malaria rather than the drug as it appears DHA-PPQ was given to seriously ill women. The study reported a 3.3% risk of perinatal death and 26% risk of parasitaemia at delivery for women who received DHA-PPQ compared to 6.6% risk of perinatal death in those treated with quinine alone and 48% risk of parasitaemia at delivery among those receiving quinine, CQ and SP.

Rijken *et al* (2008) conducted a pilot study of DHA-PPQ used as rescue treatment in fifty Karen pregnant women with recurrent *P. falciparum* infections on the Thailand-Cambodia border and neither reported deteriorations under treatment nor a single episode of vomiting. The day 63 PCR-corrected efficacy was 92.2 %. All babies were live born with a 4.4% prevalence of premature deliveries. Two infants showed congenital abnormalities; one Patau's (trisomy 13) syndrome and one had an umbilical hernia. Occurence of umbilical herniae and other genetic disorders were also reported in an assessment of the safety of AL and SP in second and third trimester pregnancies in Zambia (Manyando *et al.,* 2010). Rijken *et al* (2008) did not assess the effect of DHA-PPQ on liver and renal biochemistry and no other haematological indices apart from haemoglobin were measured.

Both reports of DHA-PPQ use in pregnancy appear to have limited study rigour in that the study by Rijken *et al* (2008) lacked a control group while the comparison groups in that of Poespoprodjo *et al* (2014) were not randomized, thus introducing limitations in the control of biases and confounders. The gaps highlight the need for a more rigourous investigation of the safety and efficacy of DHA-PPQ as treatment for uncomplicated malaria in pregnancy.

2.8 Dosaging of Antimalaria Drugs in Pregnancy

Physiological changes associated with pregnancy including increased volume of distribution and cardiac output, increased renal blood flow, decreased gastrointestinal motility and increased plasma protein-binding have long been suspected to reduce bioavailability of drugs including antimalarials (Dawes and Chowienczyk, 2001) and the need for an upward adjustment in doses of antimalarial drugs in pregnancy has been debated.

While some studies have demonstrated reduced concentrations of antimalarial drugs like atovaquone, proguanil, arthemether-lumefantrine, sulphadoxine and pyrimethamine in pregnant women (McGready *et al.,* 2003; McGready *et al.,* 2006; Nyut *et al.,* 2010) with potential implications for poor cure rates and selection of resistant parasite strains, others report no effect of pregnancy at all on the absorption, distribution, metabolism and excretion of antimalarials (Green *et al.,* 2007; Rijken *et al.,* 2011b).

Various studies have reported no relevant differences in pharmacokinetic properties of AQ, desethyl-AQ, DHA and PPQ between pregnant and non-pregnant women (Rijken *et al.,* 2011a; Rijken *et al.,* 2011b; Hoglund *et al.,* 2012; Tarning *et al.,* 2012a) but Tarning *et al* (2012b) reported a reduced total exposure to DHA and increased

clearance of PPQ in pregnancy which could negatively impact on the efficacy of DHA-PPQ. Pharmacokinetic evaluations in other ACTs are on-going and will shed more light on the issue but the consensus now seems to be that there is no justification for dose adjustments in the pregnant population.

2.9 Molecular markers of antimalarial drug resistance

Case management and intermittent preventive treatment of PAM are direct drug-based interventions that remain key to PAM control in stable transmission settings especially in sub-Saharan Africa (WHO, 2012a). These interventions depend on the continual effectiveness of antimalarials such as ACTs, SP and quinine which is used for treatment of uncomplicated PAM in the first trimester. Prior to its designation for IPTp and IPTi (intermittent preventive treatment of malaria in infants), SP was used to treat uncomplicated malaria in the general population including pregnant women.

However, there have been reports of reduced *P.falciparum* susceptibility to these drugs (Hyde, 1990; Kublin *et al.,* 2002; Dondorp *et al.,* 2009) and it is important to monitor their continuous effectiveness. Molecular markers that correlate with resistance to antimalarials present practical, simple and cheap tools for surveillance of resistance and changing parasite drug susceptibility over time following changes in malaria drug treatment policy (Kublin *et al.,* 2003; Plowe *et al.,* 2007). Molecular markers may not always predict treatment failures as this depends on other factors including immunity and drug compliance (Omar *et al.,* 2001; Djimde *et al.,* 2003).

2.9.1 Sulphadoxine-pyrimethamine resistance markers

Sulphadoxine-pyrimethamine is still the only drug recommended for IPTp despite reports of high level *P. falciparum* resistance and declining protective efficacy of IPTp-SP notably in Eastern Africa (Harrington *et al.,* 2009; WHO, 2012b; Minja *et*

al., 2013). Sulphadoxine-pyrimethamine was used for treatment of uncomplicated malaria much earlier on in Eastern Africa and this could possibly explain why SP resistance is very marked relative to West Africa.

Resistance to SP is mediated by SNPs in the *P. falciparum* dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthetase (*Pfdhps*) genes resulting in amino acid changes mainly at codons N51I, C59R, S108N/T, I164L and in the *Pfdhps* gene at codons AS436F, A437G, K540E, A581G and A613S (Wang *et al.,* 1997). The K540E and A581G mutations appear to be especially relevant for IPTi and IPTp respectively as the WHO does not recommend IPTi where the prevalence of K540E exceeds 50%. There is however no such cut-off point for IPTp.

Accumulation of *Pfdhfr* mutations are thought to be stepwise and occurs first in codon 108 followed by mutations in codons 51/59 while for *Pfdhps,* mutations begin in codons 437, then 540, 581 and 613 (Wang *et al.,* 1997). Increasing numbers of mutations in *Pfdhfr* and *Pfdhps* genes accentuate SP resistance (Hyde, 1990; Kublin *et al.,* 2002). A high prevalence of *Pfdhfr* triple mutations involving codons 51, 59 and 108 or a combination of the triple mutation and *Pfdhps* mutations is reported to better predict SP resistance (Omar *et al.,* 2001).

The triple *Pfdhfr* mutation (N51I, C59R and S108N) is commonly found across Africa (Gregson and Plowe, 2005). The *Pfdhfr/Pfdhps* quintuple mutation (N51I, C59R, S108N/ A437G, K540E) is more common in East Africa and underlies most of the greater burden of SP resistance in the East African region (Mutabingwa *et al.,* 2001; Omar *et al.,* 2001). This haplotype is however still rare in West Africa (www.drugresistancemaps.org). In West Africa, the prevalence of SNPs at codons 540, 581 and 613 in the *Pfdhps* gene is generally low but codon 437 SNPs are

prevalent. It would appear that the "determinant" mutation in West Africa is K540E such that if prevalences of it are low, then there is a reduced likelihood of detecting A581G or A613S.

Recent reports have shown increased prevalence of the A581G mutation in East Africa (Alifrangis *et al.,* 2009; Harrington *et al.,* 2009; Minja *et al.,* 2013). This observation has been associated with increased parasitaemia, concurrent intense placental inflammation and inclination towards lower median birth weights in women taking IPTp-SP (Harrington *et al.,* 2009; Minja *et al.,* 2013). However, recommendation for IPTp-SP has been reinforced recently with increased number of doses (WHO, 2012b).

2.9.2 Artemisinin-combination treatment resistance markers

With respect to case management, the ACTs are used to treat uncomplicated PAM in line with WHO recommendations. They are effective against multidrug-resistant *P.falciparum* parasites and replaced CQ and SP as first line treatment of uncomplicated malaria due to development of high grade resistance against the latter two drugs. The ACTs reduce or delay emergence of resistant parasites (White, 1999; Egan and Kaschula, 2007).

In Ghana, the ACTs used to treat uncomplicated malaria in Ghana are ASAQ, AL and DHA-PPQ but the latter is not as yet cleared for use in pregnancy. To date, there have been no reports of ACT resistance in Africa.

Ariey *et al* (2014) have recently reported that the *P.falciparum* K13-propeller gene is strongly associated with artemisinin tolerance or resistance. Single nucleotide polymorphisms at this gene are now used as ACT resistance markers. However, SNPs in the *P.falciparum* chloroquine resistance transporter gene (*Pfcrt*) and the

P.falciparum multidrug resistance gene 1(*Pfmdr*1) may contribute to reduced falciparum susceptibility to the ACTs.

Single nucleotide polymorphisms at codons 72-76 of *Pfcrt* underlie CQ resistance (Djimde *et al.,* 2001). Specifically, SNPs at codon 76 (K76T) is deemed critical for CQ resistance as it is present in all CQ-resistant falciparum isolates (Fidock *et al.,* 2000; Djimde *et al.,* 2001; Gadalla *et al.,* 2010). Wild type parasites carry K76 and mutant types carry 76T. Resistance conferred by *Pfcrt* mutations is also modulated by the *Pfmdr1* gene (Reed *et al.,* 2000) and SNPs in the Pfmdr1 gene have implications for the susceptibility of a number of antimalarials.

CQ-sensitive parasites have the CVMNK haplotype while the more commonly reported resistant haplotypes are CVIET and SVMNT (Sa *et al.,* 2009; Gadalla *et al.,* 2010). The CVIET haplotype is almost the only mutant type found in Africa. Its prevalence was high when CQ was used as first line treatment and resistance was spreading but in the absence of drug pressure now, its prevalence has gone low such that parasites populations carrying the CVIET haplotype are outcompeted by those carrying CVMNK and which eventually become predominant (Sa et al., 2009). A number of studies have reported this finding across sub-Saharan Africa (Mwai *et al.,* 2009; Kublin *et al.,* 2003; Ndiaye *et al.,* 2012; Malmberg *et al.,* 2013; Mohammed *et al.,* 2013).

Reduced *P.falciparum* susceptibility to the ACT partner drugs; AQ, PPQ and pyronaridine have been reported to be due to cross-resistance with CQ on account of similarities in structure and mechanism of drug action (Foley and Tiley, 1998; Pradines *et al.,* 1998; Raynes, 1999; Brice *et al.,* 2010).

Sisowath *et al* (2009) suggested that SNPs in the *P.falciparum* multidrug resistance gene (*Pfmdr 1*) are involved in ACT resistance/tolerance. *Pfmdr*1 SNPs at codons 86, 184, 1034, 1042 and 1246 and copy number variations (repetitive copies of the whole *Pfmdr*1 gene rather than single nucleotide base changes) alter parasite susceptibility to the aminoquinoline and arylaminoalcohol antimalarial drug classes though they are structurally unrelated. This could explain varying *P. falciparum* susceptibility to a variety of antimalarials including lumefantrine, amodiaquine, mefloquine, quinine, halofantrine and the artemisinins (Reed *et al.,* 2000; Sidhu *et al.,* 2005; Holmgren *et al.,* 2006; Folarin *et al.,* 2011; Pillai *et al.,* 2012).

Arthemeter-lumefantrine possibly selects for wild- type *Pfcrt* K76 and *Pfmdr1* N86, Y184F and D1246 alleles as evidenced in increasing prevalence of parasites carrying the NFD haplotype observed in recrudescences following treatment with AL (Malmberg *et al.,* 2013). The alternative YYY haplotype is associated with decreased sensitivity to the 4-aminoquinolones such as AQ (Froberg *et al.,* 2012). Parasites carrying N86 and Y184 genotypes reportedly show less sensitivity to lumefantrine, arthemeter and DHA compared to those carrying N86Y and Y184F genotypes (Nkhoma *et al.,* 2009). Artesunate is suspected to have selected for N86 and Y184F in a study in Thailand (Mungthin *et al.,* 2010).

There is a dearth of current information regarding prevalence of antimalarial molecular markers of resistance in pregnant women in Ghana though many such assessments have been conducted in children. The prevalence of *Pfcrt* K76T mutations was 46%-98% between 1998 and 2002, 81% of samples surveyed in 2007/2008 had mutations in the *Pfmdr1* gene and there has been a significant increasing trend for *Pfdhfr/ Pfdhps* mutations between 2003 and 2010 (Erhardt *et al.,* 2007; Duah *et al.,* 2007; Alam *et al.,* 2011; Duah *et al.,* 2013). It is important to fill

this knowledge gap to ascertain whether prevalences in pregnant women may be different, at least, compared to children and also now when IPTp coverages are increasing. Early detection of markers through monitoring will help turn attention to strategies that will delay drug resistance.

A search of published literature showed no prevalence data on single nucleotide polymorphisms at the *Pfdhps* gene in pregnant women in Ghana but 74%-96% of isolates in children and healthy adults in a perennial transmission area carried the A437G mutation between 2001 and 2004 (Marks *et al.,* 2005; Owusu-Agyei *et al.,* 2009). Close to 80% of *P.falciparum* isolates in pregnant women carried the core *Pfdhfr* mutation S108N in a perennial transmission zone in Ghana in 1998 and this increased to 93% in a survey in the same area in 2001/2002 (Mockenhaupt *et al.,* 2001; Mockenhaupt *et al.,* 2007).

Ghana introduced IPTp-SP in 2005 and by 2006 the proportion of isolates carrying the *Pfdhfr* triple mutation (N51I, C59R, S108N) had doubled from 36% in 1998 to 73% in 2006 among pregnant women (Mockenhaupt *et al.,* 2001; Mockenhaupt *et al.,* 2008). This increase may not have been due to IPTp alone as pregnant women constitute only a small population. It is also possibly due to continuing SP use for uncomplicated malaria in the general population with transmission of parasites carrying SP resistance mutations. Prevalence of *Pfmdr*1N86Y mutation was 66% among pregnant women in 1998 (Mockenhaupt *et al.,* 2001) and 42%-95% in children between 1998 and 2000.

Prior to withdrawal of CQ in 2005, the prevalence of the *Pfcrt* K76T mutation in infected pregnant women was 69% in the middle forest belt of Ghana (Mockenhaupt *et al.,* 2001). *Pfcrt* K76 prevalences have, however, been observed to be increasing in

Ghana following withdrawal of CQ in the general population though a recent study reported a rather high 76T prevalence of 58.6% across six region (Duah *et al.,* 2013; Afoakwa *et al.,* 2014).

2.10 Participation of Pregnant Women in Clinical Trials; Experiences and Willingness

Pregnant women have generally been excluded from drug trials and inferences regarding safety and efficacy of drugs used in pregnant women have been extrapolated from data gathered from children and young male adults. Drugs that have not passed through the rigour of scientific research in pregnant women have been and continue to be used, rendering most cases of drug use in this population an unmonitored experiment. (Berlin and Ellenberg, 2009; Lyerly *et al.,* 2012).

The risk of teratogenicity and mutagenicity to the developing foetus have led to the exclusion of pregnant women from clinical trials but enrolling and retaining pregnant women in clinical trials is imperative for gathering scientific evidence relating to drug use in pregnancy (Macklin, 2010). Contemporary research guidelines are oriented towards including pregnant women in clinical trials (Macklin, 2010) but ethical committees still keep an understandably increased alertness towards research protocols involving pregnant women.

Antimalarial treatment options in pregnancy are likely to be thrown wide open with the many antimalarials in development (Jagoe, 2009) and these ought to be trialled directly in pregnant women to determine not only potential risks and benefits but also to ascertain whether and how differences in pharmacokinetics and pharmacodynamics impact on efficacy in this population compared to children and adult males. Investigators have a responsibility to conduct research in a way that is acceptable to

pregnant women and ensure a successful informed consent process where relevant information on risks and benefits is presented and understood within the existing cultural contexts.

Pertinent to these objectives is a need for researchers to appreciate the social considerations surrounding participation of pregnant women in clinical studies. This can be obtained through a deeper understanding of pregnant women's personal experiences of participating in clinical trials and the factors that shape willingness to participate in such studies.

There is considerable literature addressing issues of willingness as well as experiences of participation in clinical trials among non-pregnant populations (Buchbinder *et al.*, 1999; Hussain-Gambles, 2004; Jones *et al.,* 2006; Catania *et al.,* 2008; Shah *et al.*, 2010; Paradis *et al.,* 2010) but an understandably limited number of studies have sought to answer this question from the perspective of pregnant women and essentially all have been in Western Europe and North America (Mohanna, 1998; Rodger *et al.,* 2003; Nechuta *et al.,* 2009; Gatny and Axinn, 2011; Lyerly *et al.*, 2012).

A call for greater respect from doctors, improving communication, trust in doctors/ research team, health benefits to both mother and foetus, apprehensions about taking research medication while pregnant, altruism, advancement of science, monetary benefits, racial discriminations and fear of injections have been some of the factors that determined willingness to participate in clinical trials among pregnant women (Mohanna, 1998; Rodger *et al.,* 2003; Nechuta *et al.,* 2009; Gatny and Axinn, 2011; Lyerly *et al.*, 2012).

There is a dearth of information regarding the experiences and meanings pregnant women attach to the inherent processes of clinical trial participation as well as of factors that drive willingness to participate in such studies in Ghana and sub-Saharan Africa generally. Only one study in Ghana that primarily examined user acceptability of IST for malaria in pregnancy compared to routine IPTp (Smith *et al.*, 2010) touched on some elements of the participants' social constructs of their involvement in the clinical trial.

The study, however, excluded investigation of factors affecting pregnant women's willingness to participate in drug trials. This is deemed an important gap that needs to be addressed for the success of clinical research planning especially where the eligible participants have not had prior exposure to any large-scale biomedical research undertaking. The present study seeks to explore and understand the different meanings pregnant women attach to experiences accrued during participation in a drug trial. It also desires to make meaning of how their perceptions of the processes involved in a hypothetical trial will inform their willingness to participate in such trials when invited to do so.

The perspectives of critical realism (Bhaskar, 1978) and social cognition (Fiske and Taylor, 1991) provide the theoretical foundation for the present study. According to Fade (2004), critical realism supposes that reality may be independent of human conceptualization and that the different meanings individuals attach to their lived experiences arise out of engagements with different facets of reality. Social cognition accepts that these differing meanings are reflected in behaviour and speech either directly or indirectly as humans go through various experiences in their natural settings.

These two theoretical perspectives inform the chosen methodological approach of interpretative phenomenological analysis (Smith *et al.*, 1999) and the nature of questions asked in interviews conducted towards achieving an understanding of participants' viewpoints. Interpretative phenomenological analysis incorporates the researcher's personal beliefs and standpoints in interpreting human experience (Fade, 2004; Idczak, 2007) as opposed to traditional Husserlian phenomenology that favours bracketing and exclusion of the investigator's preconceived notions.

CHAPTER THREE

METHODS

3.1 Introduction

This chapter describes the study settings, population and design as well as specific activities carried out during field and laboratory work to obtain data aimed at achieving the study objectives.

3.2 Study Area

The study was conducted in two contiguous administrative areas; Bekwai municipality and Bosomtwe district in the Ashanti region of Ghana. The two areas are about 24km apart and lie within the middle forest belt where malaria transmission is perennial and moderate-to-high in intensity. Climate and ecology are similar in the two areas with mean annual rainfall between 1600-1800mm. The major rainfall season is from March to July and the minor from September to November. Relative humidity ranges between 70-80% in the dry season.

Bekwai municipality shares boundaries with Bosomtwe district in the North, Adansi North district to the South, Bosome Freho district in the East and Amansie Central district to the West. Bosomtwe district shares borders with the Ejisu-Juaben municipality and Kumasi Metropolis to the north, Bosome-Freho district in the East, Atwima-Kwanwoma district to the west and Bekwai municipality in the south.

The geographic location of the study sites is shown in Figure 3.1 while Table 3.1 shows the 2011 population estimates and distribution of health facilities in the two areas. The Bekwai Government hospital in Bekwai municipality and St. Michael's Catholic hospital in Bosomtwe are the largest health facilities in the two areas and

they served as the study recruitment centers. Both hospitals provide emergency obstetric care.

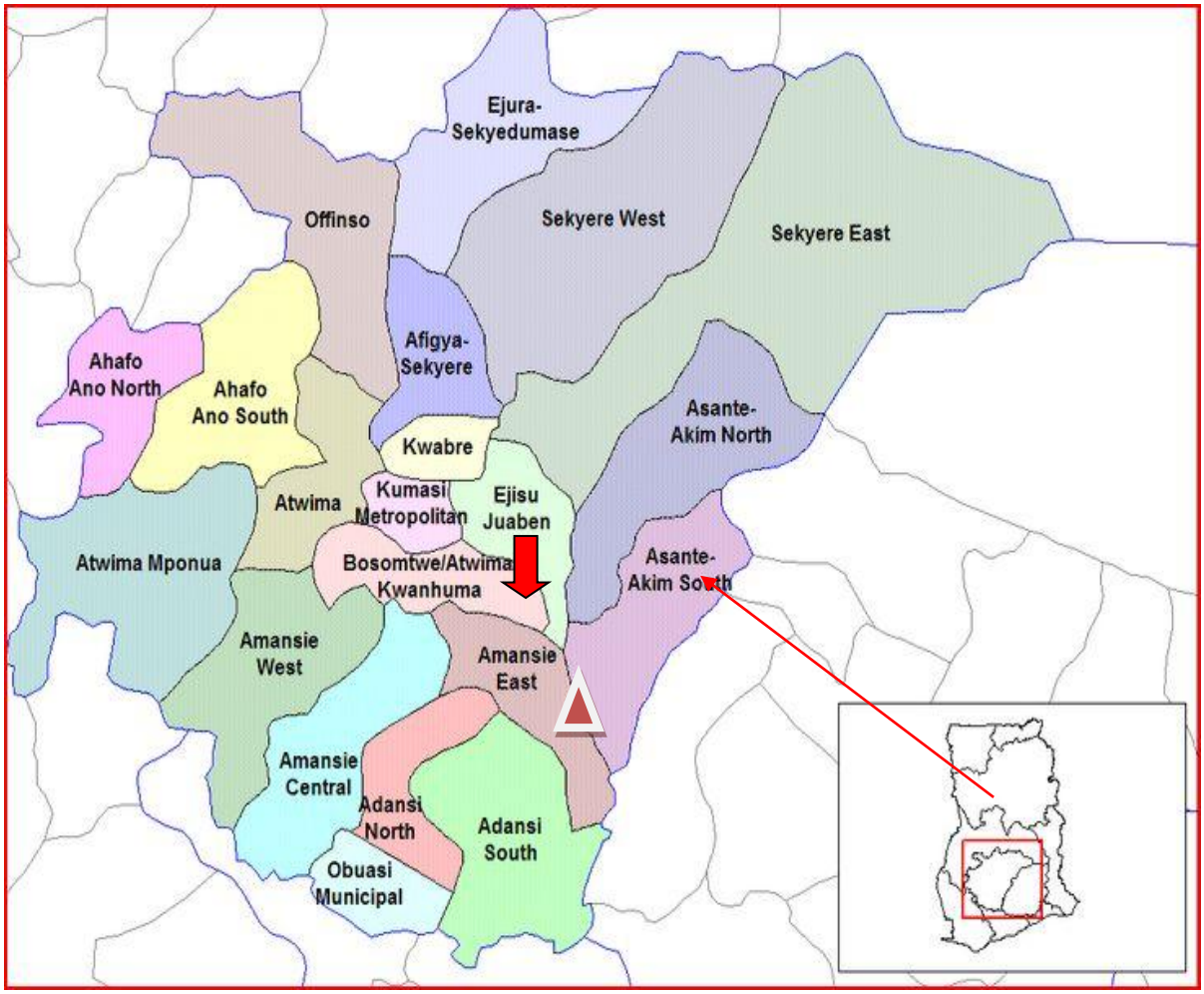


Fig 3.1: Map of Ashanti Region and its districts. Insert is the map of Ghana highlighting Ashanti Region

(Source: en.wikipedia.org/wiki/Amansie_West_District)

Bosomtwe district was carved out of the Bosomtwe-Atwima-Kwanwoma distict in 2008.

Bekwai Municipality was carved out of the Amansie-East district in 2008

Table 3.1 Population estimates and distribution of health facilities in the study areas

	Bekwai	**Bosomtwe**
Estimated Population in 2011	148, 529	103, 363
Total number of health facilities	10	16
Government health facilities	3	4
Mission-based health facilities	3	7
Private health facilities	4	5
Child welfare outreach clinic sites	102	52

Compiled from 2012 Annual Reports for Bekwai and Bosomtwe health directorates

Malaria is the leading cause of OPD attendance in both areas but there is no current data on entomological inoculation rate (EIR). Afrane *et al* (2004) reported monthly EIR of 10.8 and 11.6 for the dry and rainy season respectively for Esereso, a community in the Bosomtwe district, in 2002 but the current relevance of these data is uncertain in light of ACT and ITN use.

Bekwai Government hospital reported malaria OPD attendance and hospital admissions of 105,886 and 3,736 respectively in 2011(Bekwai Municipality Health Directorate Annual Report, 2012) and it is suspected that the vast majority were presumptively diagnosed. Malaria test positivity rate was reported to be 46%-50% between January and October in 2012 in Bosomtwe district (Ghana Urban Malaria Study, 2013). Laboratory confirmed malaria increased from 13.8% of diagnosed cases in 2010 to about 30% in 2012 at St. Michael's hospital (Biostatistics Unit, St Michael's Hospital, 2013). Farming of crops and trading are the leading occupations. The majority of roads in the two areas are untarred and make transport challenging especially in the rainy season.

3.3 Study Population

The study population was made up of pregnant women attending antenatal clinics (ANC) at St. Michael's hospital and Bekwai Government hospital in the two study areas.

In addition, routine delivery data covering 2010 and the first half of 2011 from the maternity ward of St. Michael's hospital was reviewed to obtain background prevalence of LBW and congenital birth abnormalities among neonates delivered there.

Five men were also interviewed as part of exploring pregnant women's experiences and willingness to participate in drug trials.

3.4 Study Design

The study was an open-label, individually randomized, non-inferiority trial with two arms. It tested the hypothesis that DHA-PPQ is non-inferior to ASAQ with respect to parasitological efficacy for malaria in pregnancy in the second and third trimesters and that the treatment difference falls within a pre-defined non-inferiority margin of 5%.

3.4.1 Study Arms and Drugs

The study consisted of a test treatment arm in which participants received a co-formulated preparation of DHA-PPQ containing 40mg of DHA and 320mg of PPQ per tablet (P-ALAXIN, Bliss GVS Pharma, India, Batch nos PX-101 and PX-134) at a total estimated dose of 6.75mg/kg of DHA and 55mg/kg of PPQ (Hasugian et al., 2007). This dose regimen amounted to 3 tablets daily for 3 days.

In the active control arm, participants received a co-blistered pack preparation of ASAQ containing 50mg of AS and 150mg of AQ (ARSUAMOON, Guilin Pharmaceutical Company, Shangai, China, Batch nos LQ 100906 A and LQ 110715 A) at a dose of 4mg/kg for AS (max. daily dose of 200mg) and 10mg/kg for AQ (max. daily dose of 600mg) in two daily doses for 3 days.

The two batches of DHA-PPQ and ASAQ were procured from Tobinco Pharmaceuticals and Vicdoris Pharmaceuticals respectively, both local pharmaceutical marketing companies. For each batch purchased, three drug packs were randomly selected and sent for analysis of quality and content using high-pressure liquid chromatography (HPLC) at the London School of Hygiene and Tropical Medicine. Tolerance limits given by the United States Pharmacopeia (USP) were used. Study drugs were stored in the air-conditioned pharmacy store rooms at both St.Michael's hospital and Bekwai Government hospital.

3.4.2 Inclusion Criteria

A pregnant woman was included in the study if;

i) She had asexual *P.falciparum* parasitaemia on both malaria rapid diagnostic testing and blood film microscopy and of any density

ii) She was resident within 20km radius of St. Michael's hospital in Bosomtwe district or Bekwai Government hospital in Bekwai municipality.

iii) She had no history of treatment with the study drugs in the preceding 2 weeks

iv) Haemoglobin level at recruitment was ≥ 7g/dl.

v) Viable foetus defined by presence of foetal heart using Pinnard stethoscope and/or ultrasonography

vi) She gave assurance of adherence to study procedures and delivery at St. Michael's hospital or Bekwai Government Hospital

vii) She gave informed consent

3.4.3 Exclusion Criteria

A pregnant woman was excluded based on the following;

i) Confirmed multiple gestations.

ii) Severe malaria or disease likely to influence pregnancy outcome eg renal/ cardiac disease, diabetes mellitus, known pregnancy induced hypertension, known human immunodeficiency virus infection.

iii) Known allergies to study medication.

iv) Positive human immunodeficiency virus (HIV) status

v) Haemoglobin level < 7g/dl

3.4.4 Withdrawal Criteria

A participant was withdrawn from the study when;

i) She withdrew informed consent

ii) There was confirmed use of an antimalarial drug outside the study protocol.

iii) A participant could not be located on two consecutive scheduled home visits (each scheduled home visit had an allowable 72 hours within which to locate the participant). Such a participant was considered lost to follow-up.

iv) The principal investigator so decided for safety reasons.

3.5 Composition of the project team

The entire study team comprised;

- A principal investigator (PI)
- 2 study physicians (one obstetrician/gynaecologist and one medical officer)
- 3 research assistants (RAs)
- 9 field workers (FWs)
- All midwives at the ANC clinics
- All midwives at the labour/ maternity wards
- 2 data entry clerks

3.5.1 Roles of the project team members

- The PI supervised all activities entailed in the study
- The study physicians attended to participants when they were ill or reported with adverse events
- The RAs were tertiary and senior high school graduates and were responsible for the following;
    - Obtaining informed consent
    - Screening and recruitment of eligible participants
    - Performing RDTs and other blood sampling procedures
    - Supervising field workers
    - Maintaining log books for blood samples returned from the field
    - Checking case report forms (CRFs) for completion and consistency

During enrolment of eligible participants, one was designated to do RDT tests and other blood sampling procedures, one supervised study drug allocation procedures

while the other administered initial doses of the allocated study drug and the baseline questionnaire.

- The FWs were also senior high school graduates and carried out the following tasks;
    - Follow-up home visits
    - Blood sampling during home visits
    - Assessment of adverse events
    - Reported to the PI on occurrence of adverse events
- The ANC midwives;
    - Identified eligible pregnant women and helped to invite them to join the study
    - Conducted physical examination on potential participants
- The labour/ maternity ward midwives;
    - Attended to participants during labour
    - Collected maternal and neonatal blood samples post-partum
    - Measured birthweights
    - Assessed for congenital abnormalities and reported to the PI
- Data entry clerks, also senior high school graduates, entered data separately from CRFs in a database created for the study

3.5.2 Training provided for the project team

All members were briefed on the study objectives and procedures using the study protocol.

- RAs and FWs were trained over 2 days in community entry and engagement by the Bosomtwe district public health nurse. This was in preparation for home visits.
- RAs and FWs were trained to administer and read results of RDTs by the PI using a training manual with illustrations from the Ministry of Health.
- RAs, FWs and labour/ maternity ward midwives were trained over 4 days to sample both venous and capillary blood and to prepare thick and thin blood films. The training was conducted by a senior laboratory technologist from St. Michael's hospital. The labour/ maternity ward midwives were trained to cut 1cm x 1cm placental blocks from the maternal surface close to the insertion of the cord. Blood from these blocks then were used to prepare placental smears. They were also trained to draw blood from the umbilical vein from the placental stump.
- Two FWs received further training in blood film preparation at the Kumasi Center for Collaborative Research (KCCR) at Asante Akyim Agogo.
- Labour/ maternity ward midwives were trained to identify gross birth defects by the PI using an illustrated manual.
- ANC midwives were given refresher training on physical examination.

3.6 Enrolment Procedures

3.6.1 Screening and Recruitment

- ANC midwives and RAs explained the study to pregnant women attending ANC at the study hospitals. These women had received voluntary counselling and testing for HIV as part of routine antenatal care service delivery and found to be negative. Prevalence of HIV among pregnant women tested at St.

Michael's Hospital was less than 1.9% in 2011 (ANC Records, St. Michael's Hospital)

- ANC midwives physically examined eligible and consenting women for pallor, jaundice, oedema, pregnancy viability and gestational age
- Pregnancy viability was based on presence of foetal heart sounds using both sonicaid and ultrasonography
- Gestational age was determined by symphysio-fundal height measurement and ultrasonography
- The PI and/or study physicians clarified any issue of uncertain physical examination findings.
- Pregnant women were screened for *P.falciparum* parasitaemia using both malaria RDT (First Response®, Premier Medical Corporation, India) and microscopy of thick and thin finger prick blood films.
- Two laboratory technologists, blinded to RDT results, performed the microscopy on different slides.
- Women with both tests positive were recruited after giving written informed consent
- Three finger prick blood spots were collected on Whatman No.1 filter paper (Whatman International, Maidstone, UK)
- The blood spots were air-dried and individually stored in air-tight zip-lock plastic bags with dessicants for later parasite genetic analysis.
- Venous blood was drawn for baseline haemoglobin concentration assessment, platelet count, total white blood cell and differential counts.

- RA administered a questionnaire to gather data on demography, obstetric and gynaecological history, ITN use and knowledge of causes and effects of malaria in pregnancy
- The questionnaire was administered in the local Asante Twi language
- Women with both tests negative went through routine ANC
- Women with only one test positive were treated by a physician according to national treatment guidelines.

3.6.2 Randomization and Study Drug Allocation

Pregnant women were numbered serially during screening and these served as identification (ID) numbers. Those enrolled were then randomized to study arms as described below;

- A statistician computer-generated a list of random numbers that were used as codes to allocate study drugs. Only the PI and RA could interpret these codes and the statistician played no further role in the study.
- This list of random numbers was cut into individual slips and forty of such slips sealed in an opaque envelope. Twenty-five of such envelopes were placed in a larger opaque envelope and kept locked by the PI.
- Enrolled pregnant women randomly picked a slip from an envelope under supervision by RA
- The pregnant woman was then allocated to the study medication coded for by the random number on the slip.
- When the slips in an envelope got finished, another envelope was randomly selected to continue the process

3.6.3 Study Drug Administration

- A research assistant, not involved in drug allocation, administered initial doses of the study drugs at the ANC.
- Participant was observed for 30 minutes and if vomiting occurred during this period, the dose was repeated.
- If the second dose was also vomited, the pregnant woman was withdrawn from the study.
- The remaining doses were given to the participants to take home with detailed instructions on how to store and when to take them.

No extra doses were provided in the event that vomiting occurred at home. Study participants did not receive IPTp-SP even if they were due for it. They resumed IPTp-SP after completion of study follow-up on condition that they had more than a month left to term or if they were withdrawn from study participation. Each participant was given a long lasting insecticide-treated net (LLIN) and encouraged to mount them as soon as they got home if they did not have one already.

3.7 Follow-up Procedures

Scheduled follow-up visits were conducted in the homes of study participants by the PI, RAs and FWs on days 1, 2, 3, 7, 14, 28 and 42 post-treatment, at delivery and 6 weeks post-partum. The residences of the participants were located in the evening of the recruitment day using directional information obtained during recruitment. Where there was difficulty, the participants' cell phone was called and the team received help with further directions.

Visit 1 / Day 1

- Assessment of adverse events (AE) was conducted;
    - Participants were asked if they had experienced any of AEs included in a checklist derived from published studies of the two study drugs.
    - Mentioned AEs not included in the checklist were recorded separately.
    - Severity of AEs was assessed subjectively by asking participants if they were able to carry out their routine household chores
    - If the study team perceived the reported AEs to be mild or moderate, the participant was reassured and encouraged to report back to the hospital if there was no resolution or if there was worsening in the next 12 hours.
    - If the team perceived the reported AE to be severe, the participant was transported to the hospital to be seen by the study physicians
- Study drug packs were inspected as a correlate of adherence
- The participants were physically examined for pallor, jaundice and oedema.

Visit 2 / Day 2

- Participants were questioned on new and persisting AEs and examined clinically as on DAY1
- Adherence to drug doses were also checked

Visit 3 / Day 3

- Same procedures were undertaken as for DAYS 1 and 2.
- Thick and thin blood films were taken for microscopy and filter paper blots prepared.

- Permission was sought to inspect whether they had started using the LLINs given to them.

Visit 4 / Day 7

- Participants were assessed for adverse events and physically examined.
- Thick and thin blood films were taken for microscopy and filter paper blots prepared.
- Venous blood was drawn and stored in ethylene diaminetetracetic acid (EDTA) bottles for assessment of haemoglobin concentration, platelet count and total white blood cell and differential counts.

Visit 5 / Day 14

- Participants were asked for any complaints they may be having and were physically examined.
- Enquiries regarding any antimalarial drug use since the last visit were made
- Thick and thin blood films and filter paper spots were prepared and venous blood drawn for haematology.

Visits 6 and 7 / Days 28 and 42

- Same procedures as for DAY 5
- Participants were asked if they had slept under the provided LLIN the previous night

Subsequent Antenatal and Unscheduled Visits

It was explained to the participants that the scheduled follow-up home visits were not meant to replace ANC attendance and they were encouraged to continue ANC attendance when due irrespective of scheduled follow-up visits. Elemental iron (200mg daily) and folic acid supplementation (4mg daily) were provided for

participants at the ANC as per national guidelines. Participants were encouraged to come to the hospital or contact the PI, study physicians, RAs or FWs all of whose cell phone numbers had been made available to them whenever they felt unwell outside the scheduled home visits.

At Delivery

- Participants were identified by study ID number on their ANC cards when they came to the hospital in labour.
- Midwives conducted the deliveries and collected 1ml of cord blood into EDTA tubes
- A placental segment, about 1cm x 1cm, was cut using a scalpel blade from the maternal surface opposite the insertion of the cord
- Maternal venous blood was sampled and stored in EDTA tubes within 72 hours of delivery
- Thick blood slides were prepared from the cord blood, placental segment and maternal blood
- Birth weight was measured twice using scales (SM-BB20, Microfield Instruments, England) and the mean recorded. Birth weight was recorded irrespective of whether there was a still or live birth
- Newborns were examined for congenital malformations and reports were reviewed by the PI for confirmation
- Gestational age was based on estimated delivery dates (EDD) from first and second trimester ultrasonography
- Newborns were categorized as preterm if they were delivered before 37 weeks.

Postpartum Follow-Up

This was conducted 6 weeks after delivery by PI and field workers in the home of participants to obtain reports of any neonatal adverse events such as neonatal jaundice or other morbidities and mortality.

3.8 Definitions of Serious Adverse Event and Adverse Events

An AE was defined as any unfavourable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom or disease temporarily associated with the use of the study drugs whether or not they were considered related to the study drugs (ICH-GCP Guidelines, 2002). A serious adverse event (SAE) was defined as any untoward medical occurrence in the study that resulted in death, a life-threatening condition, persistent or significant disability/incapacity, hospitalization or any condition requiring an intervention to prevent any of the mentioned outcomes. Congenital/ birth defects were also considered SAEs.

The severity of AEs was assessed as;

i) Mild----------awareness of sign or symptom but tolerated

ii) Moderate------discomforting enough to cause interference with routine activity

iii) Severe--------inability to perform routing activity

The relationship of AEs/ SAEs to study drug was assessed as;

i) Definitely unrelated--------those events which occurred prior to drug administration or those events which cannot be even remotely related to study participation

ii) Unlikely----------There was no reasonable temporal association between the study drug and the event.

iii) Possible----------the event may or may not have followed a reasonable temporal sequence from study drug administration but seems to be the type of reaction that is not easily dismissed

iv) Probable---------the event followed a reasonable temporal sequence from study drug administration, abated upon discontinuation and cannot be easily explained by the known clinical state of the participant

v) Definitely related------events for which there was no doubt about their relationship to study drug administration

3.9 Study Outcome Measures

3.9.1 Primary Outcome Measure

The primary outcome was to demonstrate non-inferiority of DHA-PPQ to ASAQ regarding PCR-corrected efficacy/ failure and safety by days 28 and 42 after start of study treatment

Parasitological failure by day 28 was defined as;

i) a need for rescue medication prior to day 28 follow-up visit

ii) Parasitaemia at day 28 without prior rescue medication

iii) Parasite density greater than baseline between day 3 and day 14

iv) Parasitaemia of any density on days 7 and 14

Similarly, parasitological failure by day 42 was defined as;

i) a need for rescue medication prior to day 42 follow-up visit

ii) Parasitaemia at day 42 without prior rescue medication

iii) Parasite density greater than baseline between day 3 and day 28

iv) Parasitaemia of any density on days 7, 14 and 28

3.9.2 Secondary Outcome Measure

- Changes in maternal haemoglobin concentration, total white blood cell and differential counts and platelet counts at days 14, 28 and 42 over baseline within and between study groups
- Proportions of maternal anaemia (Hb<11g/dl) at days 28 and 42 by study group
- Proportions of severe maternal anaemia (Hb≤ 8g/dl) at days 28 and 42 by study group
- Proportions of adverse and severe adverse events by study group
- Proportions of adverse pregnancy outcomes (spontaneous abortions, preterm deliveries, stillbirths and neonatal mortalities) by study group
- Comparison of mean birth weights in both study arms
- Proportions of neonates with low birth weights (birth weight≤ 2.5kg).
- Proportions of placental malaria (as defined by placental blood film microscopy).
- Proportions of cord blood parasitaemia.
- Sensitivity, specificity, positive and negative predictive values and area under the receiver operating characteristic curve for the First Response® malaria RDT compared to peripheral film microscopy
- Proportions of pregnant women with *P.falciparum* isolates showing SNPs in the *Pfcrt*, *Pfmdr1*, *Pfdhfr* and *Pfdhps* genes.
- A description of pregnant women's experiences and interpretations accrued during participation in the present clinical trial
- A description of factors influencing pregnant women's willingness to participate in clinical trials

3.10 Sample Size Calculation

The treatment efficacy of the active control compared to placebo is needed in calculating sample size for a non-inferiority trial. However, there is no study reporting on efficacy of ASAQ compared to placebo in pregnant women as CQ and SP were available as treatment options for uncomplicated malaria at the time ACTs were introduced.

Treatment efficacy of ASAQ compared to SP was used as a proxy measure; especially as SP is failing due to increasing parasite resistance. The treatment efficacy of ASAQ compared to SP in pregnant women was estimated to be 95.5% over a 28-day follow-up period based on two studies in Tanzania and Ghana (Mutabingwa *et al.*, 2009; Dr. Harry Tagbor, personal communication, 2010). It was determined that an alternative antimalarial drug will still be clinically useful if its treatment efficacy was no more than 5% less than that of ASAQ. A non-inferiority margin of 5% was assumed based on this clinical judgement.

Using the formula below (Pocock, 2003) at 2.5% significance level, the sample size generated had 90% power to demonstrate that the PCR-corrected parasitological treatment efficacy at days 28 and 42 in the DHA-PPQ arm will be non-inferior to that of ASAQ within a 5% non-inferiority margin.

The test treatment, DHA-PPQ, was to be judged non-inferior to ASAQ if the upper limit of the 97.5% confidence interval (based on a 2.5% significance one-tailed test) constructed around the treatment difference between ASAQ and DHA-PPQ is less than the pre-defined non-inferiority margin of 5% (one-tailed 97.5% confidence interval is equivalent to a two-tailed 95% confidence interval).

$$2n = \frac{4x\ 10.5\Pi\ (100-\ \Pi)}{\Delta^2}$$

Where;

Π is the parasitological efficacy (the primary end point for assessment of non-inferiority) of the standard treatment which is 95.5%

Δ is the non-inferiority margin of 5% and **n** is the sample size per arm

$$2\mathbf{n} = \frac{4x\ 10.5x\ 95.5x\ 4.5}{5\ x\ 5}$$

$$2\mathbf{n} = 721.98$$

$$\mathbf{n} = 360.99$$

A sample size of at least 361 pregnant women per arm was needed to demonstrate non-inferiority of DHA-PPQ treatment efficacy compared to ASAQ within a non-inferiority margin of 5%. Assuming a 20% loss to follow-up, the minimum sample size required per arm was calculated to be 452 and the total sample size 904. The chosen margin of loss to follow up was based on an assumption of appreciable attrition, especially due to the tendency of Ghanaian pregnant women to travel to their family homes usually during the last month of pregnancy in preparation for delivery.

Varying sample sizes per study arm for different non-inferiority margins is presented in Table 3.2. A non-inferiority margin greater than 5%, at the same level of power and significance could lead to the conclusion that the test treatment was not inferior to the active control when actually it may be inferior. On the other hand, a margin less than 5% will have resulted in sample sizes too large to be recruited within the time frame of the study (see Table 3.2).

Table 3.2: Sensitivity analysis of sample size with varying non-inferiority margins

Non-inferiority Margin	Resulting sample size per study arm
6%	251
5%	361
4%	564
3%	1003
2%	2256

3.11 Laboratory Procedures

3.11.1 Venous Blood Sample Collection

Sample EDTA tubes were labelled with the date of sampling, day of follow-up and participant's ID number. A tourniquet was applied above the cubital fossa. The area of the vein to be punctured was cleaned with alcohol swabs and 5ml of blood was drawn using a syringe and needle. Sterile cotton was applied to the puncture site and the needle discarded into a biohazard container. The blood was dispelled into the EDTA tube with gentle shaking to mix the blood with anticoagulants.

3.11.2 Rapid Diagnostic Test

A HRP-2-based RDT (First Response®, Premier Medical Corporation, India) was used. The RDTs were performed according to manufacturer's instructions. Briefly, the finger tip was cleaned with an alcohol swab, gently squeezed and pricked with a sterile lancet. The first drop of blood was wiped off with sterile cotton and 5µl of blood added to the sample well using the sample pipette provided in the kit. Two drops equivalent to 60 µl of assay buffer was added to the buffer well. The RDT cassette was placed on a flat surface at room temperature and the results read after 20 minutes.

A positive result was concluded when two colour bands (control and test lines) appeared. A negative result was concluded when only the control line showed without the test line. A test was deemed invalid when the control line did not appear and was repeated.

3.11.3 Haematology

The 5 ml of venous blood in EDTA tubes drawn at enrolment and follow-up visits was used for haemoglobin concentration, total white blood cell and differential count and platelet count determination using an automated haematology analyser (Sysmex Automated Analyser, KX-21N, Sysmex Corporation, Japan).

3.11.4 Liver and Renal Biochemistry

Liver and kidney function tests were not done in the study. Based on literature review, it was determined that the chance of the study drugs causing abnormal liver and renal biochemistry results was minimal. They were therefore planned to be conducted if there were clinical indications such as jaundice, oedema or complaints of oliguria. However, none of these indications were encountered during the trial.

3.11.5 Blood Film Preparation and Microscopy

Thick and thin blood films were prepared on the same frosted glass slides by field staff and midwives at recruitment, on days 3, 7, 14, 28, 42 and at delivery. The frosted ends of the slides were labelled with the date, the follow-up day and the participant's ID number. Thick films were prepared by placing two or three drops of blood obtained either from venepuncture or finger pricks in one half of the microscope slide and using a cover slide to spread them so that it was possible to view print through it.

Thin films were prepared by applying a spreader to a drop of blood. The thin film was fixed for 30 seconds with absolute methanol taking care not to expose the thick film

to the alcohol. The slides were air-dried and stained with 10% Giemsa stain for 30minutes.

A laboratory technologist, blinded to both RDT result at recruitment and study drug allocation, read thick and thin blood films under high power field using oil immersion. One hundred (100) high power fields were examined before a slide was declared negative. Parasite density was determined by counting the number of asexual *P.falciparum* parasites against 200 white blood cells (WBC), assuming a WBC count of 8,000/µl of blood;

Parasite density/µl = Parasite count x 40

For quality control, 15% of all slides were randomly selected and sent for expert microscopy at the Kumasi Center for Collaborative Research in Tropical Medicine (KCCR), Kwame Nkrumah University of Science and Technology, Kumasi. The expert microscopist's reading was taken as final where there were discrepancies.

3.11.6 Filter Paper Blot Preparation

Filter paper blots were prepared by field staff at recruitment and on days 3, 7, 14, 28 and 42. The filter paper (Whatman No.1, Maidstone, England) was cut into strips measuring 2cm x 5cm. One end of the filter paper strip was labelled with the date, participant's ID and follow-up day. Finger prick blood was taken with a sterile lancet after cleaning with alcohol. Three drops of blood were applied to the filter paper strip.

The filter paper strips were air-dried and stored individually in plastic zip-lock bags with silica gel. The bags were in turn kept in a plastic container and stored in an air-conditioned environment at temperatures 16°C-25°C.

3.11.7 DNA Extraction

For the prevalence of molecular resistance markers, only day 0 filter paper blots were used while for assessing the status of recurrent infections, day 0 and follow-up samples were used. Extraction of DNA from filter paper blots was performed according to the Chelex-100 method as described by Wooden *et al* (1993) with some modifications described by Pearce *et al* (2003). Briefly, about a half sector of the filter paper spot was cut out and incubated in 0.5% saponin (SIGMA^{TM}) in 1X phosphate buffered saline (PBS) at room temperature overnight. The supernatant was discarded and the spots washed in 0.8ml of PBS. Extraction of DNA was done by the Chelex-100 resin method. The resulting DNA supernatant was transferred out to a 96-well PCR plate and stored in a freezer at -20°C until further use.

3.11.8 Polymerase chain reaction

Polymerase chain reaction amplifications were performed in 96-well PCR plates in a thermal duo-cycler (VWR-Bie, Berntsen, Denmark). To the PCR products in each well, 4µl of a solution of biomarker and gel green stain was added. The final solution was run on 1.5% or 2% agarose gel and visualized under ultraviolet illumination in a gel imaging system (Geldoc System, Bio-Rad Laboratories, Denmark). Laboratory parasite strains 3D7, HB3, FCR3, DD2 and blood samples obtained from Danish citizens who have never been exposed to malaria were used as controls.

3.11.8.1 Amplification of *msp2* and *glurp* genes

Polymerase chain reaction amplification of the *msp2* and *glurp* genes was carried out according to methods described by Snounou *et al* (1999). The 20µl/sample outer *msp2* mixture comprised 10µl of TEMPase mastermix (Ampliqon, VWR-Bie, Berntsen, Denmark) containing 2.5mM Magnesium Chloride, 0.2mM of each dNTP Polymerase, 1.25 units of DNA Polymerase, 8µl of distilled water, 1µl 0.06µM outer

primer mix and 1µl of template DNA. Outer and nest PCR products were stored in a freezer at -20°C until further use. The amplification conditions and primer sequences are provided in the appendix.

3.11.8.2 Amplification of *Pfcrt, Pfmdr-l, Pfdhfr* and *Pfdhps* genes

Amplification of segments of the *Pfcrt* gene was done by a nested PCR procedure as described by Djimde *et al* (2001). The 20ul *Pfcrt* outer PCR mixture comprised a TEMPase buffer containing 2.5mM Magnesium Chloride and 0.2mM of each dNTP Polymerase, 1µM concentration of primers, 1.25 units of TEMPase DNA Polymerase and 1µl of template DNA. Nested PCR used similar volumes and conditions except for a reduction in the number of cycles to 30 and 48°C annealing temperature. The nest PCR primer was biotinylated at the 5` end at manufacture (MWG Biotech, Denmark).

For *Pfmdr1* codons 86, 184 and 1246, outer and nested PCR was performed as described by Duraisingh *et al* (2000) and Humphrey *et al* (2007). Similarly, amplification at the *Pfdhfr* and *Pfdhps* genes were conducted as described by Alifrangis *et al* (2005). One of the primers used in the nested PCR for *Pfdhfr* was also biotinylated at manufacture. Outer and nest PCR products were stored in a freezer at -20°C until further use. The amplification conditions and primer sequences are provided in the appendix.

3.11.9 Sequence Specific Oligonucleotide Probe---Enzyme-linked Immunosorbent Assay (SSOP-ELIZA) for *Pfcrt, Pfdhfr* and *Pfdhps*

Analysis of SNPs at the *Pfcrt, Pfdhfr* and *Pfdhps* genes was performed according to the method described by Alifrangis *et al* (2005) with slight modifications. ELISA plates (Maxisorp; Nunc, Roskilde, Denmark) pre-coated with streptavidin in

phosphate-buffered saline (PBS) (1 μg/mL) were washed three times in washing buffer (1× PBS containing 0.05% Tween 20).

The nested PCR products were diluted (1:3 for *Pfcrt* and 1:10 for *Pfdhf*r/ *Pfdhps*) in water in a 96-well PCR plate, denatured at 95°C for 5 minutes, and immediately thereafter cooled to 4°C until use. One hundred microliters of washing buffer and 2 μL of the diluted PCR products (4μl for *Pfdhfr* codon 50/51) were added to each well. Replicate ELISA plates were made to enable simultaneous probing with SSOPs targeting the full panel of *dhfr*, *dhps*, and *Pfcrt* SNP/haplotypes. The plates were incubated at room temperature for one hour and washed three times in washing buffer.

Digoxigenin-conjugated probes (MWG Biotech) were diluted in tetra-methyl ammonium chloride solution (TMAC) (Sigma Aldrich Chemie, Seelze, Germany), heated to 53°C, and 100 μL was then added to each well. The probe-TMAC solution was prepared by adding 24μl of 2μM probes in 12ml of 4nM TMAC (probe concentration was 10μM for *Pfdhfr* condon 50/51).

The plates were then incubated in a hybridization oven (Continental Lab Products, USA) at 53°C on a shaking device for one hour and washed three times in washing buffer. This was followed by two rounds of washing and incubation (10 minutes per round) in TMAC solution Peroxidase-conjugated anti-digoxigenin antibody in dilution buffer (1:1,000) (Roche Diagnostics, Mannheim, Germany) was added to each well.

After incubation for one hour at room temperature, the plates were washed three times in washing buffer and 3 tablets of 1, 2-phenyldiamine dihydrochloride (Dako, Glostrup, Denmark), 12ml of distilled water and 5μl of 30% hydrogen peroxide, was added to each well of each ELISA plate for visualization The reaction was stopped

after 30 minutes by adding 50µl of 2.5M M H_2SO_4 and the optical density (OD) at 492 nm measured in an ELISA reader (Versamax Absorbance ELISA reader, Molecular Devices, USA).

Positive and negative control OD values were compared to define a threshold for positive or negative results. Samples were interpreted as a single haplotype when either only CVMNK or CVIET was present at OD values above the positivity threshold. Samples were considered mixed but with a majority haplotype when the OD value of the weakly reacting SSOP was less than half the OD value of the strongly reacting SSOP. Samples were considered to have no dominant haplotype if the OD value of the weakly reacting SSOP was higher than half the OD value of the strongly reacting SSOP.

Samples with negative results were repeated with regards to amplification and SSOP –ELISAs. Data was entered in Microsoft Excel 2007 and analysis made based on simple proportions for prevalences and frequencies.

3.11.10 Determination of SNPs at *Pfmdr1* codons 86, 184 and 1246 by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)

Detection of SNPs at the specified codons was performed by restriction enzyme digestion of nest *Pfmdr1* PCR products in total volumes of 22µl/sample as described in Thompsen *et al* (2011). Nested *Pfmdr1* PCR products were initially run (electrophoresis) on 1.5% agarose gel and the strength of the bands, as visualized by ultraviolet light illumination, was used to determine the volume of nest *Pfmdr1* products to be used in the digestion process.

Less of the nested products were used for strong-looking bands. The restriction enzymes used were Afl111 and Apo-1 for codon 86, EcoRV and BGL11 for codon 1246 and DRA-1 for codon 184 (New England Biolabs, Ipswich, MA).

3.12 Pregnant Women's Perceptions of Clinical Trial Participation

Indepth interviews were conducted with three different groups of participants. Group 1 comprised of 20 women from the Bosomtwe district who had participated in the clinical trial. They were purposively selected based on easily accessible residence. Women who had delivered at least 2 months before the interview were chosen on the assumption that they had gone through the entirety of the clinical trial processes and so were in a position to give their opinions/ perceptions on all stages of the trial. This group of women were visited and interviewed in their homes. Women from Bekwai municipality were excluded from the qualitative study on account of logistical challenges.

Group 2 was made up of 20 pregnant women, irrespective of age, gravidity or gestation, conveniently selected and interviewed at the ANC clinic at St. Michael's hospital. The women in this group were presented with a verbal description of the ***aims, procedures, risks and benefits*** of the drug trial and asked what would inform their decisions to either join or refuse to join such a study. None of these women were recruited to participate in the trial.

Group 3 was made up of a convenient sample of husbands/ partners of the first five women from group 1. Where a husband/ partner was not available or declined to be interviewed, that of the next woman was chosen. Husbands/ partners were interviewed to obtain their opinions of their wives/ partner's participation in the study.

This is relevant as the consent of household heads may sometimes be required for participation in trials.

In-depth interviews were conducted from October to December, 2012. Interview guides for the different groups were developed in accordance with literature and cultural context and addressed themes pertaining to reasons for willingness to participate in the trial, knowledge about malaria and experiences and interpretations constructed of the trial processes such as home visits and blood draws at home.

The interviews were conducted by two interviewers (male and female) with previous experience with qualitative research methods. The PI and two social scientists at the Department of Community Health, Kwame Nkrumah University of Science and Technology trained (seven days) the interviewers on the objectives and inherent procedures in the trial, community entry and use of the interview guides. Three pilot interviews were conducted outside Bosomtwe to help refine the interview guide.

The interviews, lasting 30-60 minutes each, were conducted in the local Asante Twi language and tape recorded with the consent of the participants. When needed questions were repeated and answers probed to ensure the right meaning was recorded. Non-verbal communication was noted in field books. The taped recordings were transcribed verbatim into English by the interviewers and transcripts later checked for accuracy of translation and retention of meaning by the social scientists.

3.13 Data Management

3.13.1 Data Collection and Entry

Data was collected by field workers and midwives at recruitment at ANC clinic, on home visits and at delivery as described earlier. The PI and research assistants

checked CRFs for completeness and consistency. Case record forms and source documents were kept confidential, identified solely by ID numbers and kept in a locked file cabinet to which only the PI and one RA had access. Data was double-entered in a Microsoft Access interphase, cleaned and cross-checked for inconsistencies and missing data. The dataset was exported into Stata/IC 12.1 (Stata Corporation, College Station, Texas) for validation and analysis.

3.13.2 Data analysis

The principal analysis of parasitological efficacy and the primary outcome (demonstrating non-inferiority of DHA-PPQ to ASAQ by day 42 and day 28) was per protocol (PP) but analysis in modified intention-to-treat populations (mITT) was also undertaken. Analysis of secondary outcomes was also "intention-to-treat". Parasitological efficacy was based on assessment of treatment successes.

The PP population included randomized participants who took, at least, 2 days course out of the 3-day course of assigned treatments, had at least 4 follow-up visits with no major protocol violation and for whom parasitological outcome was recorded for days 28 and 42. The mITT populations included all randomized participants who took, at least, the first dose of the assigned treatment under observation at recruitment and who had any outcome including parasitological outcome recorded at day 28 or day 42 post-treatment with study drugs.

The justification for using both PP and mITT populations for the primary analysis lies in the fact that per protocol analysis alone is associated with biases while analysis in the intention-to-treat population (ITT) alone often leads to dilution of treatment effects and subsequent observation of smaller between-group differences with considerable risk of falsely concluding non-inferiority (ICH, 1998; Jones et al., 1996).

Demonstration of non-inferiority in both populations increases confidence in the results obtained.

Baseline demographic and clinical characteristics of study participants were summarized by treatment group using frequencies, means and proportions. Means and proportions were computed as point estimates with 95% confidence intervals or standard deviations while median measurements were described using the range of observations for a particular variable. Study variables were assessed for normality using histograms with superimposed normal curves. Parametric and non-parametric statistical methods were then applied depending on the distribution observed. Relationships between binary exposure and outcome variables were analysed with Chi-square tests of independence and 2-sided p-values presented with significance at 5% level.

Paired t-tests were used to compare means within study arms over time while unpaired t-tests were used to compare means between study arms. The differences of means were provided with 95% confidence intervals and 2-tailed p-values. Univariate logistic regression was used to generate odds ratio (OR) for associations between binary outcome variables and continuous or categorical exposure variables. The Mann-Whitney U test was used to compare non-normally distributed variables between groups.

The hypothesis tested was that the test treatment DHA-PPQ is not worse than the active comparator ASAQ within a defined margin of 5% with respect to parasitological efficacy by days 28 and 42. The primary concern was to establish that the treatment difference between ASAQ and DHA-PPQ arms supported a conclusion of non-inferiority within the pre-specified 5% margin.

The upper limit of the two-sided 95% confidence interval constructed around the treatment difference was compared to the non-inferiority margin of 5% and it was concluded there was evidence to support non-inferiority of the test treatment if it was less than 5%.

Parasitological efficacy analysis was restricted to PCR-uncorrected estimates as differentiation of new and recrudescent infections was unsuccessful. Cumulative parasitological efficacy for DHA-PPQ and ASAQ were based on simple proportions of treatment success by day 28 and day 42. Demonstration of non-inferiority was to be based on PCR-corrected parasitological efficacy at day 42 but this could not be reported as indicated earlier.

3.13.2.1 Safety Analysis

Reported SAEs/ AEs were analysed by treatment group on days 3, 7 and 14 after start of treatment and chi-square tests of independence was used to assess significance of differences in proportions of AEs. Changes in the means of haemoglobin and total white blood cell/ differential counts at days 14, 28 and 42 over baseline within and between study arms were analysed descriptively and differences tested for significance.

3.13.2.2 Molecular Analysis

For the molecular markers of resistance, simple proportions were calculated for prevalence and frequency of occurrence of the single nucleotide polymorphisms assayed for. Prevalence included while frequency excluded mixed infections. An infection was categorized as mixed when more than one haplotype or allele occurred at a gene locus or codon of a gene. Prevalence and frequency of alleles and haplotypes were presented in graphs and tables.

3.13.2.3 Analysis of Qualitative Data

The transcripts of in-depth interviews conducted were manually coded independently by the PI and one social scientist. Analysis was based on a deductive framework method approach (Gale *et al*., 2013) involving the use of pre-defined themes based on previous literature and pre-conceived notions mainly. Novel themes/ concepts representing unexpected participant experiences and social constructs were also taken into congnisance. Disagreements over meanings were settled by discussion and consensus building. Themes were examined for associations and meanings in terms of relevant contexts. The framework method was deemed suitable for analysis of the qualitative data as similar key topics were discussed with the participants and it allowed for categorization of codes and/or responses.

3.14 Ethical Considerations

Ethical approval for the study was granted by the Committee for Human Research and Publication Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science and Technology with the initial approval reference number CHRPE/190/10. A protocol amendment for the combined purposes of including the additional recruitment site, conducting parasite genetic analysis and the qualitative study was subsequently approved with the reference number CHRPE/AP/236/12. The study had clinical trials registration number NCT01231113.

The management teams of Bosomtwe district and Bekwai municipality health directorates gave permission for the study to be conducted in their respective areas. The management teams of St.Michael’s and Bekwai Government hospitals gave written consent to the use of their ante-natal clinics for recruitment.

Eligible pregnant women had the study aims, risks and benefits explained to them in the local Asante Twi language by the study staff. It was emphasized that they were not under any obligation to participate and that refusal to participate was not going to attract any punitive measures, especially in relation to accessing health care from the two hospitals.

It was also explained that even if they agreed to participate, they were still free to withdraw from the study at any time without having to give reasons and without reprisals.

Participants were given a participant information leaflet (PIL) and written informed consent was obtained by signing or thumb printing. Each study participant was given one long-lasting insecticide-treated bednet and accessing health care in the event of clinical illness was facilitated by study physicians at the two recruitment sites.

Data collected was kept confidential and participants were only identified by ID no. / randomization numbers. Participants were provided with the mobile phone numbers of the principal investigators and study team members and every effort was made to address concerns from participants, their husbands/ sexual partners and other relatives as they ensued.

CHAPTER FOUR

EFFICACY AND SAFETY ASSESSMENTS OF DIHYDROARTEMISININ-PIPERAQUINE AND ARTESUNATE-AMODIAQUINE IN PREGNANT WOMEN

4.1 Introduction

This chapter presents primarily the results of analysis of data relating to the efficacy and safety of DHA-PPQ and ASAQ. Safety assessment was based on clinical adverse events and haematological evaluation. Efficacy assessment incorporated parasitological efficacy, mean birth weight/ proportion of low birth weights and changes in maternal haemoglobin level at days 28 and 42 over baseline. The chapter also includes an assessment of the diagnostic accuracy of the First Response RDT used at screening.

4.2 Screening, enrollment and follow up

A total of 3464 pregnant women in the second and third trimesters were screened at the two study sites using First Response® malaria RDT and peripheral blood film microscopy between July 2011 and October 2012. Of these, 3047 did not meet inclusion criteria for various reasons including negative RDT results, refusal to give consent and twin pregnancies. In all 588 had positive RDT tests giving an RDT-based *P.falciparum* prevalence of 17%. Two hundred and eighty-two (282) had positive blood films but negative RDT results.

A total of 417 pregnant women satisfied inclusion criteria, were enrolled and randomized to receive ASAQ (205) and DHA-PPQ (212). Participant flow through the initial 42-day period is shown in Fig 4.1. By day 28, the number of participants had decreased to 382 and to 371 by day 42 due mainly to losses to follow-up.

About 88% (368/417) of the enrolled participants constituted the per protocol population for primary efficacy analysis over the 42-day follow-up period. Randomized pregnant women who had outcomes at day 28 or day 42 constituted modified "intention-to-treat" populations for analysis of parasitological efficacy in addition to the per protocol analysis. Only 56.8% (237/417) and 42.2% (176/417) of the enrolled participants were available for assessments at delivery and at completion of the 6-week post-partum period respectively.

The overall prevalence of parasitaemia as defined by both RDT and blood film microscopy was 12% (417/3464). Regarding quality control for blood film microscopy, there was 89% agreement between the study and expert microscopists resulting in a kappa statistic of approximately 0.6 suggesting moderate agreement.

Baseline demographic and clinical characteristics of the study participants are presented in tables 4.1a and 4.1b respectively. Over 80% of participants had formal education up to the primary level at least while more than 50% were either primigravidae or secundigravidae. Close to a third of the participants were aged 20-24 years and about 60% owned ITNs. About 65.3% (252/386) of the pregnant women were anaemic (Hb<11g/dl) at baseline.

Randomization was largely successful as there were no differences between study arms with respect to almost all variables assessed. The exceptions with statistically significant differences were age categorizations (p=0.05), haemoglobin concentration categorization (p=0.04) and mean total white blood cell count ($6.3x10^3$ vs $6.6x10^3$; p=0.04). The percentage of women with haemoglobin range 7.0-8.9g/dl in the DHA-PPQ arm was close to twice the percentage in the ASAQ arm.

About 84.4% had baseline parasite density <500/µl. Baseline parasite density was associated with parity (p=0.003), haemoglobin concentration (Hb) categorized as shown in Table 4.1b (p=0.02) and ownership of ITNs (p=0.04). Parasite density was independent of age (p=0.09), gestational age (p=0.80) and gravidity (p=0.14). Gestational age was associated with Hb concentration (p=0.03).

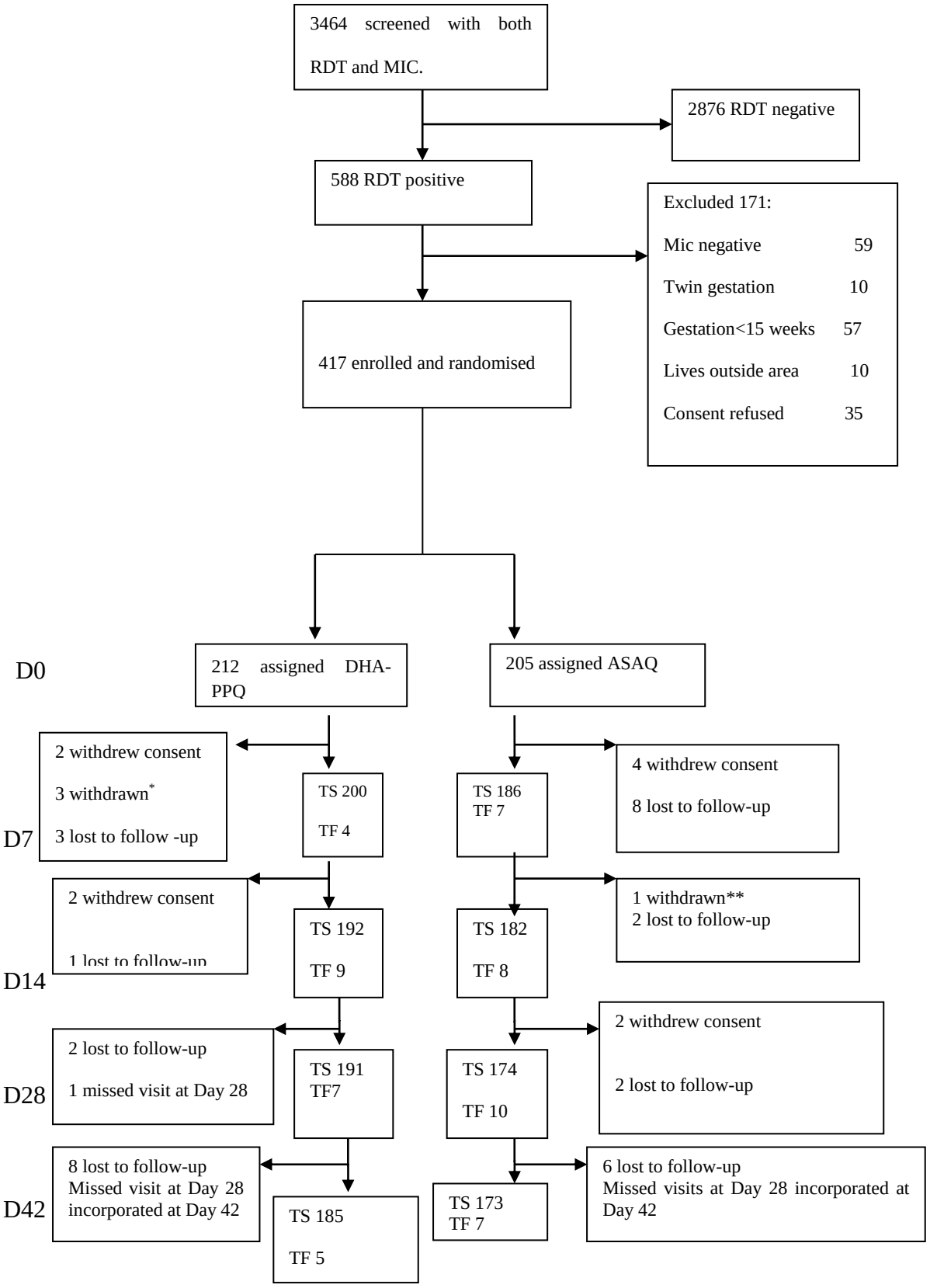


Fig 4.1: Participant flow through the initial 42-day follow- up period

*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.

Table 4.1a: Demographic characteristics of study participants at enrolment

Variable	ASAQ (%)	DHA-PPQ (%)	p
Education (N=415)			0.91
None	24 (11.8)	20 (9.5)	
Primary	29 (14.2)	32 (15.2)	
[a]Junior High School	129 (63.2)	135 (64.0)	
[b]Senior High School	19 (9.3)	19 (9.0)	
Tertiary	3 (1.5)	5 (2.4)	
Occupation (N=413)			0.93
Traders	72 (35.3)	72 (34.5)	
Artisans	58 (28.4)	56 (26.8)	
Teachers	6 (2.9)	8 (3.8)	
Farmers	68 (33.3)	73 (34.9)	
Age in years (417)			0.05
15-19	40 (19.5)	29 (13.7)	
20-24	56 (27.3)	77 (36.3)	
25-29	56 (27.3)	67 (31.6)	
≥30	53 (25.9)	39 (18.4))	
Mean Age (SD)	25.5 (6.0)	25.0 (5.2)	0.34
Gestational Age (N=405)			0.61
<24 weeks	111 (56.4)	112 (53.9)	
≥24 weeks	86 (43.7)	96 (46.2)	
Mean Gestational Age (SD)	22.2 (4.7)	22.7 (4.9)	0.33
Gravidity (N=416)			0.33
1	48 (23.4)	55 (26.1)	
2	52 (25.4)	66 (31.3)	
(3&4) combined	69 (33.7)	56 (26.5)	
≥5	36 (17.6)	34 (16.1)	
Parity (N=416)			0.12
Nulliparous	54 (26.3)	74 (35.1)	
Primiparous	46 (22.4)	47 (22.1)	
Multiparous	105 (51.2)	90 (42.7)	
ITN Ownership (403)			0.84
Yes	117 (58.8)	122 (59.8)	
No	82 (41.2)	82 (40.2)	
Slept in ITN night before survey (N=340)			0.19
Yes	61 (36.8)	76 (43.7)	
No	105 (63.3)	98 (56.3)	

[a] Junior High School; 3 years on top of primary school. [b] Senior High School; 3 years on top of Junior High School.

Table 4.1b: Clinical characteristics of study participants at enrolment

Variable	ASAQ (%)	DHA-PPQ (%)	p
Parasite Density (N=405)			0.99
<500/µl	169 (84.5)	173 (84.5)	
≥500/µl	31 (15.5)	32 (15.5)	
Geometric Mean Parasite Density	238 (95%CI;199, 285)	236 (95%CI; 193, 289)	0.28
Haemoglobin (g/dl)(N=386)			0.04
7-8.9	15 (7.8)	28 (14.5)	
9-10.9	115 (59.6)	94 (48.7)	
≥11	63 (32.6)	71 (36.8)	
Mean Haemoglobin (g/dl)	10.1 (95%CI; 9.9, 10.2)	10.0 (95%CI; 9.8, 10.2)	0.44
[a]**Total WBC ($x10^3$)**	6.3 (95%CI; 6.0, 6.5)	6.6 (95%CI; 6.3, 6.8)	0.04
Platelet Count ($x10^3$)	167.8 (95% CI; 159, 176.5)	179.7 (95%CI; 166.4, 193)	0.14
Neutrophils (%, SD)	(64.0, 12.4)	(64.1, 13.4)	0.92
Lymphocytes (%, SD)	(27.0, 9.0)	(27.0, 10.4)	0.99
[b]**Pre-enrolment Complaints**			
Anorexia	64 (31.5)	73 (34.9)	0.46
Nausea	48 (23.7)	52 (24.9)	0.77
Vomiting	49 (24.1)	45 (21.5)	0.53
Abdominal Pains	120 (58.8)	120 (57.4)	0.77
Diarrhoea	25 (12.4)	25(12.0)	0.90
Dizziness	64 (31.4)	65 (31.1)	0.95
Headaches	135 (66.5)	133 (63.6)	0.54
General Body Weakness	110 (53.9)	108 (51.7)	0.65
Fever	76 (37.4)	75 (35.9)	0.74
Heartburns	53 (26.2)	54 (25.84)	0.93
Itching	43 (21.2)	41 (19.62)	0.69
Palpitations	62 (30.5)	68 (32.7)	0.64
Cough	50 (24.6)	51 (24.8)	0.98

[a] Total white blood cell and differential counts are presented as means. [b] refer to complaints/ symptoms women had experienced and judged most important over the month preceding enrolment and are presented as number and percentage. Individual pregnant women mentioned more than pre-enrolment complaint. This explains why total percentages for the various complaints (in brackets) total more than 100% in each study arm

4.2.1 Diagnostic accuracy of the First Response® RDT used at screening

First Response® RDT results of 1800 randomly selected women were compared with the results of expert microscopy of corresponding peripheral blood films prepared at the time the RDTs were conducted. The random selection was done using the randomization facility in STATA 12. The expert microscopist was blinded to RDT results. A total of 1664 blood films were determined to be of good quality and were included in analysis.

Fig 4.2 shows the study profile for the First Response® RDT diagnostic accuracy assessment. About 96% (91/95) of microscopy positive films showed *P.falciparum* for which parasite density ranged 40/µl -69680/µl. The remaining four (4) positive blood films showed *P.malariae* and *P.ovale* and these were excluded from analysis. The geometric mean parasite density was 1587/µl (95%CI; 961, 2618).

Table 4.2 shows the performance of First Response® malaria RDT compared to expert peripheral microscopy in the diagnosis of malaria infection in pregnant women. First Response® RDT showed a sensitivity of 82.1% (95%CI: 72.9, 89.2), specificity 82.1% (95%CI: 80.0, 84.0) and positive and negative predictive values of 21.7% (95%CI: 17.6, 26.4) and 98.7% (95%CI: 97.9, 99.2) respectively. The area under the receiver operating characteristic (ROC) curve was 0.82 (95%CI: 0.78, 0.86).

Of the 17 slides corresponding to false negative First Response® RDT results, 5 had parasite density 40/µl, 8 had 80/µl and one each had parasite density 120/µl, 160/µl, 240/µl and 760/µl respectively.

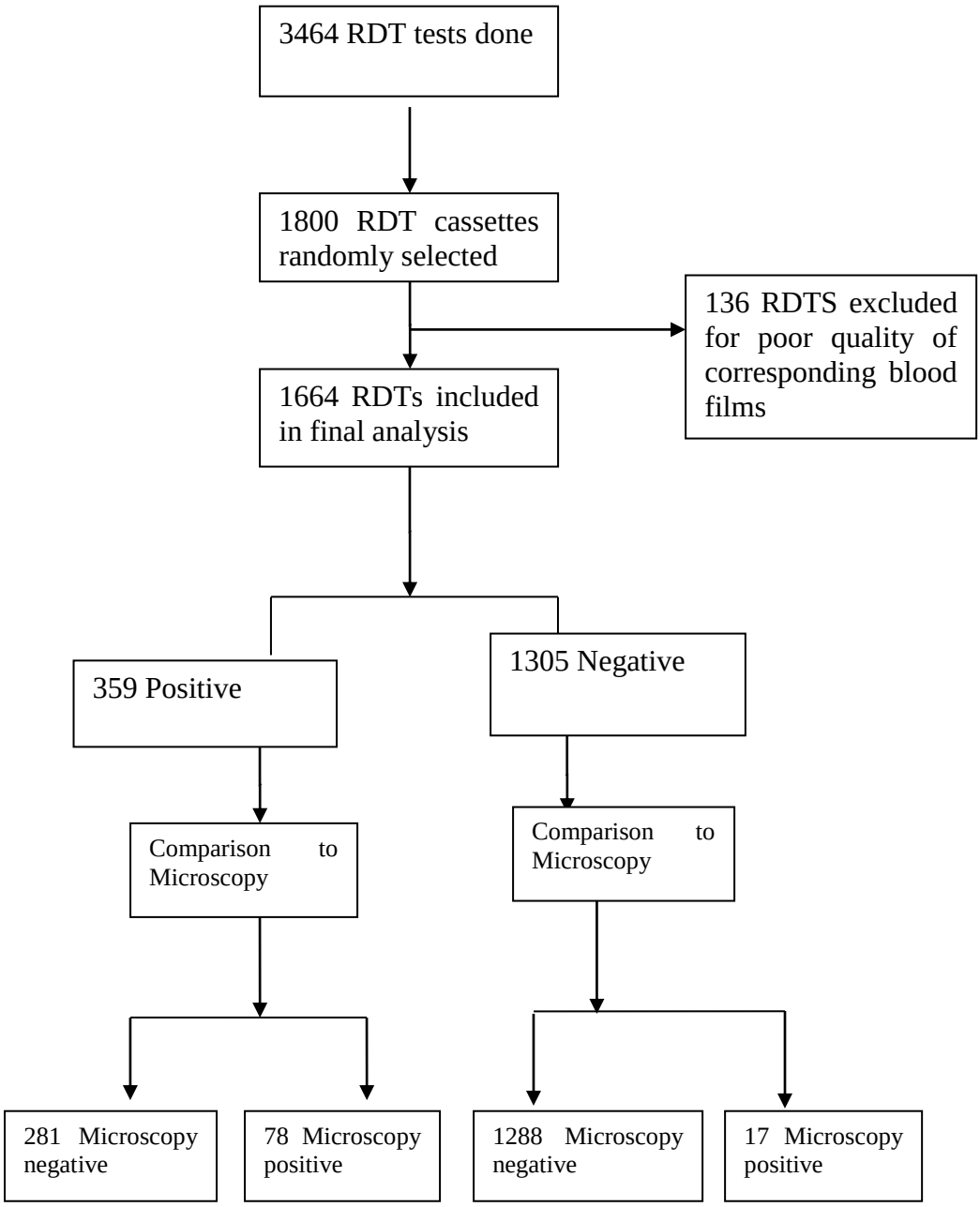


Fig. 4.2 Study profile for First Response® RDT diagnostic accuracy assessment

Table 4.2: Performance of First Response® RDT compared to peripheral microscopy for detecting malaria infection in pregnant women

	Microscopy		
	Negative	Positive	Total
First Response® RDT			
Negative	1288	17	1305
Positive	281	78	359
Total	1569	95	1664

Variability of diagnostic accuracy between sub-groups of participants was not assessed because there were only a few positive blood films and sub-groups would have been too small in number for any meaningful analysis. For instance, only 6 out of the 91 participants with *P.falciparum* on microscopy had parasite density <50/µl while 15 had parasite density <100/µl. In addition, variability of diagnostic performance according to season was also not assessed but the rainy season seemed to favour positive RDT outcomes as most positive RDT results (264/359) were recorded during that period (late March to November). However, of a total of 1567 tests conducted in the rainy season, less than a fifth (264/1567) were positive while almost all tests (95/97) conducted in the dry season were positive.

Pregnant women with parasite density >500/µl were more likely to have a positive RDT result compared to those with densities ≤500/ µl (RR 5.2, 95%CI: 4.7, 5.8; p<0.0001). In contrast, multiparous women (RR 0.63, 95%CI: 0.45, 0.87; p=0.006) and those aged ≥30 years (RR 0.29, 95%CI: 0.22, 0.38; p<0.0001) were less likely to show positive RDT results compared to nulliparous women and participants aged 15-19 years. There was no significant difference between primiparous and nulliparous women regarding the likelihood of a positive RDT outcome (RR 0.69, 95%CI: 0.47, 1.01; p=0.06).

4.3 Parasitological efficacy assessment

Parasitological efficacy (defined by treatment success) was assessed first by peripheral blood film microscopy to determine treatment success by days 28 and 42 and subsequent PCR genotyping of paired baseline/ treatment failure samples to adjust for recrudescence and reinfections. Analysis was done in the per protocol and modified intention-to-treat populations that have been defined under the section on data analysis (3.13.2).

4.3.1 Analysis according to protocol

Table 4.3 shows PCR-unadjusted cumulative parasitological efficacy estimates for DHA-PPQ and ASAQ by days 28 and 42 with 95% confidence intervals in the per protocol population. The efficacy estimate for DHA-PPQ by day 28 was 91.6% (95%CI: 86.7, 95.1). This dropped to 89.0% (95%CI: 83.6, 93.0) by day 42. Artesunate-amodiaquine showed similar PCR-uncorrected efficacy to DHA-PPQ on both days. Treatment failure rates were 8.4% (16/190) and 11.0% (21/190) by days 28 and 42 respectively for DHA-PPQ and 10.7% (19/178) and 13.5% (24/178) for ASAQ. Estimates for differences in treatment efficacy and the 2-sided 95%CI (equivalent to a one-sided 97.5%CI) around the differences are also provided and show sufficient evidence to support non-inferiority of DHA-PPQ to ASAQ. The upper limits of the 2-sided 95% CI are less than the pre-defined non-inferiority margin of 5%.

Though not required per the study protocol, non-inferiority of DHA-PPQ to ASAQ was also demonstrated by days 28 and 42 for crude efficacy estimates and estimates adjusted for baseline maternal characteristics (Table 4.4) based on 90% and 99% confidence intervals around the noted risk differences. The risk differences and their

various confidence intervals showing non-inferiority or otherwise are illustrated graphically in Fig. 4.3.

None of the baseline variables; age, gestational age, gravidity, parasite density and haemoglobin concentration was associated with parasitological outcome by days 28 and 42 (Table 4.5). At both time points, secundigravidae appeared to have about twice the risk of treatment failure compared to primigravidae but these were not statistically significant; {adjustedRR 2.43 (95%CI; 0.92, 6.40), p=0.07} by day 28 and {adjustedRR 1.92 (95%CI; 0.89, 4.17), p=0.55} by day 42.

Table 4.3: PCR-unadjusted parasitological efficacy estimates by days 28 and 42

	ASAQ (N=178)	**DHA-PPQ (N=190)**	**Risk Difference for Parasitological efficacy**	**95%CI of Risk Difference**
Day 28				
Treatment success (n)	159	174		
Parasitological efficacy (95%CI)	89.3% (83.8, 93.5)	91.6% (86.7, 95.1)	-2.3	(-8.3, 3.8)
Day 42				
Treatment success (n)	154	169		
Parasitological efficacy (95%CI)	86.5% (80.6, 91.2)	89.0% (83.6, 93.0)	-2.5	(-9.1, 4.3)

Table 4.4: 90%, 95% and 99% Confidence Intervals around treatment efficacy differences between ASAQ and DHA-PPQ by days 28 and 42

	Risk Difference (RD)	[&]90% CI (RD)	95% CI (RD)	99% CI (RD)
Day 28 crude	-2.3	(-7.3, 2.8)	(-8.3, 3.8)	(-10.2, 5.7)
Day 28 adjusted[*]	-3.5	(-7.7, 0.7)	(-8.5, 1.5)	(-10.0, 3.1)
Day 42 crude	-2.5	(-8.1, 3.2)	(-9.1, 4.3)	(-11.3, 6.4)
Day 42 adjusted	-3.9	(-9.4, 1.6)	(-10.4, 2.7)	(-12.5, 4.7)

[&]90% and 99% Confidence Intervals, though not required, are presented to show that non-inferiority is also demonstrated with them

[*] 'adjusted' refers to efficacy/ risk difference estimates adjusted for baseline maternal characteristics; age, gestational age, gravidity, haemoglobin concentration and parasite density in binomial regression

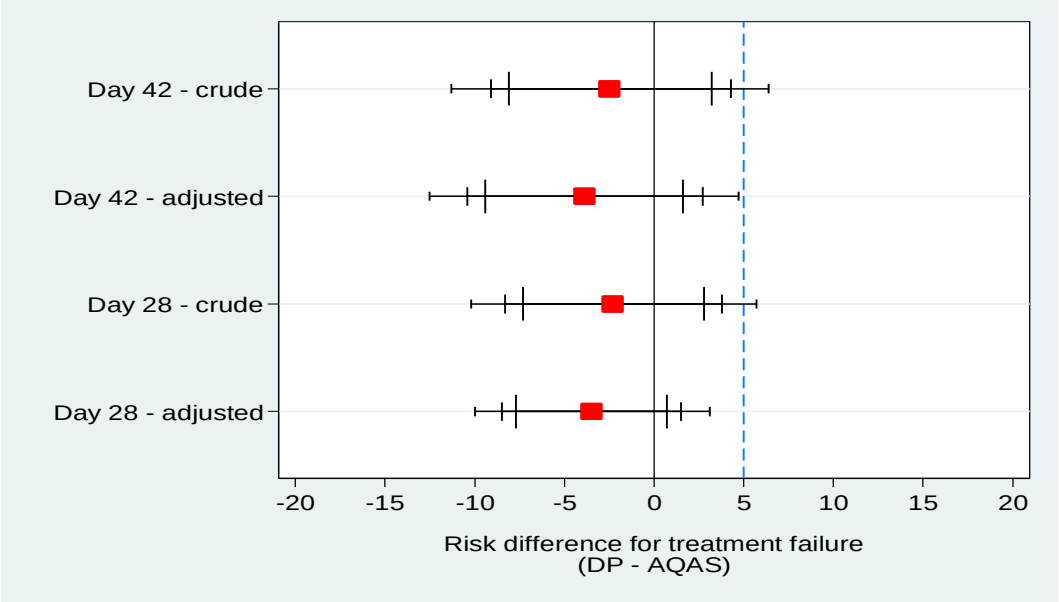


Fig 4.3. Illustration of non-inferiority of DHA-PPQ compared to ASAQ based on 90%, 95% and 99% CI of risk differences for crude and adjusted efficacy estimates by days 28 and 42.

(The blue line indicates the pre-defined non-inferiority margin of 5%. For confidence intervals, long bars represent 90%CI, short bars 95%CI and the smallest bars 99%CI)

Table 4.5: Baseline factors assessed for association with parasitological outcome by days 28 and 42

	Day 28			Day 42		
variable	**Adjusted RR**	**95% CI**	**p-value**	**Adjusted RR**	**95% CI**	**p-value**
Age	1.02	(0.96, 1.07)	0.60	1.00	(0.95, 1.05)	0.95
Gestational age	1.00	(0.93, 1.07)	0.95	1.00	(0.95, 1.06)	0.86
Gravidity	1.02	(0.89, 1.21)	0.82	0.95	(0.81, 1.12)	0.55
Parasite Density	1.50	(0.69, 3.27)	0.30	1.50	(0.77, 2.94)	0.24
Haemoglobin level	1.09	(0.64, 1.87)	0.74	0.87	(0.55, 1.37)	0.55

4.3.2 Analysis in modified intention to treat populations

Parasitological efficacy analysis was also carried out in modified "intention to treat" populations (mITT). The mITT populations were constituted by all randomized participants who took the first dose of assigned treatment and had outcome on day 28 or day 42.

Efficacy estimates obtained were similar to results observed in the "per protocol" analysis. Crude cumulative efficacy by day 28 in the DHA-PPQ arm was 90.4% (95%CI: 85.5, 94.2) and was similar to that in the ASAQ arm; 89.1% (95%CI: 83.7, 93.2). Estimates for day 42 were also similar between the study arms as shown in Tables 4.6. Cumulative treatment failure rates were 9.6% (19/199) and 12.6% (24/191) by days 28 and 42 respectively for DHA-PPQ while for ASAQ, they were 10.9% (20/184) and 13.3% (24/180)

Non-inferiority of DHA-PPQ was demonstrated for day 42 adjusted but inconclusive for day 42 crude. Treatment difference by day 28 showed DHA-PPQ was non-inferior to ASAQ based on the 2-sided 95% confidence intervals around the respective treatment differences (see Tables 4.7 and Fig 4.4). Again, parasitological outcome by days 28 and 42 was not associated with maternal age, gestational age, gravidity or baseline parasite density in the "modified intention to treat" analysis (see Table 4.8).

Table **4.6** PCR-unadjusted parasitological efficacy estimates for mITT populations by days 28 and 42

	ASAQ (N=184)	**DHA-PPQ (N=199)**	**Risk Difference for Parasitological efficacy**	**95%CI of Risk Difference**
Day 28				
Treatment success (n)	164	180		
Parasitological efficacy (95%CI)	89.1% (83.7, 93.2)	90.4% (85.5, 94.2)	-1.3	(-7.4, 4.8)
	ASAQ (N=180)	**DHA-PPQ (N=191)**	**Risk Difference for Parasitological efficacy**	**95%CI of Risk Difference**
Day 42				
Treatment success (n)	156	167		
Parasitological efficacy (95%CI)	86.7% (80.8, 91.3)	87.4% (81.9, 91.8)	-0.7	(-7.6, 6.1)

Table 4.7: 90%, 95% and 99% confidence intervals around treatment efficacy differences between ASAQ and DHA-PPQ by days 28 and 42 for mITT populations

	Risk Difference (RD)	90% CI (RD)	95% CI (RD)	99% CI (RD)
Day 28 crude	-1.3	(-6.4, 3.8)	(-7.4, 4.8)	(-9.3, 6.7)
Day 28 adjusted	-3.3	(-8.0, 1.4)	(-8.9, 2.3)	(-10.6, 4.1)
Day 42 crude	-0.7	(-6.5, 5.0)	(-7.6, 6.1)	(-9.8, 8.2)
Day 42 adjusted	-2.1	(-7.8, 3.7)	(-8.9, 4.8)	(-11.1, 7.0)

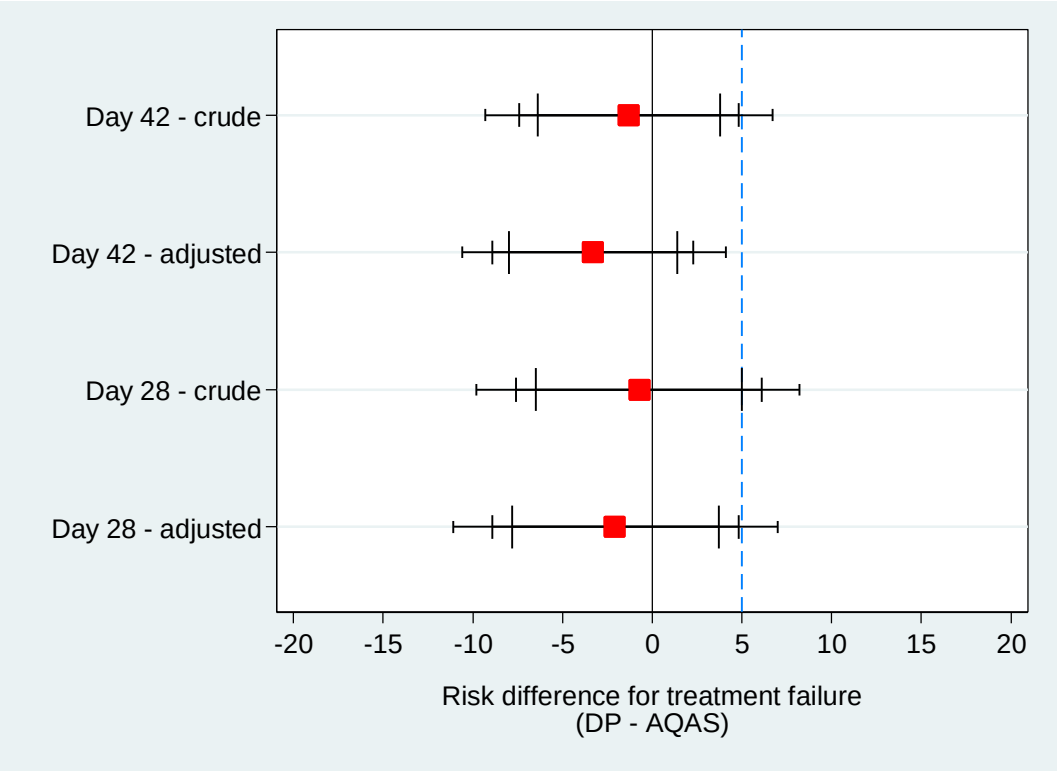


Fig 4.4. Non-inferiority of DHA-PPQ to ASAQ based on 90%, 95% and 99% CI around risk differences for crude and adjusted efficacy estimates by days 28 and 42 in the mITT populations

(Blue line represents non-inferiority margin of 5%. For confidence intervals, long bars represent 90%CI, short bars 95%CI and the smallest bars 99%CI)

Table 4.8: Baseline factors assessed for association with parasitological outcome by days 28 and 42 in mITT populations

	Day 28			**Day 42**		
variable	Adjusted RR	95% CI	p-value	Adjusted RR	95% CI	p-value
Age						
20-24	0.93	0.36, 2.40	0.88	0.88	0.39, 1.37	0.75
25-29	1.13	0.45, 2.82	0.79	0.97	0.44, 2.16	0.94
30+	1.08	0.40, 2.87	0.88	0.94	0.40, 2.19	0.88
Gestational age						
≥24 weeks	1.05	0.57, 1.92	0.87	1.14	0.67, 1.95	0.62
Gravidity						
2	2.08	0.84, 5.15	0.11	0.78	0.09, 1.66	0.08
Combined (3+4)	1.40	0.53, 3.61	0.50	0.27	0.65, 1.18	0.57
5+	1.62	0.57, 4.58	0.37			
Parasite Density						
>500/µl	1.48	0.72, 3.05	0.29	1.42	0.73, 2.75	0.30

4.4 Birth weight

Mean birth weight and prevalence of low birth weight (birth weight <2.5Kg) were assessed in 162 participants with birth weight records available (Table 4.9). The mean birth weights were not different; 2.95Kg in the ASAQ arm and 2.94Kg in the DHA-PPQ arm (p=0.87). The overall prevalence of low birth weight was 9.3% (15/162) with borderline higher occurrence in the DHA-PPQ group [4.2% (3/71) vs 13.2% (12/91); p=0.05]. Baseline factors such as maternal age (OR 1.07 95%CI: 0.69, 1.67; p=0.74), gestational age (OR 1.42 95%CI: 0.77, 2.62; p=0.27) and gravidity (OR 1.27 95%CI: 0.82, 1.99; p=0.29) were not associated with birth weight in univariate logistic regression analysis

4.5 Haemoglobin concentration assessments at days 28 and 42

There were significant increases in mean haemoglobin concentration (Hb) in both study arms comparing baseline and days 28 and 42 respectively. By day 28, the ASAQ arm showed an increase of 0.7g/dl (10.1g/dl vs 10.8g/dl; $p<0.0001$) while a similar increase of 0.6g/dl was observed in the DHA-PPQ arm (10.0g/dl vs 10.6g/dl; $p=0.0001$).

The increments over baseline were apparently comparable at day 28 as there was no significant difference in the mean Hb between study arms (10.8g/dl vs 10.6g/dl; $p=0.25$). At day 42, however, pregnant women in the ASAQ arm showed a significantly higher mean Hb compared to those treated with DHA-PPQ (11.2g/dl vs 10.8g/dl; $p=0.01$) as shown in Table 4.9. Figure 4.5 illustrates changes in the percentage of women with severe anaemia ($Hb<8.0$g/dl) and moderate anaemia ($8.0\geq Hb\leq 10.9$g/dl) at enrolment and at days 28 and 42.

Both study arms had comparable grades of severe and moderate anaemia at days 28 and 42 (see Table 4.9 as well). Figure 4.5 also shows there are increases in the proportion of women with normal haemoglobin levels ($Hb\geq 11.0$g/dl) over baseline at both days 28 and 42 but this is more prominent at day 42.

Table 4.9: Comparison of haemoglobin levels at days 28 and 42 and birth weight between study arms

	Day 28			Day 42		
	ASAQ(N=158)	**DHA-PPQ**(N=167)	**p**	**ASAQ**(N=151)	**DHA-PPQ**(N=162)	**p**
Haemoglobin (g/dl)						
<8.0g/dl n(%)	4 (2.5)	7 (4.2)	0.70	1 (0.7)	1 (0.6)	0.27
8.0-10.9g/dl n(%)	90 (57.0)	95 (56.9)		70 (46.4)	90 (55.6)	
≥11.0g/dl n(%)	64 (40.5)	65 (38.9)		80 (53.0)	71 (43.8)	
Mean (95%CI)	10.8 (10.6, 10.9)	10.6 (10.4, 10.8)	0.25	11.2 (11.0, 11.4)	10.8 (10.6, 11.0)	0.01
		ASAQ (N=71)		**DHA-PPQ (N=91)**		**p**
Birth weight (Kg)						
<2.5 n (%)		3 (4.2)		12 (13.2)		0.05
≥2.5 n (%)		68 (95.8)		79 (86.8)		
Mean (95%CI)		2.95 (2.86, 3.04)		2.94 (2.82, 3.06)		0.87

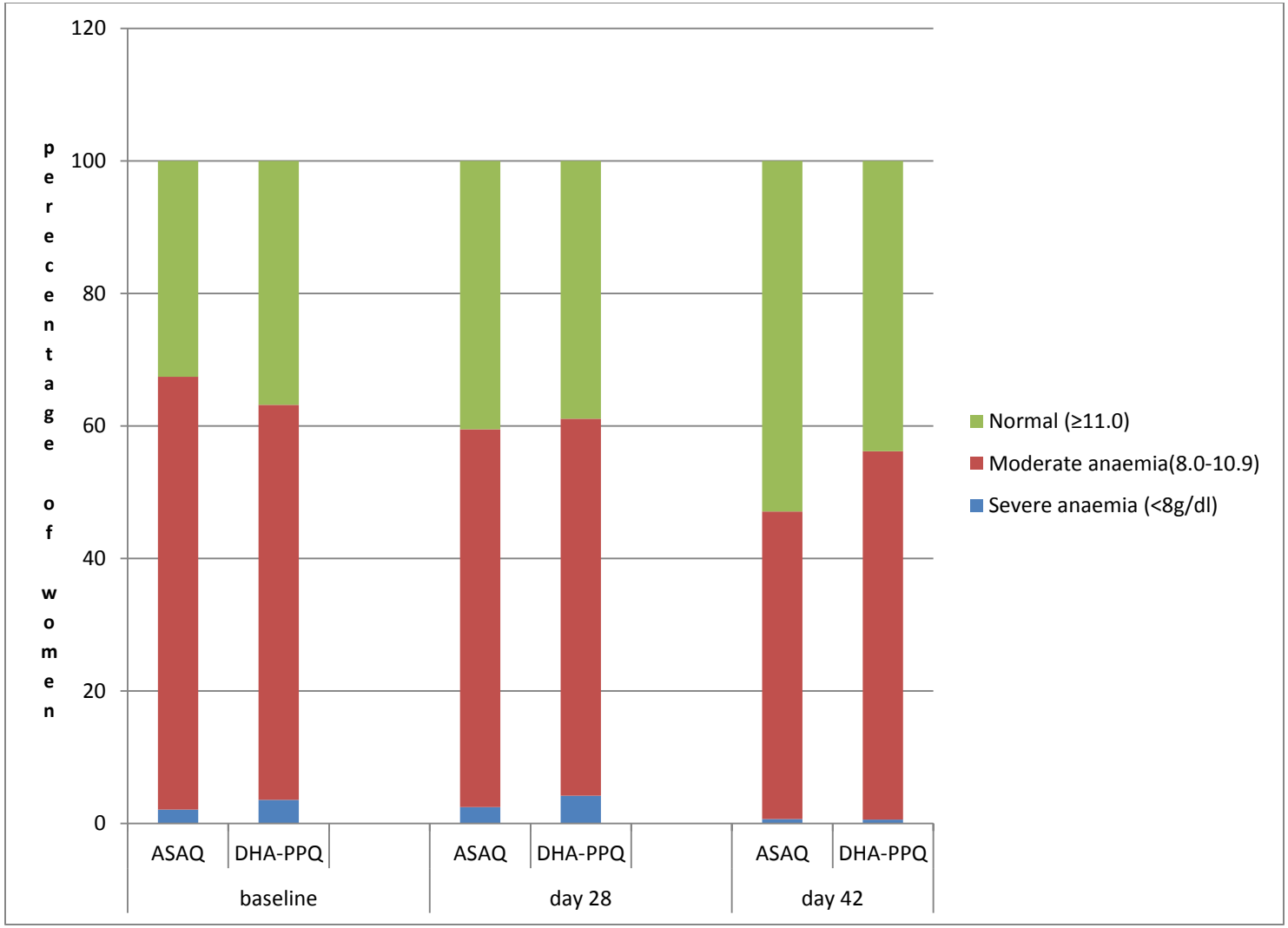


Fig 4.5 Changes in percentage of women with anaemia at days 28 and 42 compared to baseline

4.6 Safety and tolerability assessments

Assessment of safety and tolerability involved monitoring for changes in white blood cell and differential counts on days 14, 28 and 42 after start of treatment. Reports of adverse (self-reported) and severe adverse events were also recorded. Liver and renal biochemistry were not assessed as there were no indications for them as defined per the protocol.

4.6.1 White blood cell and differential count assessment

There were statistically significant within-study arm reductions in the means neutrophil count and increments in the means of lymphocyte count over baseline at days 14, 28 and 42 after starting study drugs. However, the changes were generally not significantly different between the treatment groups as white blood cell and differential counts were largely comparable between treatment groups on days 14, 28 and 42 (Table 4.10). The exception was a significantly higher lymphocyte count in the ASAQ arm on day 14 (34.3% vs 31.1%; p=0.02).

Significant changes in platelet count were observed on days 14 and 42 in the ASAQ arm and only on day 14 in the DHA-PPQ arm (179.7 vs 207.8; p=0.02) (see Tables 4.11 and 4.12).

Table 4.10 Comparison of white blood cell and differential counts between study arms on days 14, 28 and 42 after start of treatment

	Day 14			Day 28			Day 42		
	ASAQ	**DHA-PPQ**	**p**	**ASAQ**	**DHA-PPQ**	**p**	**ASAQ**	**DHA-PPQ**	**p**
[a]WBC{[b]N, mean(sd)}	159, 6.4(2)	164, 6.8(2)	0.06	158, 6.4(1.5)	166, 6.7(1.8)	0.06	157, 6.2(2.3)	165, 6.5(2.2)	0.36
[c]Neut{N, mean(sd)}	143, 57.5(13.3)	149, 59.1(15.1)	0.34	146, 59.4(10.9)	148, 61(11.6)	0.23	137, 59.3(12.8)	141, 57.6(11.7)	0.26
[d]Lymph{N, mean(sd)}	151, 34.3(11.6)	154, 31.1(11.8)	**0.02**	156, 33.1(10.3)	163, 31(9.1)	0.06	148, 32.9(10)	160, 33.6(10)	0.54
[e]Platelets{N, mean(sd)}	156, 199.5(72.3)	164, 207.8(126)	0.47	157, 176.6(61.1)	166, 183.8(54.6)	0.26	149, 183.3(62.9)	161, 189.8(59.1)	0.35

[a]WBC is Total White Blood Cell Count (x 10^3) [b]N is the number of participants assessed for the outcome [c]Neut is Neutrophil Count (%) [d]Lymph is Lymphocyte Count (%) [e]Platelet Count (x 10^3)

Reference ranges for the Sysmex KX21N Autoanalyzer are as follows; TWBC (5.0-15.0), Hb (8.0-17.0), Neutrophils (45.0-95.0), Lymphocytes (5.0-55.0), Platelets (50-400). These reference values apply to Tables 4.11 and 4.12 as well.

Table 4.11 Changes in white blood cell and differential counts within the ASAQ study arm

	Baseline	**Day 14**	[f]**p**	**Day 28**	[g]**p**	**Day 42**	[h]**p**
[a]WBC{[b]N, mean(sd)}	191, 6.3(1.5)	159, 6.4(2.0)	0.6	158, 6.4(1.5)	0.5	157, 6.2(2.3)	0.63
[c]Neut{N, mean(sd)}	178, 64(12.4)	143, 57.5(13.3)	<0.0001	146, 59.4(10.9)	0.001	137, 59.3(12.8)	0.001
[d]Lymph{N, mean(sd)}	187, 27(9)	151, 34.3(11.6)	<0.0001	156, 33.1(10.3)	<0.0001	148, 32.9(10)	<0.0001
[e]Platelets{N, mean(sd)}	183, 167.8(60)	156, 199.5(72.3)	<0.0001	157, 176.6(61.1)	0.18	149, 183.3(62.9)	0.02

[a]WBC is Total White Blood Cell Count (x 10^3) [b]N is the number of participants assessed for the outcome [c]Neut is Neutrophil Count(%) [d]Lymph is Lymphocyte Count (%) [e]Platelet Count (x 10^3)) [f]p,[g]p and [h]p are p-values for significance of differences between baseline/Day14, baseline/Day28 and baseline/day42

Table 4.12 Changes in white blood cell and differential counts within the DHA-PPQ study arm

	Baseline	**Day 14**	[f]**p**	**Day 28**	[g]**p**	**Day 42**	[h]**p**
[a]WBC{[b]N, mean(sd)}	190, 6.6(1.6)	164, 6.8(2.0)	0.3	166, 6.7(1.8)	0.6	165, 6.5(2.2)	0.63
[c]Neut{N, mean(sd)}	183, 64.1(13.4)	149, 59.1(15.1)	0.002	148, 61(11.6)	0.03	141, 57.6(11.7)	<0.0001
[d]Lymph{N, mean(sd)}	191, 27(10.3)	154, 31.1(11.8)	0.001	163, 31(9.1)	0.001	160, 33.6(10)	<0.0001
[e]Platelets{N, mean(sd)}	183, 179.7(91.1)	164, 207.8(126.1)	0.02	166, 183.8(54.6)	0.62	161, 189.8(59.1)	0.23

[a]WBC is Total White Blood Cell Count (x 10^3) [b]N is the number of participants assessed for the outcome [c]Neut is Neutrophil Count(%) [d]Lymph is Lymphocyte Count (%) [e]Platelet Count (x 10^3) [f]p,[g]p and [h]p are p-values for significance of differences between baseline/Day14, baseline/Day28 and baseline/day42

4.6.2 Adverse Events

Adverse events were treatment emergent and their incidence recorded on days 1, 3 and 7 after start of study treatment are shown in Tables 4.13, 4.14 and 4.15 respectively. General weakness, vomiting and abdominal pains were prominent on day 1 with frequencies of 37%, 23% and 21% respectively. There was no statistically significant difference in frequency of AEs between study arms (Table 4.13).

Adverse events following treatment appeared to peak on day 3. Most common AEs reported on day 3 were general weakness 50.0% (192/384), abdominal pains 26.3% (101/384), vomiting 24.2% (93/384), dizziness 20.3% (76/384) and anorexia 17.0% (65/384). More statistically significant differences between study arms were observed on day 3 than on day 7. Women in the ASAQ arm experienced higher frequencies of anorexia (22.3% vs 12.0%; p=0.007), nausea (2.6% vs 0%; p=0.02), vomiting (29.4% vs 19.5%; p=0.02), dizziness (26.6% vs 14.5%; p=0.003) and general weakness (62.5% vs 38.5%; p<0.0001) (see Table 4.14)

The difference in frequency of occurrence of general weakness observed on day 3 was still present on day 7 (36.8% vs 25.7%; p=0.02) against an apparent trend of resolution of AEs with obliteration of study group differences. On day 7, there were significantly more reports of sleeplessness (19.8% vs 11.2%; p=0.02) and nightmares (11.0% vs 4.3%; p=0.02) among pregnant women who received ASAQ compared to the DHA-PPQ arm (Table 4.15). Figure 4.6 illustrates first the differential occurrence of some selected AEs between ASAQ and DHA-PPQ arms on days 3 and 7 and the reduction in frequency of AEs from day 3 to day 7

Abdominal pains and general weakness were present to appreciable extents with prevalence of 20.2% (70/345) and 17.4% (60/345) respectively but with no significant

difference between arms on day 14 (table not shown). There were no reports of nausea on day 7 but 29.3% (101/345) of participants reported it on day 14 (not shown). Tolerability was high as no participant left the trial or refused to continue the medications because of adverse events.

Table 4.13 Frequency of adverse events on first day after start of study drugs

DAY 1			
Adverse events	**ASAQ (N=198)**	**DHA-PPQ (N=210)**	**p**
Anorexia *n(%)	23 (11.6)	28 (13.3)	0.52
Nausea	18 (9.1)	28 (13.3)	0.18
Vomiting	40 (20.2)	56 (26.7)	0.13
Abdominal pain	42 (21.2)	44 (21.10	0.97
Diarrhoea	12 (6.1)	18 (8.6)	0.33
Dizziness	27 (13.6)	36 (17.1)	0.33
Sleeplessness	20 (10.1)	22 (10.5)	0.89
Nightmares	18 (9.1)	14 (6.7)	0.37
Visual disturbance	8 (4.0)	7 (4.0)	0.71
Tinnitus	13 (6.6)	16 (7.7)	0.67
General weakness	79 (39.9)	74 (35.2)	0.33
Itching	23 (11.6)	21 (10.1)	0.55

* Adverse events presented as number reporting adverse event and percentage. Occurrences of particular adverse events were not mutually exclusive as individual pregnant women mentioned more than one adverse event. This applies to Tables 4.14 and 4.15 for adverse events on days 3 and 7 respectively.

Table 4.14 Frequency of adverse events 3 days after start of study drugs

DAY 3			
Adverse events	**ASAQ (N=184)**	**DHA-PPQ (N=200)**	**p**
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 number reporting adverse event and percentage &for nausea (ASAQ=194, DHA-PPQ=209)

Table 4.15 Frequency of adverse events 7 days after start of study drugs

	DAY 7		
Adverse events	**ASAQ (N=182)**	**DHA-PPQ (N=187)**	**p**
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 number reporting adverse event and percentage

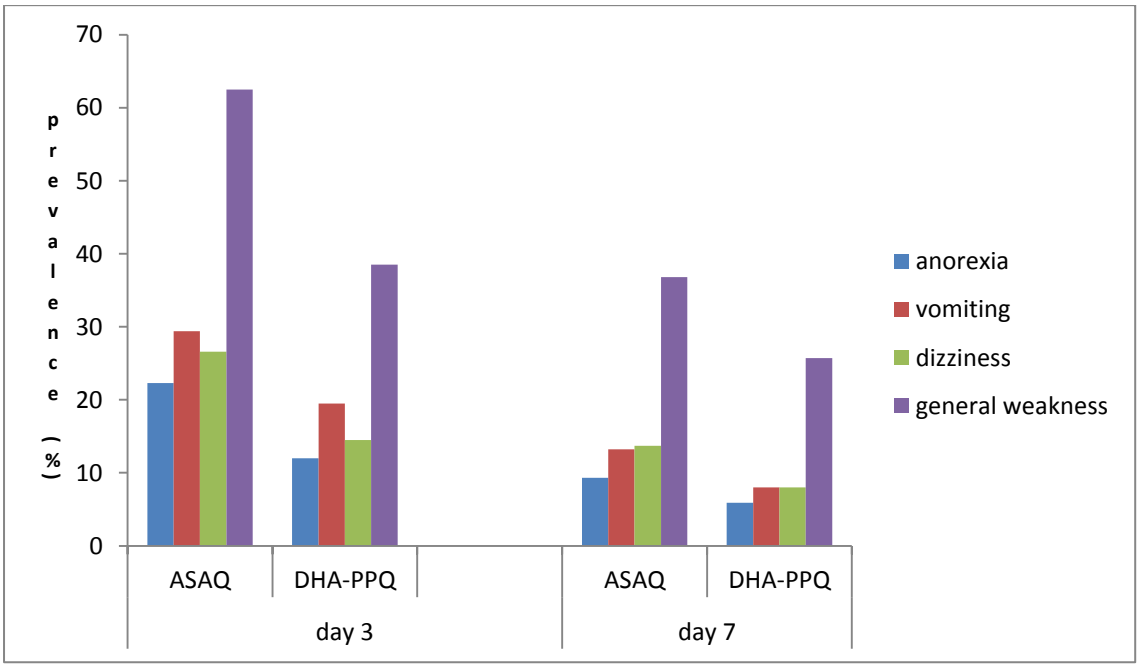


Fig 4.6 Graph illustrating decreased incidence of selected adverse events comparing days 3 and 7 after start of study treatment

4.6.3 Serious Adverse Events

One participant who received DHA-PPQ developed severe anaemia (Hb 3.8g/dl) three weeks after she had completed treatment. She was 36 years old, had 6 children and

was pregnant for the seventh time. She was enrolled at a gestational age of 26 weeks with a haemoglobin concentration of 8.0g/dl. She was transfused 3 units of blood on admission and also treated for a concomitant urinary tract infection with oral co-formulated amoxicillin and clavulanic acid. She was monitored on the ward for six days and made good recovery. She was discharged with a supply of haematinics and eventually delivered uneventfully.

Another participant, 32 years of age, gravida four and 30 weeks pregnant who also received DHA-PPQ developed pregnancy-induced hypertension (P1H) three days after starting treatment. She presented at St. Michael's hospital with complaints of marked dizziness and on examination, the blood pressure was 160/110 mmHg. There was however no microalbuminuria on urine examination. It was realized from her medical records that she had had a previous history of PIH. She was admitted to the maternity ward, managed on daily Nifedipine 30mg and discharged when the blood pressure was adequately controlled. She was withdrawn from the study and referred to be seen by the resident obstetrician/gynaecologist.

A case of antepartum haemorrhage due to placental abruption occurred in a 28-year old woman, gravida four, at 32 weeks gestation. She had noticed minimal vaginal bleeding in the early hours of the third day after starting DHA-PPQ. She presented at St. Michael's hospital later in the morning when the bleeding apparently increased in volume and she was seen by the PI and the study physician in the Bosomtwe study center.

Ultrasonographic examination showed the foetus was viable and an emergency caesarean section was done. The baby was delivered alive but died about 2-3 weeks

later. The woman was transfused 5 units of blood intra- and post-operatively and recovered uneventfully.

One woman presented with severe abdominal pains at 29 weeks gestation about two and a half months after taking ASAQ. She was managed with antibiotics on an impression of appendicitis/ cholecystitis in pregnancy. She recovered uneventfully.

Three women in the ASAQ arm and one in the DHA-PPQ arm developed moderately severe diarrhoea with prostration between days 2-5 after start of treatment. They were managed with intravenous fluids and oral rehydration salts on the ward and all discharged within 24 hours. No maternal deaths were recorded during the trial.

There were 3 babies with minor birth defects at delivery (all cases of polydactyly), 2 cases of neonatal deaths (1 mentioned above and a baby girl with early neonatal sepsis/ jaundice who was referred to Komfo Anokye Teaching Hospital where she died) and one baby with a swelling in the upper gum detected 6 weeks post-partum in the DHA-PPQ arm. In addition, there were two events of stillbirth and one event of a miscarriage in the DHA-PPQ arm. In the ASAQ arm one case of a stillbirth, one miscarriage and one intrauterine death at 24 weeks gestation (occurring six weeks after completing study medication) were recorded.

4.7 Delivery and 6-week postpartum assessment

The overall prevalence of preterm deliveries was 5% but there was no significant difference in its occurrence between study arms (5.2% vs 4.9%; p=0.92). Similar to observations made at day 42, the ASAQ arm showed a higher mean maternal haemoglobin concentration at delivery but this was not significant (Table 4.16).

There was a significantly lower prevalence of peripheral parasitaemia in the DHA-PPQ arm compared to women in the ASAQ arm (37.1% vs 21.7%;p= 0.012).

Of neonates followed up by the 6-week postpartum assessment day, one whose mother took DHA-PPQ had a birth defect which was not detected at birth (a minor swelling on the gum). Two babies from each arm had neonatal jaundice

Table 4.16: Comparison of delivery assessments between study arms

	ASAQ	**DHA-PPQ**	**p**
Neonatal outcomes			
[a]Gestation			0.92
≥37 weeks * n(%)	91 (94.8)	116 (95.1)	
<37 weeks	5 (5.2%)	6 (4.9%)	
Congenital malformation			0.12
[&]Yes	0	3 (2.7)	
No	89 (100.0)	110 (97.3)	
Maternal outcomes			
[$]maternal Hb (95% CI)	12.0 (11.5, 12.4)	11.5 (11.2, 11.9)	0.10
Peripheral parasitaemia			0.012
Yes	36 (37.1)	26 (21.7)	
No	61 (62.9)	94 (78.3)	
Placental parasitaemia			0.43
Yes	19 (21.1)	30 (25.9)	
No	71 (78.9)	86 (74.1)	
Cord parasitaemia			0.90
Yes	26 (30.6)	33 (31.4)	
No	59 (69.4)	72 (68.6)	
6 weeks follow-up assessments	**ASAQ (N=81)**	**DHA-PPQ (N=95)**	
Detected malformations	0 (0)	1 (1.1)	
Jaundice	2 (2.5)	2 (2.1)	
Death	0 (0)	2 (2.1)	

*neonatal and maternal outcomes at delivery are presented as frequency and percentage [&]polydactyly was the only congenital malformation seen [$]maternal Hb is mean maternal haemoglobin concentration in g/dl

[a]the number assessed for outcomes in the table are as follows;

gestation (ASAQ=96 / DHA-PPQ=122) congenital malformation (ASAQ=89 / DHA-PPQ=113)

maternal Hb (ASAQ=74 / DHA-PPQ=87) peripheral parasitaemia (ASAQ=97 / DHA-PPQ=120)

placental parasitaemia (ASAQ=90 / DHA-PPQ=116)

cord parasitaemia (ASAQ=85 / DHA/PPQ=105)

CHAPTER FIVE

POLYMORPHISMS AT THE CHLOROQUINE RESISTANCE TRANSPORTER, MULTIDRUG RESISTANCE 1, DIHYDROFOLATE REDUCTASE AND DIHYDROPTEROATE SYNTHETASE GENES IN *P.FALCIPARUM* ISOLATES FROM INFECTED PREGNANT WOMEN

5.1 Introduction

This chapter presents the prevalence/ frequency of SNPs at the *Pfcrt*, *Pfmdr1*, *Pfdhfr* and *Pfdhps* genes. A total of 199 randomly selected baseline filter paper blots were analysed by methods described in chapter 3, found evaluable and included in analysis. It also incorporates secondary prevalence data from previous studies in Ghana to help describe trends in the prevalence of these SNPs in Ghana.

5.2 Distribution of *Pfcrt* haplotypes

Successful *Pfcrt* haplotyping was achieved in 83.9% of samples (167/199). Infections carrying the CVMNK haplotype were predominant with prevalence and frequency of 88.6% (148/167) and 88.1% (140/159) respectively. Frequency excludes while prevalence includes mixed haplotype infections where a majority haplotype could not be determined. For the CVIET haplotype, the prevalence was 16.7% and the frequency 11.9%.

5.3 Distribution of SNPs at the *Pfmdr1* gene.

Successful genotyping at the *Pfmdr1* gene was achieved in 82.4% (164/199), 74.9% (149/199) and 77.9% (155/199) of samples at codons 86, 184 and 1246 respectively. The prevalence and frequency of individual SNPs at the various codons are presented in Table 5.1. Wild type infections were predominant at codons 86 and 1246 at frequencies of 87.9% and 98.7% respectively while for codon 184, they were under 50%. Only minor differences between frequency and prevalence measures were observed.

5.4 Frequency of *Pfmdr1* Haplotypes

Construction of the *Pfmdr1* SNPs at codons 86, 184 and 1246 into haplotypes showed that infections carrying the single mutant N86-184**F**-D1246 (NFD) and N86-Y184-D1246 (NYD) haplotypes were equally common with a frequency of 43.2% (54/125) each (see Fig 5.1). There were no *P.falciparum* isolates with the triple mutant 86**Y**-184**Y**-1246**Y** haplotype.

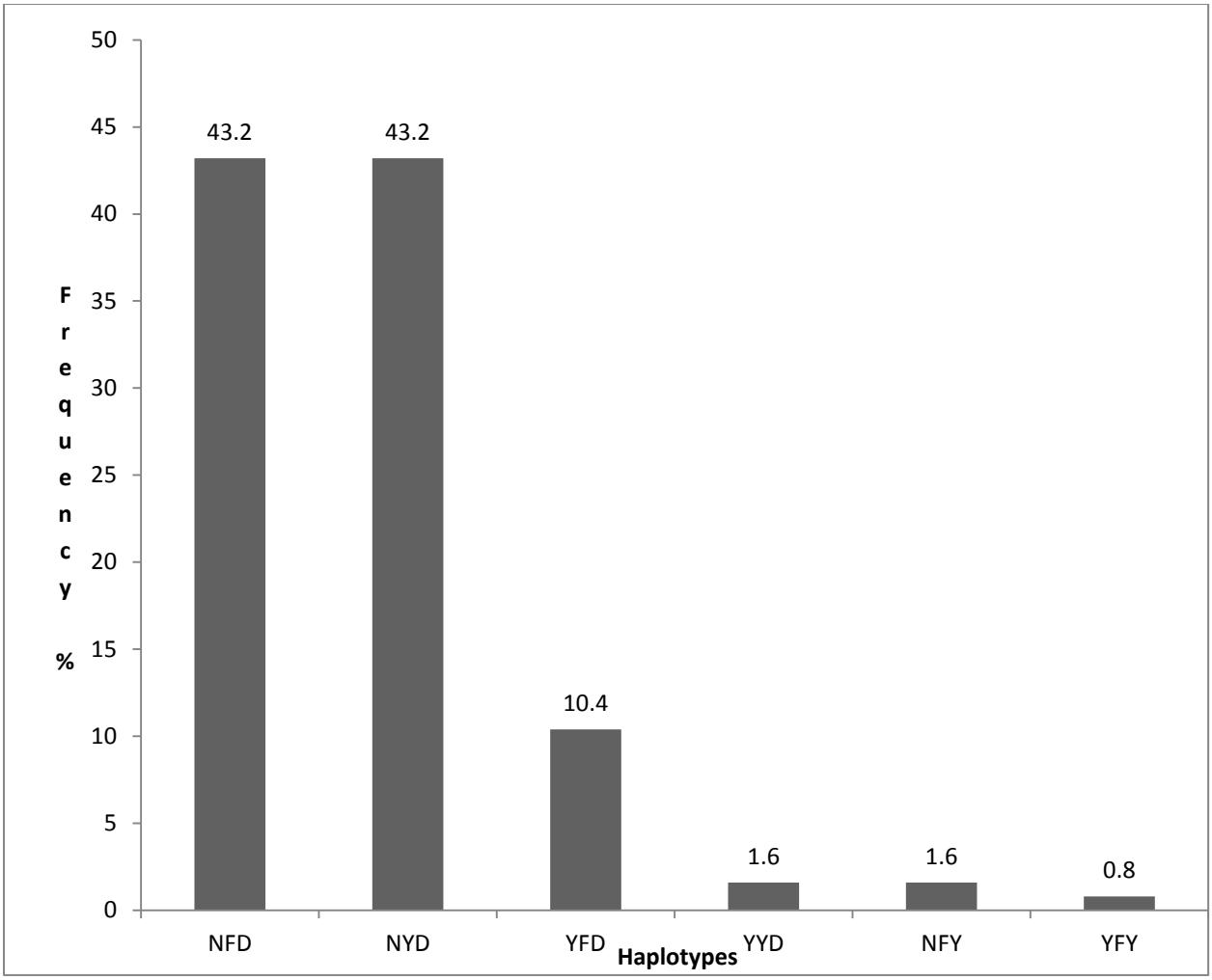


Fig 5.1 Frequency of *Pfmdr1* haplotypes constructed from SNPS at codons 86, 184 and 1246

5.5 SNPs in the *Pfdhfr* and *Pfdhps* genes

At the *Pfdhfr* gene, the proportions of evaluable samples were 71.4% (142/199) at codon 50/51, 73.4% (146/199) at codon 59, 69.8% (139/199) at codon108 and 66.3% (132/199) at codon 164. Mutant parasites were in the majority at codons 51, 59 and 108 with frequencies of 75.7% (103/136), 85% (119/140) and 87.8% (122/139) respectively. Neither the S108T nor the I164L mutations were observed. The full complement of frequencies and prevalence of *Pfdhfr* SNPs are shown in Table 5.2.

Successful genotyping at the *Pfdhps* gene was observed in 73.9% (147/199), 71.8% (143/199), 76.4% (152/199) and 74.4% (148/199) of study samples respectively at codons 436/437, 540, 581 and 613. At codon 436/437, AG and SG haplotypes were predominant with respective frequencies of 67.4% and 29.1%. Combined, the frequency of the 437G mutation was 96.5%. Only one evaluable sample that was a mixed infection showed the K540E mutation, otherwise only wild types at codon 540 were observed. The frequencies of A581G and A613S mutations were 2.7% and 6.2% respectively. The frequencies and prevalence of the various SNPs at the *Pfdhps* gene are shown in Table 5.3.

Table 5.1 Frequency and Prevalence of *Pfmdr1* SNPs at codons 86, 184 and 1246

	Prevalence			**Frequency**		
Pfmdr1 SNPs	Evaluable Samples **(N)**	Number with specified SNP **(n)**	**% (n/N x 100)**	Evaluable Samples **(N)**	Number with specified SNP **(n)**	**% (n/N x 100)**
N86	164	144	87.8	157	138	87.9
86Y	164	25	15.2	157	19	12.1
184F	149	88	59.1	137	76	55.5
Y184	146	48	46.6	137	61	44.5
D1246	155	153	98.7	155	153	98.7
1246Y	144	2	1.4	144	2	1.4

*Frequency excludes mixed infections while prevalence includes mixed infections

Table 5.2 Prevalence and Frequency of *Pfdhr* SNPs

Pfdhfr codon	SNPs	Prevalence			Frequency		
		Evaluable Sample (N)	Number with specific SNP (n)	**% (n/Nx100)**	Evaluable Sample (N)	Number with specific SNP (n)	**% (n/Nx100)**
50/51	CN	142	36	25.5	136	33	24.3
	CI	142	106	75.5	136	103	75.7
59	C	146	24	16.4	140	21	15.0
	R	146	122	83.6	140	119	85.0
108	S	139	17	12.2	139	17	12.2
	N	139	122	87.8	139	122	87.8
	T	0		0			
164	I	132	132	100.0	132	132	100.0
	L	0		0			

Table 5.3 Prevalence and frequency of SNPs at the *Pfdhps* gene

Pfdhps codon	SNPs	**Prevalence**			**Frequency**		
		Evaluable samples (N)	Number with specific SNP (n)	**% (n/Nx100)**	Evaluable samples (N)	Number with specific SNP (n)	**% (n/Nx100)**
436/437	AA	147	3	2.0	141	3	2.1
	AG		95	64.6		95	67.4
	SA		3	2.0		1	0.7
	SG		45	30.6		41	29.1
	FA		1	0.7		1	0.7
540	K	143	142	99.3	141	141	100.0
	E		1	0.7	0		
581	A	152	147	96.7	150	146	97.3
	G		5	3.3		4	2.7
613	A	148	138	93.2	146	137	93.8
	S		10	6.8		9	6.2

5.6 Distribution of *Pfdhfr* and *Pfdhps* haplotypes

Single nucleotide polymorphisms at the *Pfdhfr* and *Pfdhps* genes were constructed into haplotypes considering only isolates having at least 2 codons successfully genotyped at the respective gene loci. Mixed infections were included in the haplotype construction as long as clear majority SNPs could be identified or else the sample was excluded from analysis.

At the *Pfdhfr* gene locus, omitting haplotypes with single mutations, only 9 isolates out of an evaluable 126 (7.1%) were pure wild type (CNCSI) while 77.0% (97/126) carried the triple mutation (C50+ **N51I**+**C59R**+**S108N**+I164) (CIRNI). Haplotypes with double mutations (C50+N51+**59R**+**108N**+I164), (C50+**51I**+C59+**108N**+I164) and (C50+**51I**+**59R**+S108+I164) constituted 15.9% (20/126) of evaluable isolates (Fig 5.2)

The distribution of *Pfdhps* haplotypes obtained combining SNPs at 436/437/540/581/613 is shown in Fig 5.3. Only 4 (3.1%) isolates out of an evaluable 130 samples were pure wild type. Of the pure wild types, 3 were the AAKAA and 1 was SAKAA haplotype. The single mutation haplotypes AGKAA and SGKAA were present in frequencies of 61.5% (80/130) and 30% (39/130) respectively. Only one isolate exhibited the triple mutated haplotype AGKGS while the double mutated haplotype (AGKAS) was present in 4.6% (6/130) of evaluable isolates.

Combining haplotypes at the two genes (shown in Fig 5.4), the *Pfdhfr/Pfdhps* quadruple mutation (51I+59R+108N/ 437G) was present in 92.6% (75/81) of evaluable isolates. Of these, 56% (42/75) were AGKAA and 44% (33/75) were SGKAA at the *Pfdhp*s gene. The quintuple mutation (51I+59R+108N/ 437G+613S) was found in 6.2% (5/81) while the sextuple mutation (51I+59R+108N/ 437G+581G+

613S) was present in 1.2% (1/81) of evaluable isolates. The quintuple mutation carrying the K540E (51I+59R+108N/ 437G+540E) was not observed.

5.7 Trends in prevalence of antimalarial resistance molecular markers in Ghana

Figures 5.5-5.8 are based on combined prevalence data from the present study and other studies from Ghana reporting such data for molecular markers of antimalaria resistance in published papers. These other studies involved pregnant women, children and non-pregnant adults but are presented in general terms without reference to the populations involved to show trends in the prevalence of *Pfcrt*, *Pfmdr1*, *Pfdhfr* and *Pfdhps* polymorphisms.

The earliest reports found regarding *Pfcrt* mutation were from studies conducted in 1998. *P.falciparum* isolates carrying the *Pfcrt* K76T mutation were generally predominant from 1998 up to 2007/2008. An unusually high prevalence was reported in the 3-4 years after chloroquine proscription in 2004. Wild type isolates increased in population from 2007/2008 onwards although one study reported a rather low prevalence in 2012 (Fig 5.5)

Regarding *Pfmdr1,* the N86 allele has recorded prevalence in excess of 80% since 2004 while the 86Y was relatively stabilized between 2004 and 2007/2008 but drastically decreased since then. The NFD haplotype appears to have stabilized between 2007/2008 and 2012 (Fig 5.6).

The *Pfdhfr* core mutation, S108N, appears to have stabilized in prevalence between 80%-95% from 1998 to 2012 with the exception of one study reporting about 60% prevalence for 2004. The *Pfdhf* triple mutation (CIRNI) shows a general increase in prevalence from 1998 and has ranged between 72%-80% between 2006 and 2012 while the pure wild type (CNCSI) did not show significant change in the decade

preceding 2012 (Fig 5.7). The *Pfdhfr/Pfdhps* quadruple mutation, on the contrary, has been on the increase from 2002-2012 (Fig 5.8).

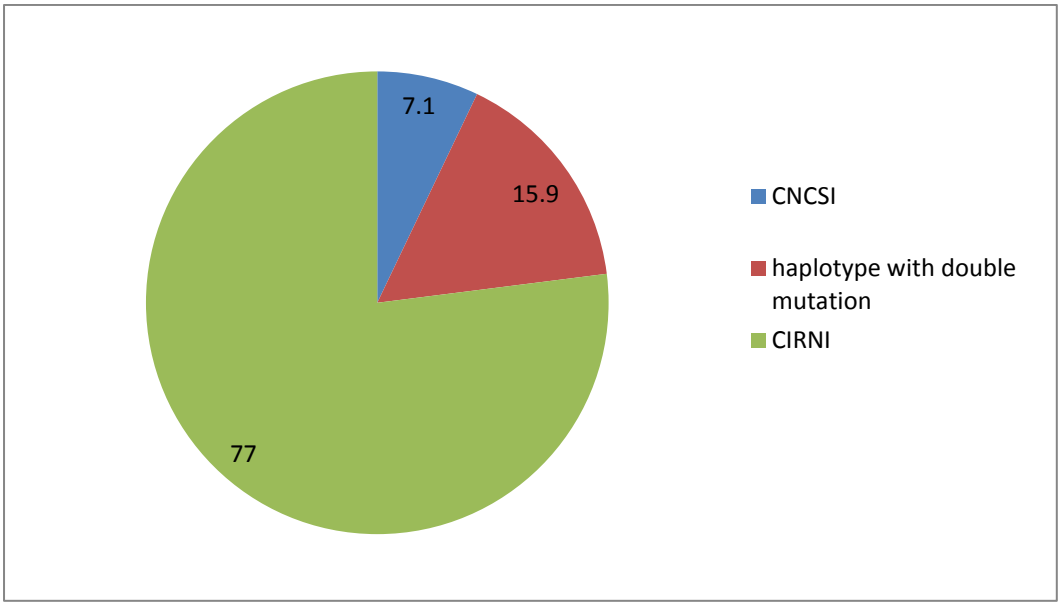


Fig 5.2 Distribution of *Pfdhfr* haplotypes in *P.falciparum* isolates from infected pregnant women

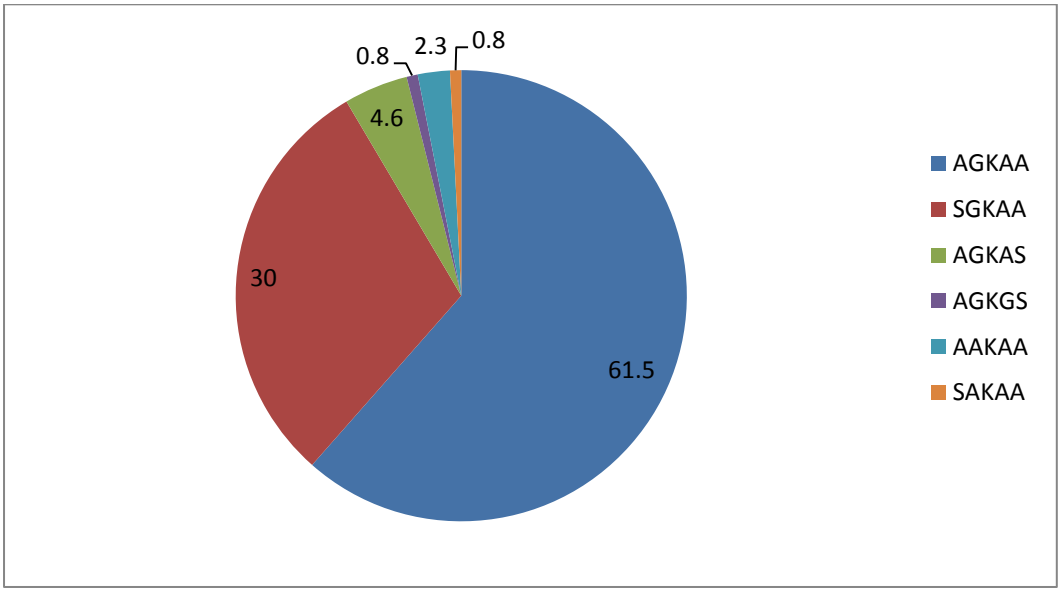


Fig 5.3 Distribution of *Pfdhps* haplotypes in *P.falciparum* isolates from infected pregnant women

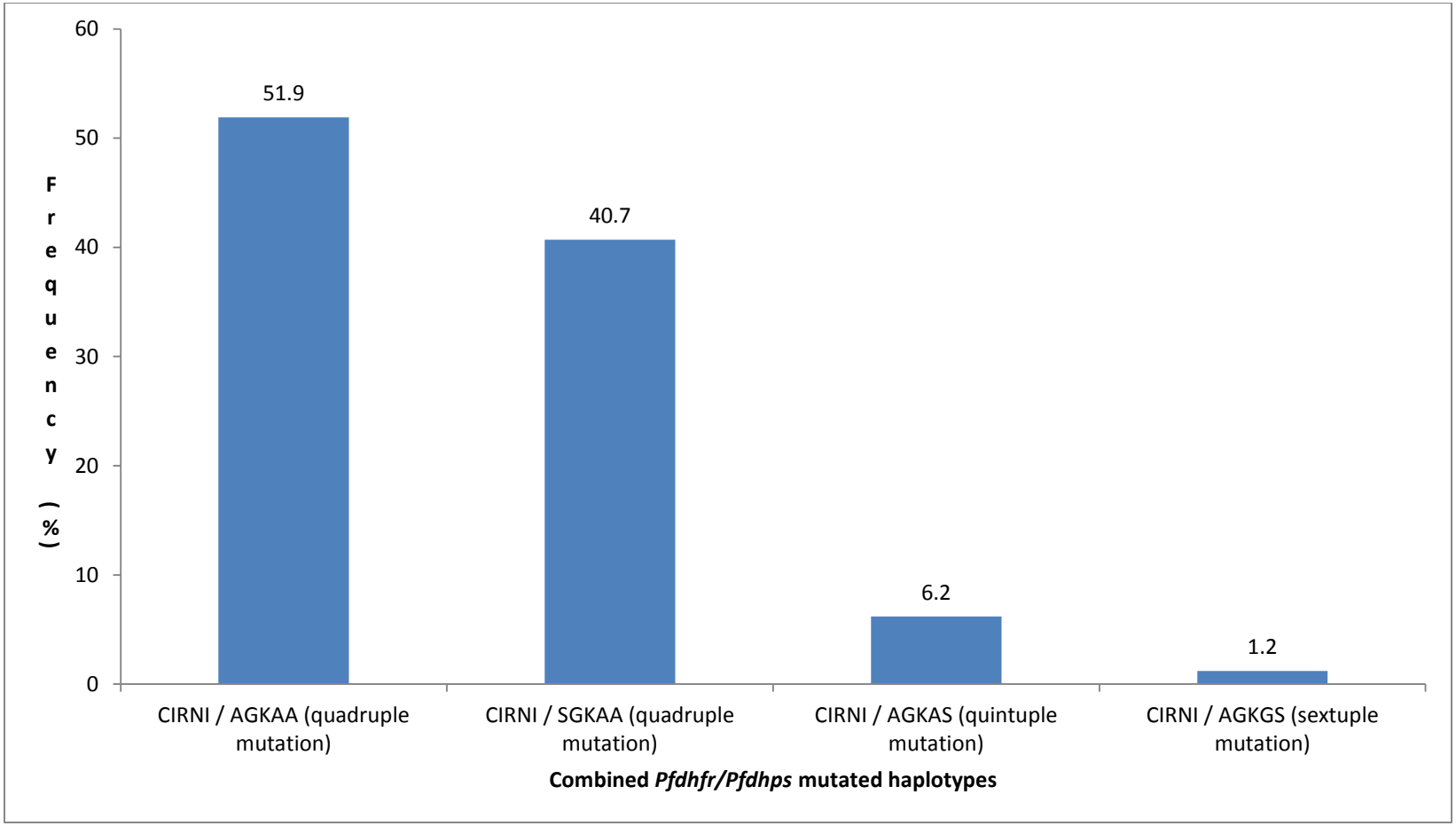


Fig 5.4: Frequency of combined *Pfdhfr/Pfdhps* mutated haplotypes.

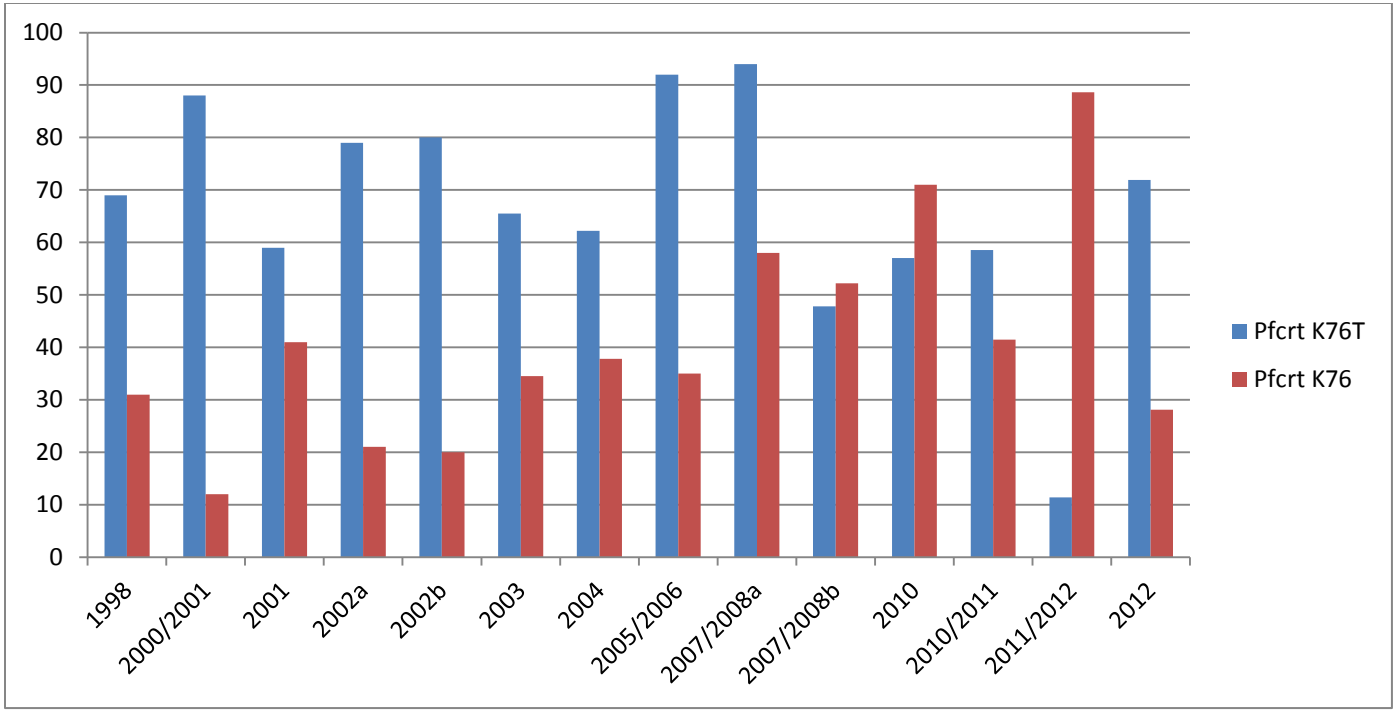


Fig 5.5 Trends and comparison of prevalence of *Pfcrt* SNPs in studies conducted across Ghana between1998 and 2012

Data was sourced from the following**; 1998** (Mockenhaupt et al., 2001), **2000/2001 (**Evans et al., 2005), **2001** (Marks et al., 2005), **2002a** (Ehrhardt et al., 2007), **2002b** (Mockenhaupt et al., 2005), **2003** ((Marks et al., 2005), **2004** (Owusu-Agyei et al., 2009), **2005/06, 2007/08a** and **2010** (Duah et al., 2013), **2007/2008b** (Alam et al., 2011), **2010/2011** (Afoakwa et al., 2014), **2011/12** (present study), **2012** (Asare et al., 2014).

{For 2000/2001, 2002a and 2012, *Pfcrt* K76 was not explicitly reported but inferred}.

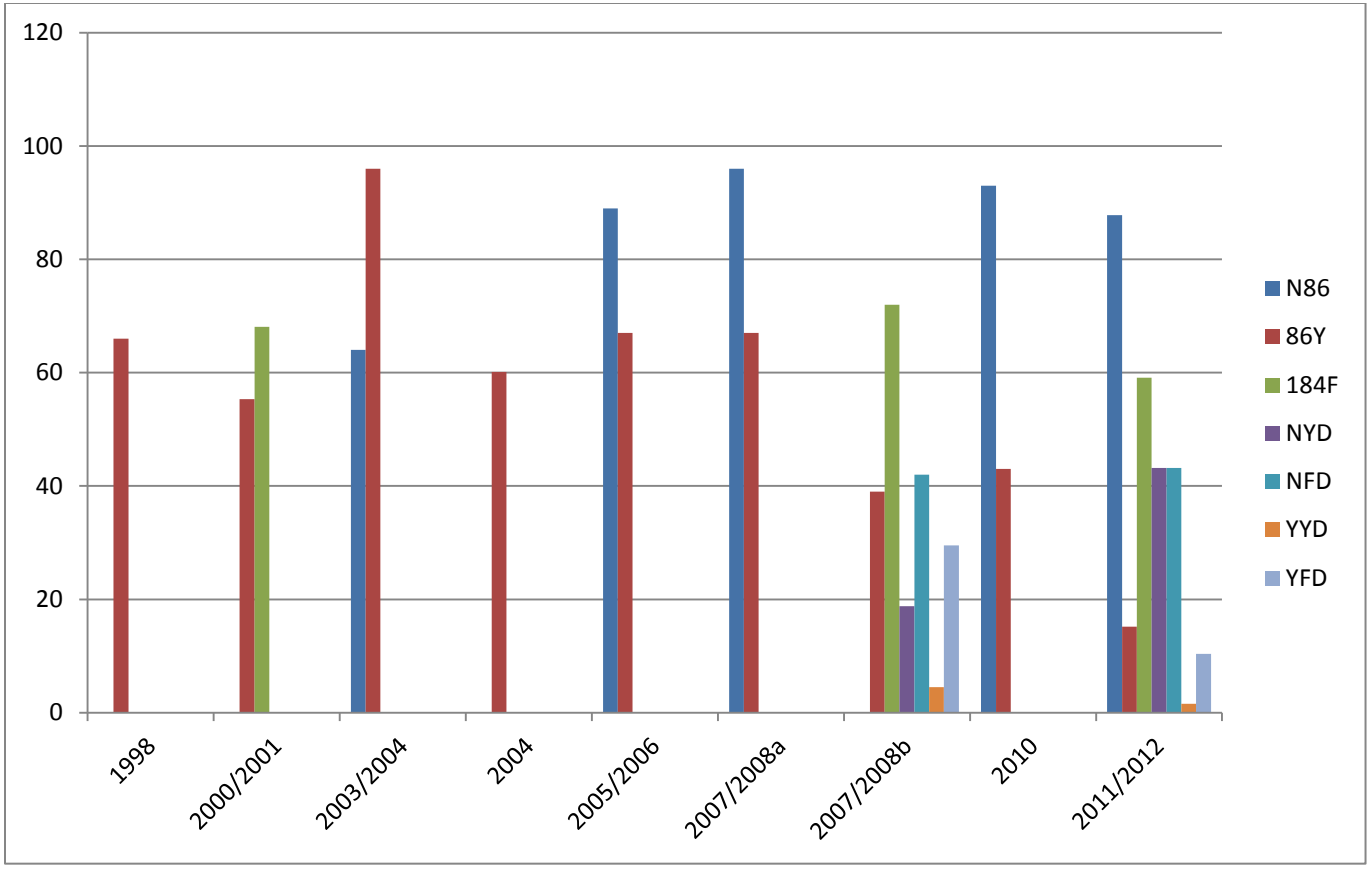


Fig 5.6: Trends and comparison of prevalence of *Pfmdr1* codon 86 alleles, 184F, and haplotypes in isolates from Ghana

Sources of Data**; 1998** (Mockenhaupt et al., 2001), **2000/2001** (Abruquah et al., 2010) **2003/04, 2005/06, 2007/08a** and **2010** (Duah et al., 2010), **2007/2008b** (Alam et al., 2011), **2004** (Owusu-Agyei et al., 2009), **2011/12** (present study).

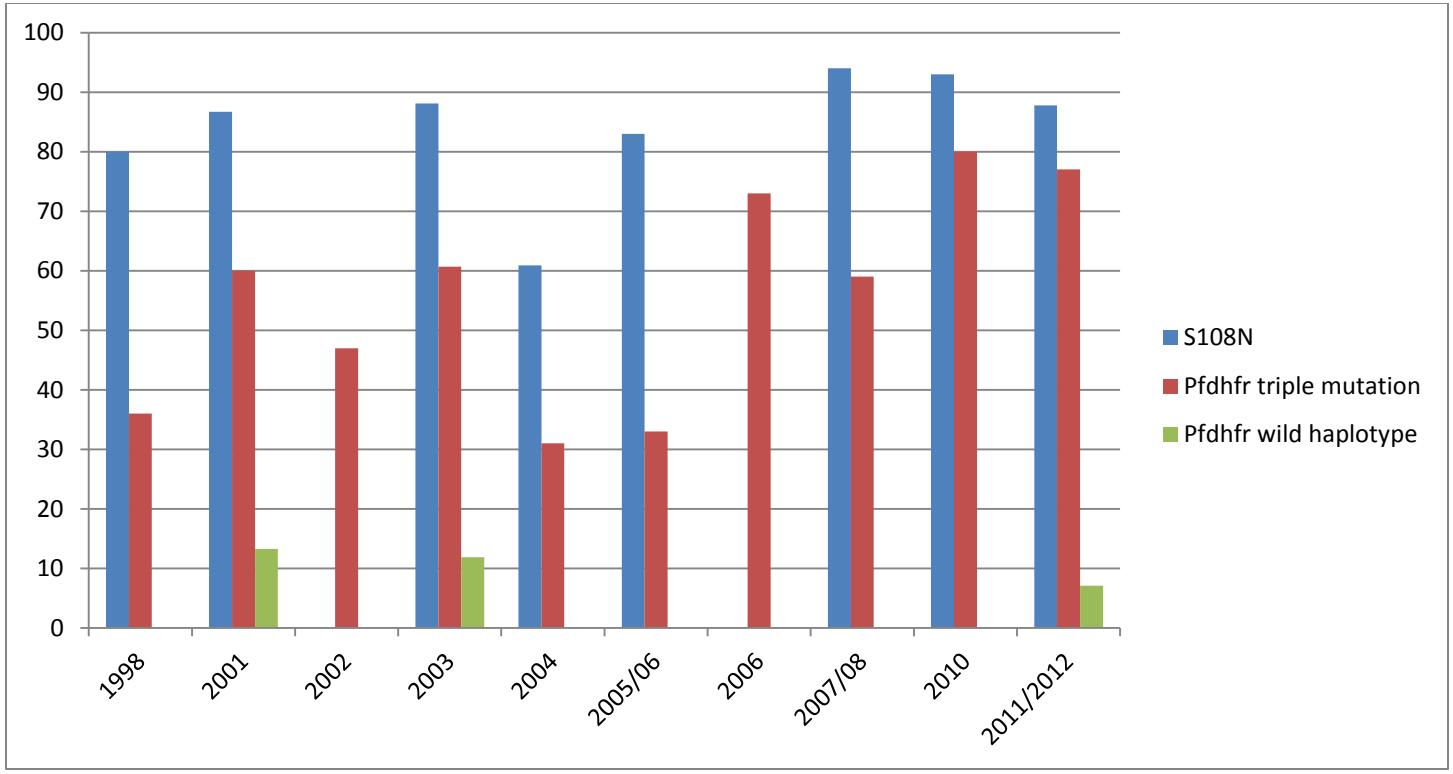


Fig 5.7 Trends and comparison of prevalence of *Pfdhfr* S108N, triple mutation (IRN) and wild type haplotype in Ghanaian isolates

Source of data; **1998** (Mockenhaupt et al., 2001), **2001 and 2003** (Marks et al., 2005), **2002** (Mockenhaupt et al., 2005), **2004** (Owusu-Agyei et al., 2009), **2006** (Mockenhaupt et al., 2008), **2007/08** (Alam et al., 2011), **2005/06 and 2010** (Duah et al., 2012), **2011/12** (present study).

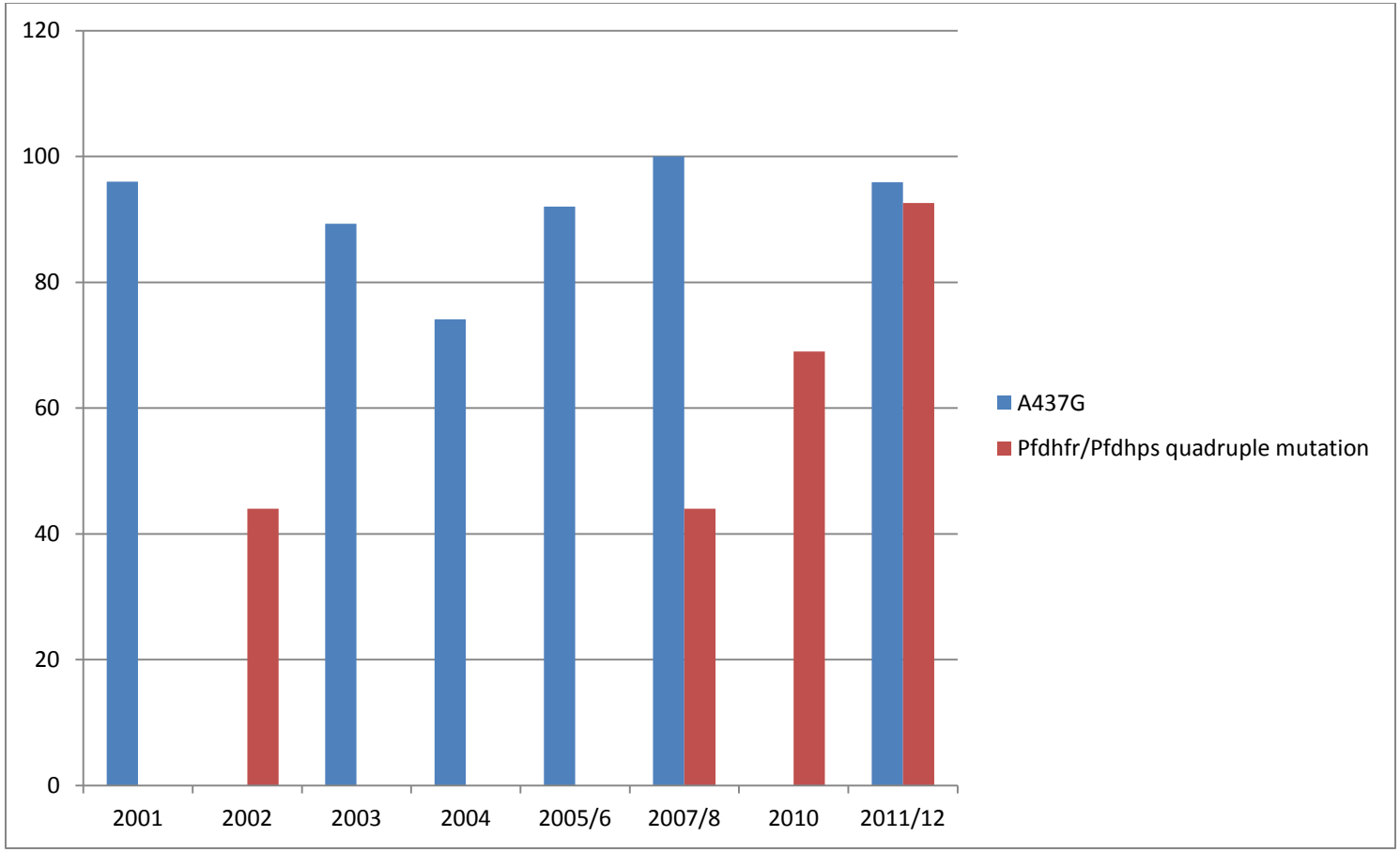


Fig 5.8 Trends in prevalence of A437G and *Pfdhfr*/*Pfdhps* quadruple mutations in isolates from Ghana

[&]Source ofdata: **2001 and 2003** (Marks et al., 2005), **2002** (Mockenhaupt et al., 2005), **2004** (Owusu-Agyei et al., 2009), **2005/06, 2007/08 and 2010** (Duah et al., 2012). Data for **2011/12** was from the present study

CHAPTER SIX

PERCEPTIONS OF CLINICAL TRIAL PARTICIPATION AMONG GHANAIAN PREGNANT WOMEN

6.1 Introduction

This chapter first presents results of in-depth interviews with a subset of trial participants (and their husbands) on their experiences and interpretations emanating from their engagement in the trial. Secondly it details the considerations of another group of pregnant women who did not participate in the clinical trial regarding willingness to participate in a drug trial.

Table 6.1 shows the demographic characteristics of the women interviewed and they were similar with respect to all characteristics. The majority of women in both groups had formal education up to Junior High School, belonged to the Ashanti tribe by virtue of the study area and were married with at least two children.

Table 6.1 Demographic characteristics of women interviewed for perceptions of clinical trial participation

	Trial Participants (N=20)	**Non-trial participants (N=20)**
Age in years (mean, range)	(27, 19-43)	(29, 19-37)
Level of Education		
Primary	3	4
[a]Junior High School	15	13
Senior High School	2	3
Occupation		
Unemployed	5	
Trader	8	12
[b]Artisan	7	8
Number of Children		
None		6
One	6	3
≥Two	14	11
Religion		
Christianity	19	18
Islam	1	2
Marital Status		
Single	1	
Married	17	19
Co-habiting	2	1
Ethnic Group		
Ashanti	18	17
Ewe	1	
Dagaarti	1	
Grusi		1
Frafra		1
Waala		1

[a]Junior High School entails 3 years of schooling beyond six years of primary education. This level of education is considerably basic especially in rural and semi-urban areas and graduates may at best be described as semi-illiterate. [b]Artisans refer to hairdressers, seamstresses, teachers, a farmer, a musician and a mobile banker (a mobile banker is a bank clerk assigned to visit traders daily at their work places to collect their savings and thus saves the traders the hustle of going to the bank).

6.2 Trial participants's experiences and perceptions

Trial participants' experiences and perceptions of the trial are presented with respect to their knowledge of causes of malaria, reasons for agreeing to participate in the study, experiences with study drugs, perceptions of home visits and blood sampling

and views on whether there should be monetary compensation for participating in the trial.

6.2.1 Knowledge of causes of malaria

An exploration of the study women's knowledge of causes of malaria was of interest in a malaria intervention study such as the present one. The role of malaria parasites was not explicitly expressed but knowledge of the link between mosquitoes and malaria and the need to sleep in ITNs was universal among participants. However, other causes of malaria were mentioned in addition to mosquitoes. These included eating certain types of food and poor hand washing practices. Malaria was also expressed as some sort of a constant feature of pregnancy.

"it is mosquitoes that cause all these malaria. I was not sleeping under the net because I feel very hot when I sleep under it" (GO-AA-010, 43 years, P 4)

"The mosquitoes bite us and that is why we get malaria. At times, the food that we eat too can contribute to it……..And the fruits that we eat of late too is a factor. The chemicals are too much…..and they say every pregnant woman has malaria" (GO-AA-002, 34 years, P3)

6.2.2 Reason for study participation

Reasons for consenting to participate in the study included underlying health benefits and trust in the health system/ research team. The reasons are categorized under three sub-themes;

<u>Fear and Anxiety</u>

It is possible some women may have joined the study out of "fear and anxiety" occasioned by presentation of study information in a manner that could potentially be interpreted as "intimidating". The content of information given was largely correct but

it appears emphasis was unduly placed on potential complications of malaria in pregnancy in some cases.

"they told me that, mosquitoes have bitten me so I have malaria……. ……. They said that if they put me on the medication, the malaria will not affect my baby. They also told me that if I don't agree for them to put me on the program, it is possible that I can lose my baby" (GO-010-AA, 43 years, P4)

"they said they wanted to check if I had malaria or else I can die if I have one and I am not treated"

(GO-EE-05, 23 years, P1)

Linking existing discomfort to malaria

The women attended ANC for routine pregnancy monitoring only. However, they accepted when they were told they had malaria parasites because they made connections between some discomforts they felt at that time and malaria. They therefore joined the study to "cure" the malaria.

"the reason why i believed was that I was having waist pains and abdominal pain a lot. it was as if i will have miscarriage. so when he said that, i believed that it was true that i have malaria parasite in my blood" (GO-AA-007, 31 years, P4)

Trust in the research team and ANC midwives

Another factor that facilitated study participation was apparent trust in the knowledge or competence of the research team and/ or ANC midwives. Advice from ANC midwives to join the study appeared important to the women in deciding whether they would join the study or not. The element of trust also seemed to underlie acceptance

of the fact that they had malaria parasites even though they were not acutely ill during screening for the trial.

"what made me join is that the nurses (ANC midwives) told us when we go and we are told there is malaria parasite in our blood, we should not worry. We will be given medication for us to be fine. They said this malaria study is on- going everywhere" (GO-EE-02, 34 years, P4)

6.2.3 Experience with study drugs

The women's experiences with study drugs are presented under two sub-themes; "acceptability of study drugs" and "taking antimalarials when one did not feel sick"

Acceptability of study drugs

The study drugs appeared to have been universally accepted among the trial participants. A wide range of adverse events including vomiting, dizziness and general weakness were described irrespective of the study drugs they were assigned. In spite of these adverse events, the women expressed willingness to take the same medication again if required because of its perceived health benefits.

"You see when I went to the hospital, I was not sick…. But when I started taking this drug, I was not feeling well at all. It has rather exposed ***all my hidden sickness****. I became discouraged. I even said that I will stop taking the drug. Because I was strong before I took the drug. After I took the drug, it revealed* ***all my hidden sicknesses in my body"*** (GO-AA-010, 43 years, P4, took DHA-PPQ)

"I will take it if they give me another one. I believed that ***my blood was very weak*** *for that drug and that was why I had all such experiences. I have to take it the next time they give me so that it clears all the malaria parasites from my blood"* (GO- AA-010, 43 years, P4).

Reason for taking antimalarial drugs when one did not feel sick

Aside the presence of malaria parasites, confidence/ trust in the health system / health worker (includes the research team) appeared to be an important factor in the decision making process with respect to accepting to take antimalarial drugs even though they knew they were not sick.

In this respect, husbands were of the opinion that one is to believe and accept whatever one is told at the hospital and therefore supported that their partners took the drugs even without clinical illness

"I took it because it was given to me by health professionals at the hospital" (GO-EE-07, 18 years, P1)

"she went for check-up and they told her that she has malaria. You need not to disagree with them when they tell you something like that… they do test blood to check whether there is any disease in it. So you cannot dispute that fact...." (G3-AA-002, 42 years, husband to GO-AA-010)

6.2.4 Perceptions and attitude to home visits

Home visits were accepted by trial participants, their families and the wider community though there was initial anxiety and surprise. The anxiety seemed to arise from unfamiliarity with home visits targeted solely at pregnant women. There were some instances, however, where some family members and neighbours alike linked the home visits to the possibility that the women were infected with human immunodeficiency virus (HIV). Subsequent visits and explanations helped to dispel this view eventually.

"my husband used to mock me by saying .....you are special because it is you alone who gets a doctor (trial field worker) *to visit you in your house. He was really surprised about their visit. ……. Everybody was happy about their visits"* (GO-AA-010, P4)

"When she said someone will visit her I was quite worried because I had not seen that before ……but I knew it was going to help" (G3-EE-01, 36 years, husband)

"they said the illness might not be malaria……they said they have not seen such a programme before… that you are sick of malaria and health workers will visit you….they said it could be HIV/ AIDS" (GO-EE-06, P8)

The home visits were also conceptualized as a sort of a government/ hospital-owned programme or service that had been introduced to promote welfare of pregnant women in Ghana. Based on this perception, they commended the government and Pramso hospital (one of the recruitment centers) obviously thinking the government was driving this "programme".

"they said I was lucky that the ***government*** *has appointed someone to come to me"* (GO-EE-04, 35 years P8)

..... they were congratulating the Pramso hospital for introducing that ***programme*** *in order to prevent the pregnant women from getting such diseases.* (GO-AA-007, 31 years, P4).

6.2.5 Perceptions and attitudes towards blood sampling

Blood sampling during scheduled home visits were uneventful and none of the women in the trial refused it. Similarly, there was no withdrawal from the trial on account of blood being taken in the house. Neither the volume of blood nor the pain

associated with needle pricks seemed important but the frequency of blood samplings (about 6 in all) and a perceived negative impact on the growing foetus' wellbeing came up as issues that bothered study women;

"At a point in time I was afraid about the taking of the blood samples. So last time, my husband called them concerning how many times….. They assured him that they are using the blood to check whether there are malaria parasites in it or not. After they finish, I will be okay. In fact, I was okay after they finished. As for the taking of the blood samples, I think you must stop. It is not good for everybody. They can test the urine to check whether the parasites are cleared or not instead of the blood" (GO-AA-008, 21 years, P2)

"I was not comfortable with the fact that they were taking my blood when I was pregnant. I wondered if it not going to affect the child……. he answered that they are taking a little so it won't affect the child. So I was okay after that answer" (GO-AA-003, 25 years, P2)

Regarding the issue of blood sampling in participants' houses during home visits, full acceptance or acceptance with misgivings appeared to have been fashioned by the following viewpoints/ perceptions;

i) <u>knowledge and understanding of what the blood was going to be used for</u>

Adequate comprehension of the purpose for which the blood sample was taken underlined acceptance of blood sampling in the house for both participants and family members. However, there were also hints of preferences for blood to be taken at the hospital because the results would get to be known faster.

"I don't have any problem if you decide to take blood samples at home. When you come for the blood, I know that it is because of the malaria that you are coming for my blood. So I don't have any problem about that" (GO-AA-003, 25 years, P2)

ii) perceived convenience

Some women interpreted home visits from the perspective that they were being saved the money and time that they would have used to come to the hospital had they been asked to do so. This interpretation of convenience contributed to their accepting blood sampling in the house.

"I like it if they take it (blood draw) home……you will not travel to any place. They will just come to the house and take it. It is easier if they come home" (GO-AA-008, 21 years, P2)

i) inadequate privacy in the house

The opinion or viewpoint was not expressed explicitly during the trial but some women and their family members had misgivings about blood sampling done at their homes because they did not think there was enough privacy secured during the procedures. They thus preferred blood sampling in the hospital.

"*taking of the blood at the hospital is better than if it is taken at home…..... if somebody sees that he (field worker) is taking her blood at home, the person might have a bad feeling about it that he is using the blood for money rituals or something. On the other hand, at the hospital, it is taken in a room and there are machines ready to test it for the results to be released. Nobody knows if they will take the blood straight to the hospital or they will take it somewhere. So the hospital is better that if it is taken at home"* (G3-AA-002, 42 years, husband to G0-AA-010)

The latter narrative also seemed to suggest a potential mistrust that the blood samples could be used for some purpose other than the noted efficacy and safety assessments. This possible mistrust did not seem to be associated with blood sampling at the hospital but with those carried out by field workers in the houses of participants.

6.2.6 Monetary Benefits

Study women interpreted trial processes, especially home visits, as an exhibition of the researchers' concern for their health. They perceived they were being helped to attain good health for themselves and their babies. This perception underscored a general viewpoint that there was no need for any form of monetary compensations.

A related opinion was that they should have rather compensated the researchers for showing "compassion" towards them. The study drugs and the long lasting insecticide-treated net (LLIN) supplied were in some cases interpreted as compensation.

> *"left to me alone, we are the ones who are supposed to compensate you"* (GO-EE-04, P8)

However, another standpoint highlighted a "justice" perspective in that some thought they should have been appropriately compensated in kind for their time and blood samples taken.

> *"yes…you should have given me something to eat to recoup the blood you took"* (GO-AA-003)

6.3 Antenatal clinic attendants' perceptions regarding willingness to participate in drug trials

Key elements emerged from interviews with non-trial participants that shaped their willingness to participate in a hypothetical trial of antimalarial drugs, the details and aims of which were verbally presented to them as described in chapter 3. These included anticipated health benefits, assurances that no harm will be done to them and conceptualizations of trial objectives, processes and potential experiences with drug adverse events and blood sampling.

6.3.1 Health benefits

Anticipated health benefits to themselves and their babies during pregnancy underlined willingness to participate in the study. This was based on an understanding of the adverse outcomes of malaria in pregnancy

*"if there are malaria parasites in my blood, I will agree to join to protect me and my baby form getting malaria"(*GT-AA-006, G2P1)

6.3.2 Conceptualization of study as an effort worthy of support

The study was interpreted as part of a bigger effort to get rid of disease. Such an effort was considered good and needed to be supported by becoming part of it or joining the study

"yes…. please I will join…it is because you are helping to get rid of disease" (GT-EE-05, G3P2)

6.3.3 Familiarity with study drugs

As part of orally presenting the study aims and procedures to the pregnant women, they were shown packs of the study drugs; ASAQ and DHA-PPQ. Recognition and

familiarity with reported effectiveness of ASAQ favoured an inclination towards participation.

"yes I would like to join… this is because I have taken one of these before (ASAQ). I know that it is good because I was healed after I took the drug" (GT-AA-005, 8/12. G1P0)

"I really like the white and yellow drug you showed me (ASAQ). *it is a very good drug…. it is very uncomfortable when you take it at the beginning. But as time goes on, you will feel better. you become very weak at the moment you take it"* (GT-AA-004, 8/12, G3P2)

6.3.4 Trust in health workers

Willingness to participate in drug trial if invited was also contingent upon granting of permission by health workers in whom trust was apparently placed. Pregnant women were ready to cede decision-making regarding participation to health workers who were perceived to know everything regarding their health and that of their pregnancy.

"so i have to ask them (nurses) for them to agree before I join. They will give me the go ahead whether I have to take the drug and whether I have to go through all the procedures you have told me about. This is because I am a pregnant women and I don't most of the time take any drug outside their advice. I am afraid. I don't want anything to happen to me…. as I was saying, they know everything so if they agree, I will come and join. if the nurses allow me to take part with my condition, I will. For example if I am assured that the taking of my blood samples will not give me any additional problem, I will". (GT-AA-03, G3P2)

6.3.5 Perceptions about taking antimalarials when one does not feel sick

Conceptualizations surrounding the use of antimalarial drugs when one did not feel sick were categorized under the following sub-themes;

i) Preventive measure

Willingness to partake in the study and take antimalarials when one did not feel sick at all seemed to be favoured by conceptualization of the activity as a preventive measure that was going to prevent malaria infections.

"I don't think I would be worried about that because I am protecting myself from the disease…. even if the sickness has not started, you are protecting yourself from it. So that, if it comes, it will not attack you severely" (GT-AA-03, G3P2)

ii) "Hidden sickness"

Willingness to take antimalarials when one did not feel sick was also linked to the conceptualization of a "sick state" that is a constant component of the human system. This 'sick state' apparently only manifests physically from time to time but is constantly present even in a state of health. In essence, one did not have to feel sick or notice symptoms before taking medications because there was "hidden sickness". Taking antimalarials when one did not feel sick was therefore perceived to be acceptable.

"Everybody is sick in any part of his or her body even though everybody is looking alright…..: everybody is sick, just that you don't see it physically… If it has not put you on bed, you will never know that you are sick" (GT-AA-02, G4P3)

6.3.6 Perceptions about prospective home visits and blood sampling

Home visit was perceived as acceptable among the pregnant women and was not deemed to be a barrier to study participation. It was interpreted as "good" and a show of "love" from the researchers.

"…if someone does not love you the person will not come to visit you. I would rather have to welcome them. This is because they are coming because of me and they have a good mission also. …" (GT-AA-02, G4P3).

"…it is a good because thing…. if it was not a good thing, I don't think anybody will waste his time to come for the visit" (GT-EE-09, G5P4)

Blood sampling at home were universally thought to be acceptable and seemed to hang on an adequate understanding of the study aims. As was found among the trial participants, blood sampling was linked to HIV. Additionally, the volume and pain associated with needle pricks came up as issues pregnant women may consider in deciding whether to participate in clinical studies.

"I don't like malaria….. So if the person comes, you must allow the person to take your blood. Right now you have explained everything to me so I will agree when they come…. If I had not been educated about it, I don't think I would agree if they come to take my blood" (GT-AA-007, 5/12, G3P2)

"I think other people may be afraid to take their blood samples because of HIV…..there are some too who are not comfortable with the one they take from the veins. That one is too much. The one from finger pricks is fine" (GT-AA-004, G3P2)

6.3.7 Perceptions of potential drug adverse events

Drug adverse events were generally perceived as a familiar phenomenon and did not appear to deter willingness to participate in the hypothetical study. Moreover, adverse events were considered discomforts that must be encountered and tolerated in order to reach an ultimate health benefit. In this wise, the discomfort seemed to be perceived as a mechanism by which the drug's effectiveness is expressed and by which the final benefit of cure is achieved.

"these discomforts are normally associated with malaria drugs. For example the SP malaria drug they give us at the antenatal is like that. Everybody complains about it. This is because, anytime you will take it, you will fall sick for 3 days… you will have headache continuously and you will also vomit any food that enters your stomach. But after 3 days, then you feel okay again. Almost all the malaria drugs are like that" (GT-AA-001, G1P0)

"yes….may be through this discomfort, the malaria parasites will get out of my system" (GT--EE—04, G3P2)

Adverse events were also conceptualized as an entity independent of study drugs such that it should be possible to isolate them so that the study drugs can become more comfortable to take.

"no I won't… as I am sitting down I am not sick…: i know that if I take the drug, it is possible that it can expose all the hidden sickness in my body... i think you must talk to the manufacturers of the drug to take out all the associated discomforts from the drug" (GT-AA-03, G3P2)

CHAPTER SEVEN

DISCUSSION AND CONCLUSIONS

7.1 Introduction

A randomised non-inferiority trial was conducted to assess the safety and efficacy of DHA-PPQ treatment of uncomplicated *P.falciparum* malaria compared to ASAQ in second and third trimester pregnant women to help address the paucity of safety data regarding DHA-PPQ use in pregnancy. Additionally, the performance of the First Response® malaria RDT was compared with that of microscopy in the diagnosis of malaria infection in pregnancy to help fill knowledge gaps on its accuracy in Ghanaian pregnant women. Furthermore, against a background of reports of declining ACT and SP-IPTp effectiveness, the prevalence of parasite mutations underlying reduced susceptibility to these antimalarials was assessed to help update knowledge in this subject. Lastly, pregnant women's perceptions of clinical trial participation were assessed to better understand factors motivating participation in biomedical research in this population.

The principal study findings are summarized under the following broad headings;

1. Efficacy of DHA-PPQ and ASAQ in pregnancy.
2. Safety and tolerability of DHA-PPQ in pregnancy.
3. Diagnostic Performance of First Response® RDT in pregnant women.
4. Prevalence of *Pfcrt*, *Pfmdr1*, *Pfdhfr* and *Pfdhps* mutations in pregnant women.
5. Pregnant women's perceptions of participation in malaria drug trials.

The discussion of these findings, the strengths and weaknesses of the study and implications for practice, policy and future research form the basis of this chapter.

7.2 Efficacy of DHA-PPQ and ASAQ in pregnancy

DHA-PPQ was non-inferior to ASAQ with respect to PCR-uncorrected parasite clearance rates by days 28 and 42 in both per protocol and modified intention-to-treat analyses. Both study arms showed statistically significant increases in haemoglobin concentration over baseline at days 28 and 42 but the ASAQ arm had a significantly higher mean concentration than found in the DHA-PPQ arm at day 42 (11.2g/dl vs 10.8g/dl; p=0.01). There was no difference in the mean birth weight. However, the DHA-PPQ arm had a significantly higher proportion of babies with LBW.

The efficacy results, however, need to be interpreted cautiously in the context of a number of limitations encountered. These include the inability to recruit the full complement of the estimated sample size, inability to report PCR-corrected efficacy estimates and use of a "modified" intention-to-treat population for analysis rather than a "pure" intention-to-treat population.

The study recruited less than 50% of the proposed sample size within the time frame available. Combined with participant losses to follow up at the efficacy end points of days 28 and 42, this had the effect of reducing the study's power. A reduced power, if the sample size is not too small, has the effect of diminishing treatment differences and favours conclusion of non-inferiority in non-inferiority trials such as this one. In this wise, non-inferiority of DHA-PPQ to ASAQ may have been observed because of the low sample size used. However, the observation of non-inferiority in both analyses populations increases confidence in the efficacy results reported.

A major reason for the low number of pregnant women recruited was the inclusion criterion of positive parasitaemia by both RDT and blood film microscopy. Only a few women, compared to the total screened, met the requirement possibly because of

a low prevalence of parasitaemia from a nationwide distribution of LLINs in 2011 at the initial stages of the study. The criterion was however necessary to ensure only pregnant women with viable peripheral parasitaemia were recruited. Additionally, there was a period of about 3 months when very little or no recruitment was done because pregnant women were not attending ANC at public health facilities. This situation emanated from protests at some health care financing reforms that had been introduced in Ashanti region at the time.

The study is unable to report PCR-corrected efficacy estimates and this limits comparison to other studies. Genotyping of paired baseline/ treatment failure samples yielded very few positives and recrudescence/ reinfections could not be differentiated at the *msp2* and *glurp* genes. The reason for this is unclear but amplification at other gene loci was largely successful.

Failure to include all randomised participants in the intention-to-treat analysis may introduce biases and limit confidence in the results obtained. However, the number excluded was about 8% of those randomized at day 28 and 11% at day 42 and could be considered to be within acceptable ranges. Moreover, the excluded participants were from both study arms and were similar in number and characteristics so that the potential impact of a selection bias is minimal and the study's internal validity is not significantly undermined from the exclusions made. Similar conclusions of non-inferiority were reached in both analyses populations and this increases confidence in the results obtained. The losses to follow up, including those at delivery and at 6 weeks post-partum, were mainly because the women had travelled outside their homes ostensibly for family support as they neared delivery or after they had delivered. This was inspite of assurances given to the contrary at recruitment.

Furthermore, there were deliveries that occurred before the days 28 and 42 end points and this contributed to the inadequacy of numbers available for analysis at these points. Losses to follow-up, with consequent non-availability of data at days 28 and 42 occasioned the use of a "modified intention to treat population" limited to those with any outcome at these endpoints. Data imputation at the defined end points may have been an alternative approach but this leaves much room for subjectivity. While the study team could not have prevented the women from travelling, the losses may have been minimized if the team had probably laid more emphasis on the need for the women to be around up to study completion.

There was only a moderate level of agreement observed between field and expert micrcoscopy and this may have impacted on parasitological outcomes and the ultimate results. However, any effect on parasitological outcomes could be either way so that its overall effect is likely to be annulled.

Information is lacking on studies comparing DHA-PPQ and ASAQ in a non-inferiority design. Observations from the present study, however, resonate with other studies where DHA-PPQ was found to be non-inferior to other ACTs such as ASMQ and AL and reported to be comparable to ASAQ (Bassat *et al.,* 2009; Naing *et al.,* 2013; Nji *et al.,* 2015).

Considering that DHA-PPQ has a good efficacy profile (Smithius *et al.*, 2006; Zongo *et al.,* 2007; Myint *et al.,* 2007; Valecha *et al.,* 2010), it is anticipated that PCR-corrected efficacy estimates would still demonstrate non-inferiority. Cumulative PCR-unadjusted parasitological efficacy was comparable between study arms by days 28 and 42 in the per protocol (PP) and modified intention-to-treat (mITT) populations. The similar efficacies of ASAQ and DHA-PPQ by day 42 suggest the longer post-

treatment prophylaxis associated with DHA-PPQ was not demonstrated. This is probably because malaria transmission in the study area was high such that there may have been considerable new infections.

The day 28 efficacy estimate for ASAQ in the PP population is similar to the PCR-uncorrected estimate for the same time point in Tanzanian pregnant women (89.3% vs 91.0%) (Mutabingwa *et al.,* 2009) while the day 42 estimate also compares favourably with those in Cameroonian and Asian children (Thanh *et al.,* 2009; Nji *et al.,* 2015). Compared to reports in Ghanaian children, ASAQ efficacy by day 28 varied from similar uncorrected findings of 74.5% and 95.3% respectively observed in 2003 and 2006 (Koram *et al.,* 2005; Adjei *et al.,* 2008).

Naturally acquired malaria immunity in the pregnant women offers a reasonable explanation for the appreciable disparity (89.3% vs 74.5%) between the present study's findings and that in children under five in Koram *et al* (2008). In Adjei *et al* (2008), inclusion of children 10-14 years old may have contributed to the appreciably high efficacy of 95.3%. There is some level of naturally-acquired immunity in this age group in high transmission intensity areas which may have contributed to parasite clearance.

It is possible the relatively lower efficacy observed for ASAQ may be underlined by poor adherence to the co-blistered preparation used but this is not very likely as drug packs were inspected for compliance on home visits. Moreover, most doses were taken under observation in the homes of the participants. Poor adherence, under the circumstances, would be minimal and not impact significantly on ASAQ efficacy reported. The co-blistered rather than a co-formulated preparation of ASAQ was used

because that was what was readily available when the study commenced. Tolerability was high as no participant left the study on account of the study drugs.

Comparing DHA-PPQ efficacy results in non-pregnant population, the day 28 DHA-PPQ efficacy observed (91.6% in the PP) was consistent with estimates of 90.0%-98.0% over the same follow-up period from other studies (Bassat *et al.,* 2009; Zwang *et al.,* 2009; Valecha *et al.,* 2010; Zani *et al.,* 2014). The day 42 DHA-PPQ efficacy observed compares favourably with assessments in children (Hasugian *et al.,* 2007) but varies widely compared to results from other studies (Smithius *et al.,* 2006; Bassat *et al.,* 2009).

Comparability between DHA-PPQ efficacy in the present study and non-pregnant populations is consistent with reports of insignificant pharmacokinetic differences in the metabolism of DHA-PPQ between pregnant and non-pregnant women (Hoglund *et al.,* 2012). This may suggest there is no need for adjusting antimalarial drug dosages in pregnancy as a result of physiological alterations suspected to reduce drug bioavailability (McGready *et al.,* 2006; Nyut *et al.,* 2010).

Aside the reduced sample size favouring a conclusion of non-inferioity, it is possible the moderate-to-high transmission intensity in the study area so much clouded the longer post-treatment prophylaxis of DHA-PPQ that there was hardly any difference in treatment success between it and ASAQ. In such a case, wider non-inferiority margins and subsequently smaller sample sizes are likely to still demonstrate non-inferiority. The observation in the present study may be an indication that a margin wider than the chosen 5% would still have been clinically relevant. *P.falciparum* prevalence by RDT was 17% while that by combined blood film microscopy and RDT was 12%.

The overall prevalence of anaemia (Hb<11.0g/dl) at baseline was consistent with results of other studies in pregnant women in Ashanti region (Bam, 2009; Tay *et al.,* 2013). The finding of a higher mean haemoglobin concentration in the ASAQ arm at day 42 is inconsistent with reports of better haematological recovery on account of a longer post-treatment prophylaxis for DHA-PPQ (Zongo *et al.,* 2007; Kamya *et al.,* 2007; Ratclif *et al.,* 2007). This finding could be explained as a run off from noted differences in haemoglobin concentration at baseline. The DHA-PPQ arm had a significantly higher proportion of women in the lowest haemoglobin concentration category of 7-8.9g/dl (14.5% vs 7.8%; p=0.04) and may thus have been disadvantaged in this respect compared to ASAQ.

Nutrition, helminthiasis and some socio-cultural factors may impact on haemoglobin levels but these were not investigated. Though it may be described as severe anaemia, a ≥7g/dl haemoglobin concentration cut-off was considered an appropriate middle ground and used in the inclusion criteria. A choice of 8g/dl would likely leave out many pregnant women who need the intervention while 6g/dl would be stretching boundaries rather far.

In addition to haemoglobin concentration, there were also differences in age and total white blood cell count at baseline. Differences in age and haemoglobin concentration were adjusted for in the efficacy analysis as they have been known to impact on treatment outcomes so that any possible effect is annulled. These differences possibly arose by chance and do not invalidate the randomization process as it was successful for other baseline variables. The efficacy analysis did not take cognisance of differences in baseline total white blood cell count as this is unlikely to confound antimalarial treatment outcome. There is no clear reason for the higher frequency of

LBW in the DHA-PPQ arm. There was no difference in preterm births, placental and cord parasitaemia between the study arms.

The overall prevalence of parasitaemia (12%), based on combined RDT and microscopy, is consistent with recent reports (Gifty Antwi, personal communication, 2013; Tay *et al.,* 2013) and follows a general trend of declining *P.falciparum* prevalence in pregnancy in Ghana (Glover-Amengo *et al.,* 2005; Tagbor *et al.,* 2006; Ofori *et al.,* 2009). The decline may be due to effectiveness and increased coverage of control tools like ITN, IPTp-SP, ACTs and possibly a declining malaria transmission. There are no recent malaria transmission studies in Ashanti region but there has been an appreciable decline from over 400 to 139 infective bites/person/year over the last decade in Kassena-Nankana district in the Upper East Region of Ghana (Victor Asoala, 2014, personal communication).

Positive RDT with negative microscopy results during screening may have been detecting placental infections. Sequestration of parasites in the placenta could lead to absent or undetectable peripheral parasitaemia at microscopy (Kattenberg *et al.,* 2011; Conroy *et al.,* 2012). It is possible the prevalence of asymptomatic *P. falciparum* may be higher than represented and that more women could have been recruited if the RDT sensitivity were higher than observed. Ultimately, it is also possible the study results could have been different with a different RDT.

7.3 Safety and tolerability of DHA-PPQ and ASAQ

Dihydroartemisinin-piperaquine was found to be safe and better tolerated than ASAQ. Only mild diarrhoea was more frequent with DHA-PPQ but this was not statistically significant. Most treatment-emergent AEs had resolved 3 days after start of study treatment. There were no excess events of SAEs including spontaneous abortions or

other adverse pregnancy outcomes in the DHA-PPQ arm. Polydactyly and a gum mass were the only congenital abnormalities observed.

Neither DHA-PPQ nor ASAQ showed overall abnormalities in differential white blood cell counts. Absence of liver/ renal biochemistry and electrocardiography assessments is a limitation and restricts comparison to studies reporting these parameters. However, the overall conclusion of DHA-PPQ safety in pregnancy is not diminished.

The better tolerability of DHA-PPQ compared to ASAQ and its association with more frequent diarrhoea have been reported in other studies (Zwang *et al.,* 2009; Katrak *et al.,* 2009; Trung *et al.,* 2009; INESS Report, 2011; Zani *et al.,* 2014).

Occurrence of significantly more frequent reports of general weakness, sleeplessness and nightmares in the ASAQ arm compared to the DHA-PPQ group 7 days after start of treatment is difficult to explain in light of expectations that most AEs would have resolved by this time. It is likely that AEs on day 7 and beyond for both study drugs may not actually be due to study drugs but rather what may be described as pregnancy-associated complaints, similar to the pre-enrolment complaints.

Of the reported SAEs, only the cases of diarrhoea are deemed to have a probable link to DHA-PPQ on account of more frequent association and the timeframe between exposure and occurrence. Though the case of placental abruption also occurred within 3-4 days after start of treatment, there is no known plausible mechanism by which DHA-PPQ may cause that condition. It is rather deemed to be within background occurrences of pregnancy complications seen in the area. For instance, in the first quarter of 2014 alone, 3 cases of antepartum haemorrhage and 18 cases of

hypertensive diseases in pregnancy were managed at the maternity ward of St. Michael's hospital (Maternity ward records, St. Michael's hospital, 2014).

Absence of excess events of spontaneous abortions or other adverse pregnancy outcomes in the DHA-PPQ arm is consistent with findings in Papua New Guinea and comparable to ASAQ and AL use in Tanzania and Zambia (Mutabingwa *et al.,* 2009; Manyado *et al.,* 2010; Poespoprodjo *et al.,* 2014). The prevalence of polydactyly observed in the DHA-PPQ arm is similar to that reported in Tagbor *et al* (2008) among pregnant women who received AQ.

Neither ASAQ nor DHA-PPQ showed significant changes in total white blood cell count over baseline on all assessment days contrary to reports relating DHA-PPQ with decreased white blood cell count (Davies *et al.,* 2005; Myint *et al.,* 2007). Both study drugs were characterized by significant neutropenia and lymphocytosis compared to baseline levels at days 14, 28 and 42. Neutropenia is consistent with reports of AEs linked to artemisinin derivatives (Nosten and White, 2007). The findings of neutropenia and lymphocytosis must be interpreted with caution as the mean values observed remained within normal ranges for the autoanalyzer used. In effect, there were no abnormalities with the differential white blood cell counts in either study arm similar to findings by Ahmed *et al* (2008). Pregnancy itself is associated with increased neutrophil and lymphocyte counts (Chandra *et al.,* 2012).

7.4 Diagnostic performance of First Response® RDT in pregnant women

First Response® RDT correctly identified 82.1% of women with *P.falciparum* parasitaemia and the same proportion of aparasitaemic women. There were false negative RDT results for slides with parasite densities ≥200/µl. Using peripheral blood film microscopy as the reference standard was a limitation because of the

element of subjectivity but there is high confidence in the results reported because the microscopy was done by an expert from a reputable research institution. Moreover, false positive RDT results may not actually have been false as they could be detecting placental infections. Placental infections were, however, not the focus of this study.

About 7.5% of randomly selected RDT cassettes were excluded on account of poor quality of corresponding blood films. Analysis was based on about 1600 RDT/ blood film pairs and this is considered an adequate number.

The study's observations were below WHO recommendations of 95% sensitivity and 90% specificity (WHO, 2009). The observed sensitivity is also lower than >90% reported for HRP-2 RDTs with microscopy as reference (Kattenberg *et al.,* 2011). First Response® RDT sensitivity was similar to that reported in a study involving pregnant women (Matangila *et al.,* 2014) but lower than that observed in another (Minja *et al.*, 2012) though both studies used PCR as reference.

Comparing to Ghanaian children, the test sensitivity was lower than reports of 100% for HRP2 RDT tests (Baiden *et al.,* 2012; Osei-Kwakye *et al.,* 2013) presumably because older age and immunity in the pregnant women limit parasite densities and predispose to lower test sensitivity. Among the study women, older age groups and higher parities were less likely to show positive RDT results and this highlights the role of immunity in RDT sensitivity. There was parity data for only about 20% of the study population but this number is sufficiently large to ensure validity of the inferences made.

Though HRP-2 tests are reportedly more sensitive than LDH tests (Karbwang *et al.,* 1996), the First Response® RDT performance was below that of an LDH test in pregnancy in the same malaria transmission zone in Ghana (Tagbor *et al.,* 2008).

Declining parasite prevalence earlier mentioned may explain this finding. However, the sensitivity was much higher than that reported for another LDH test also in pregnant women in northern Ghana (Ayeh-Kumi *et al.,* 2012). Transmission in northern Ghana is markedly seasonal and the low prevalence of *P.falciparum* parasites at the time the study was conducted may have impacted on the poor performance of the LDH test.

The relatively lower sensitivity and specificity observed could be from a generally low parasite density distribution in the study women. The observations agree with a reported RDT sensitivity of about 75% for parasite densities 100/µl -1000/µl (Msellem *et al.,* 2009) and ≥ 80% of the study women had parasite density <500/µl at baseline. However, densities ≥200/µl are the detection threshold for RDTs irrespective of geographical location (WHO, 2009).

The variability in performance of First Response® RDT can also be attributed to possible polymorphism and deletion of the HRP-2 gene. These phenomena have not been investigated in Ghana but have been reported in West Africa (Wurtz *et al.*, 2013). Sub-optimal storage conditions and misclassification of RDT results are other possible reasons but these are unlikely because the RDTs were stored in an air-conditioned environment and used in ambient temperatures presumably below 40°C (the maximum temperature at which the RDT remains stable). Furthermore, study staff were trained over five days to correctly perform the tests.

Persistence of HRP-2 antigen from previous infections in the pregnant women presumably is one of the reasons for the >200 false positive RDT results. This phenomenon is well documented in literature (Karbwang *et al.,* 1996; Moody, 2002; Humar *et al.*, 1997; Kattenberg *et al.,* 2012). Though unlikely, the large number of

false positive RDT results could have been due to false negative microscopy results in some cases.

False negative test results are often attributed to parasite densities below the RDT's detection threshold. However, parasite densities from some microscope slides corresponding to false negative RDT results were as high as 240/µl and 760/µl. This finding is similar to some studies (Birku *et al,* 1999; Dyer *et al.,* 2000; Bisoffi *et al.,* 2010) that found false negative RDT tests with parasite densities ≥1000/µl.

It is possible the parasite isolates that could not be detected lacked the gene for HRP-2 or produced polymorphic forms that could not be detected. The prozone effect (Gillet *et al.,* 2009) may also explain this finding but its influence is not very clear with regards to parasite densities under 1000/µl as observed in the present study.

Two slides with *P.malariae* alone showed positive RDT results when it is known HRP-2 is produced solely by *P.falciparum* in all its blood-stage life cycles (Hayward *et al.,* 2000). It is possible the RDTs in this particular instance were detecting HRP-2 from a concomittant *P.falciparum* placental infection or they were detecting persistent HRP-2 from a previous falciparum infection.

The positive predictive value of 21.7% is most likely due to the low prevalence of infection (5.5% by microscopy) among the women analysed for the diagnostic accuracy and is not likely to have adverse implications for clinical practice.

7.5 Prevalence of *Pfcrt, Pfmdr1, Pfdhfr* and *Pfdhps* mutations in pregnant women

Close to 90% of *P. falciparum* isolates in the study women were wild type at codon 72-76 of the *Pfcrt* gene. A similar prevalence was observed for the *Pfmdr1* N86 allele while the N86-184**F**-D1246 (NFD) and N86-Y184-D1246 (NYD) haplotypes had

equal prevalence of 43.2%. The *Pfdhfr* triple mutation (N51I+C59R+S108N) was present in 77% of evaluable samples. The quintuple mutation (51I+59R+108N/ 437G+540E) suspected to contribute immensely to SP resistance was not observed in the study.

A limitation to the study was the low proportion of evaluable samples at some codons especially in the *Pfdhfr* and *Pfdhps* genes and it is possible prevalence data may be higher than reported.

The prevalence of *Pfcrt* 76K observed is potentially the highest reported in Ghana since changing from CQ as first line treatment of uncomplicated malaria in 2004.

It is consistent with studies reporting repopulation of wild-type *P.falciparum* parasites when CQ was withdrawn in Malawi (Kublin *et al.,* 2003), Kenya (Mwai *et al.,* 2009), Senegal (Ndiaye *et al.,* 2012), Tanzania (Mohammed *et al.,* 2013; Malmberg *et al.,* 2013) and Mozambique (Thomsen *et al*., 2013). Moreover, the resistant haplotype found was only CVIET and this agrees with commonly-made observations for CQ-resistant isolates from Africa (Venkatesan *et al*., 2014).

Such a high frequency of *Pfcrt* wild type isolates suggests a lack of use of CQ for the past years or at best, scanty use. In contrast, a study conducted in 2012 in southern Ghana reported 72% prevalence of the *Pfcrt* K76T mutation in participants older than 6 months (Asare *et al.,* 2014). The high prevalence of *Pfcrt* mutant parasites in that study was attributed to sustained CQ use as evidenced from its presence in urine samples from 17% of the study participants. The use of CQ among pregnant women in the present study was not ascertained but it could not have been significant judging from the high frequency of wild type parasites reported.

The predominance of wild-type parasites reported in the present study, despite the use of ASAQ since 2005 in second and third trimester pregnancies, appears to support the position that AQ does not select for K76T mutation to any significant extent (Sarr *et al.,* 2008; Danquah *et al.,* 2010; Ly *et al.,* 2012) although this remains an unsettled question. It is therefore unlikely that ASAQ use contributed to the high prevalence of 76T mutations in Asare *et al* (2014)

The study findings contrast with close to 90% prevalence of *Pfcrt* mutants in children with severe malaria (Abruquah *et al.,* 2010). It is not clear when this particular study was conducted but believed to be around 2001/2002 when CQ was actively used.

Compared to more recent studies, the low prevalence of 76T mutation observed (11.4%) also contrasts sharply with 51% prevalence (Kwansa-Bentum *et al.,* 2011) and 58.54% prevalence (Afoakwa *et al.,* 2014) reported in studies conducted in 2010/ 2011 among outpatient department attendants in six regions spanning the coastal/ mangrove and forest epidemiological zones in Ghana. Malaria transmission is perennial in these areas with peaks in the rainy seasons. The reason for the difference is not clear as a high proportion of participants in the two studies were adults similar to the pregnant women in the present study which was conducted in 2011/ 2012. The likelihood of CQ use in addition to AQ in these populations cannot be completely ruled out.

A lack of CQ use aside, another explanation for the predominance of *Pfcrt* wild-type *P.falciparum* isolates observed is selection by AL. They are predominant in recrudescent samples observed after AL use (Sisowath *et al.,* 2005; Thomsen *et al*., 2013). The present study did not assess for *Pfcrt* and *Pfmdr1* selection owing to too

few positives in the follow-up days. This is likely due to a high efficacy of the study drugs.

The study findings suggest there is prospect for reintroducing CQ for case management in pregnancy in the event that ACTs begin to fail but this is not likely to be in the very near future. Chloroquine is safe throughout pregnancy (Wolfe and Cordero, 1985; Villegas *et al*., 2007) and was given as weekly malaria chemoprevention before IPTp-SP was introduced. Any future use of chloroquine must be in combination with another drug to help protect it from early re-emergence of resistance. Its use ought to be restricted to pregnant women who have tested positive by either rapid diagnostic test or microscopy. In combination with azithromycin (AZ), CQ has been tried for treatment with varying efficacy in non-pregnant populations (van Eijk and Terlouw, 2011).

In reintroducing CQ, considerations must be given to dealing with adverse events such as pruritus. Furthermore, since a re-emergence of resistance will be expected, it will be necessary to ensure CQ is not sold in the private sector again and secondly to conduct surveillance of molecular resistance markers in pregnant women.

The high prevalence of *Pfmdr1* N86 compares favourably with 71%-100% prevalence reported in Ghanaian children in 2007-2010 (Duah *et al.,* 2013). The predominance of this allele is explained by selection following AL use as has been demonstrated in a number of studies including a review (Sisowath *et al.,* 2005; Lekana-Douki *et al.,* 2011; Venkatesan *et al.,* 2014). The high prevalence of N86 could be due to a lack of CQ use but the high prevalence of 184F in the present study supports the impact of AL. The prevalence of *Pfmdr1* mutation Y184F was within the range of 40%-80%

reported for children across the country in 2010 (Duah *et al.*, 2013). 15.1% prevalence for N86Y mutation is consistent with a decreasing trend in Ghana (see Fig. 5.6).

The prevalence of NFD haplotype observed is similar to that reported for Ghanaian children in 2007/2008 (Alam *et al.*, 2011). There have been reports of increasing prevalence of the NFD haplotype following introduction of AL (Humphreys *et al.*, 2007; Malmberg *et al.*, 2013; Thomsen *et al.*, 2013). A higher prevalence than observed was expected but it is possible low drug pressure in pregnant women has kept selection of NFD low. No triple c86-184-1246 mutant YYY haplotype was observed in contrast to a reported 5% in Ghanaian children in 2007/2008 (Alam *et al.*, 2011). This finding agrees favourably with a study in Zanzibar that reported significant decreases in prevalence of the YYY haplotype seven years after using ASAQ (Froberg *et al.*, 2012).

The frequency of the *Pfdhfr* triple mutation is comparable to previous reports in pregnant women in West Africa (Mockenhaupt *et al.*, 2008; Bertin *et al.*, 2011; Moussiliou *et al.*, 2013). In pregnant women, it seems to have stabilized since the last reported survey in 2006 (Mockenhaupt *et al.*, 2007) compared to appreciable increases seen in Ghanaian children over a similar time period (Duah *et al.*, 2012). Intermittent preventive treatment of malaria in pregnancy with SP is obviously not provoking further significant increases in the frequency of the triple mutation over the 5-6 years preceding the survey. The observed prevalence of *Pfdhps* 437G mutation is consistent with other study reports (Marks *et al.*, 2005; Bertin *et al.*, 2011; Duah *et al.*, 2012) and underlies the equally high frequency of the quadruple *Pfdhfr/Pfdhps* mutation (N51I+C59R+S108N/ A437G).

The K540E mutation was not found in the present study and is in line with other studies reporting its absence or very low prevalence in West Africa (Marks *et al.,* 2005; Duah *et al.,* 2012; Mousiliou *et al.,* 2013). Together with the minor prevalence of 581G and 613S mutations reported, it may be argued that IPTp-SP in Ghana (West Africa) is not likely to be associated with lower mean birthweights, increased parasitaemia and placental inflammation reported in other places where higher levels of these mutations occur (Harrington *et al.,* 2011; Minja *et al.,* 2013). The limited prevalence of these mutations may explain the good efficacy of SP, PCR-uncorrected cure rate of 85%, in pregnant women (Dr. Harry Tagbor, personal communication, 2010) in a district adjoining the study area.

The quadruple *Pfdhfr/Pfdhps* mutation (N51I+C59R+S108N/ A437G) was present in over 90% of evaluable isolates, consistent with reports in pregnant women from Benin (Bertin *et al.,* 2011; Moussiliou *et al.,* 2013). *Pfdhfr/Pfdhps* quintuple mutation (N51I+C59R+S108N/ A437G+A613S) and sextuple mutation (N51I+C59R+S108N/ A437G+A581G+A613S) were present in very few samples in contrast to a widespread occurrence in East Africa where they include the 540E mutations (Minja *et al.,* 2013).

The implication is that while IPTp-SP may still be useful in West Africa, there are questions over its effectiveness in East Africa. The WHO has recently recommended increased doses of SP for IPTp even in areas where high frequencies of *Pfdhps* 540E and 581G occur (WHO, 2012) and it is important to monitor how implementation will impact on selection of *Pfdhfr/ Pfdhps* mutations correlated with low birth weight, maternal anaemia and placental malaria/ histology.

7.6 Pregnant women's perceptions of participation in malaria drug trials

Study women alluded to both biomedical and locally-construed notions of malaria causation. Trust in health workers/ researchers and anticipated health benefits mainly appeared to be the motivation for trial participation among the study women. Treatment-emergent adverse events were interpreted to mean the study drug in question was efficacious and study women were willing to endure them for the welfare of their pregnancies. Home visits and blood draws during the trial were linked to HIV/AIDS. Lastly, while there were no suggestions of cultural barriers to blood draws at home, it appears there is a preference towards hospital-based blood draws.

The strength of the study lies in combining experiences and perceptions of trial participants with those of another group of pregnant women who have not participated in trials previously. The dichotomy between "what is" and "what could be" in this context is thus diminished.

None of the trial participants interviewed suffered a serious adverse event, withdrew from the trial or was lost to follow-up. The interviewees were also generally of a low socio-economic status. It is possible participants who had the mentioned experiences or pregnant women with higher educational status may have views different from those obtained. Nevertheless, the views expressed are valid as they include dissenting opinions on various issues.

Simultaneously holding accepted biomedical and locally-construed notions of causes of malaria is commonly reported (Espino *et al*., 1997; Ahorlu *et al.*, 1997; Asante et al., 2010). Incongruence between local perceptions of malaria or any other disease condition and recognized biomedical causes can pose challenges for disease control as recommended interventions may not be accepted (Heggenhougen *et al.*, 2003). This

leads to delays in seeking appropriate treatment with subsequent increased morbidity and mortality. In the context of recruiting and retaining study participants in drug trials, it may be easier for participants to understand study information if their concept of disease causation aligns with known biomedical causes.

Malaria was conceptualized as an integral part of pregnancy presumably because most pregnancy-related complaints are similar to malaria symptoms. Study participants were knowledgeable about the consequences of malaria in pregnancy presumably from study information given and health education at ANC. Relating malaria to poor hand washing habits is very likely the result of a mix-up of information given to pregnant women. Health education on various disease conditions is given at ANC and they may have mistaken causes of cholera or other diarrhoeal diseases with those of malaria.

The main reasons noted for consenting to participate in the trial; underlying health benefits and trust in the health workers/ researchers are in line with other studies (Mohanna, 1998; Rodgers *et al.*, 2003; Nechuta *et al.*, 2009; Gatny and Axinn, 2011; Lyerly *et al.*, 2013). Among the trial participants, the need to pursue health benefits is perceived on the part of investigators to have been reinforced by elements of "fear and anxiety" that possibly emanated from the orientation of study information given. This perception was informed by non-verbal expressions as well. Research assistants were trained on appropriate delivery of study information but they may have "over-emphasized" the complications of malaria in pregnancy. Participants may have interpreted this "over-emphasis" to mean they had to join the study to avert these outcomes. Such a situation could have amounted to diminished autonomy for the women.

The informed consent process serves to equip potential study participants with the information required to decide to participate in a study or otherwise. Pivotal to the written informed consent in clinical trial is a description of anticipated risks derived from the intervention or activities associated with the intervention (Nijhawan *et al*., 2013). Perhaps the risk associated with not receiving the intervention also ought to be given attention as it may hold equal importance in some instances. Given the adverse effects of malaria in pregnancy, it may be argued that an emphatic explanation of the consequences of harbouring malaria parasites was justifiable and may not have been an "over-elaboration" on the part of the study workers conducting the informed consent process.

Nevertheless, the fact that the investigator perceives some respondent narratives suggested "fear and anxiety" is itself an indication that the consenting process could have been better done. There were other pregnant women who were given study information in a similar manner but who refused to consent to study participation. These were treated according to national guidelines. The argument for diminished autonomy is thus weak.

Trust in the research team, particularly the midwives, may be interpreted from the perspective of unequal power relations. Recruitment into the trial was conducted in a hospital setting with the involvement of antenatal clinic midwives who have delivered routine care to most of these participants previously. These factors could have led to an unequal power balance between the research team and the participants (Holloway and Wheeler, 1999; Karnieli-Miller *et al.*, 2009) such that women may have had diminished autonomy. They may have participated in the study more to please the antenatal midwives than out of a decision arrived at by carefully weighing risks and benefits that the informed consent process sought to achieve.

Contrary to findings from other studies (Mohanna, 1998; Jones *et al.,* 2006; Catania *et al.,* 2008), altruism and advancement of science were not important facilitators of trial participation. Advancement of science in particular is more likely to be given as a reason for study participation by well educated people.

In spite of various experiences of drug adverse events, the study women were willing to complete courses of study drugs and repeat treatment if required because of perceived health benefits; first for their pregnancies and secondly for themselves. The need to protect their pregnancies was important as previously reported (Roger *et al.,* 2003; Smith *et al.,* 2010)

Study medications were not perceived to be direct causes of adverse events but vehicles by which pre-existing sickness or "hidden sickness" was expressed. Adverse events were also blamed on one having "weak blood". In this sense, study drugs were perceived as powerful and overwhelming for a comparatively weak "blood/ body system".

The concept of "strong or weak blood" also finds expression in other situations in the study area. For instance, a male or female with infertility can be described as having "weak blood". Also, when a newborn baby shows a strong physical resemblance to one parent, that parent is said to have "strong blood". The concepts of "weak blood" and "hidden sickness" point to a 'body system' that is not sufficiently strong or does not measure up to some standard. While no direct reference was made to pregnancy being an underlying factor for "hidden sickness" and "weak blood", it is not unreasonable to presume the pregnant women made these descriptions with their pregnancies in mind.

Discomfort/ adverse events associated with study drugs are also perceived to relate to efficacy. This reflects a local belief system that the more bitter a drug is or makes one feel uncomfortable, the better it cleans or purges one's body of disease. Based on this belief, some herbal potions used locally have the acronym "bitters"

While study women understood home visits to be integral components of the trial, their family members and the community at large conceptualized the visits/ research as a government programme or service. This is similar to findings in a study on concomitant treatment of HIV and malaria in Tanzania (Reynolds *et al.*, 2013) where the study was referred to as "a project". The link with government may have been premised on home visits conducted during national immunization days (NID) when community health nurses and trained volunteers immunize children. Also, in 2011 when the trial had begun, there was a national programme of ITN distribution which involved home visits by trained volunteers. Both campaigns had government involvement.

Linking home visits and blood draws during the study to HIV/AIDS is similar to findings reported in Reynolds *et al* (2013). This perception may be based on a development project implemented in the study area over a decade ago. This project provided nutritional supplementation and income-generation skills to people living with HIV/AIDS and included home visits. While discretion would have been exercised not to disclose the status of the beneficiaries to the public, it is possible some information got out of hand. This may have subsequently helped to shape the association between home visits to particular people and the suspicion that they have HIV/AIDS. Participants and family members alike were concerned over possible HIV/AIDS stigmatization which is still a problem in Ghana. It is also possible blood

draws for HIV testing following voluntary counselling at the ANC clinics impacted on the perceptions that linked blood draws on home visits to HIV.

The present enquiry is one of the first to address perceptions surrounding blood draws conducted on pregnant women in their homes and contrary to preconceived notions, there were no cultural barriers to blood draws at home. However, there were hints of mistrust with respect to the true destination and purpose of the blood samples taken. In line with local belief systems, some participants and relatives expressed fears that blood samples could be "spiritually manipulated" to produce money for the researchers at the risk of harm to the donor. Such beliefs underscored preferences for blood draws to be done at the hospital.

Similar interpretations of blood draws in the context of research have been expressed in other studies and poor understanding of study aims play immense roles (Wong *et al.,* 2004; Fairhead *et al.,* 2006; Masiye *et al.,* 2008; Newton et al., 2009; Boahen *et al.,* 2013; Grietans *et al.,* 2014). Mistrust associated with blood draws in other studies was, however, devoid of spiritual connotations (Wong *et al.,* 2004; Limkakeng *et al.,* 2013). Perceptions surrounding blood draws in research reflect local social orientations.

There were also concerns over the frequency and volume of blood draws in the study. Similar complaints were made by parents concerning blood draws from their children in a malaria vaccine trial in Ghana (Febir *et al.,* 2013).

7.7 Conclusions/ Implications

The study makes an original contribution to knowledge on the safety and efficacy of DHA-PPQ use in pregnancy. Involving over 400 pregnant women, the study found that dihydroartemisinin-piperaquine is non-inferior to artesunate-amodiaquine for the

treatment of uncomplicated malaria in pregnancy with respect to safety and efficacy. However, this result has to be interpreted with caution against a background of limitations including an inability to recruit the full sample size with consequent reduced power that favours conclusion of non-inferiority, inability to report PCR-corrected efficacy estimates and use of a "modified intention-to-treat" analysis population. The observation of a higher occurrence of low birthweight in the DHA-PPQ arm is unlikely to detract from its safety.

Results from the study may have policy implications for WHO and national malaria control programmes (NMCPs) in endemic countries. The evidence provided by this study and others within the framework of the Malaria in Pregnancy Consortium are likely to inform the policy process regarding introducing DHA-PPQ for use in pregnancy in malaria endemic areas. Better tolerability and a single daily dosage regimen for DHA-PPQ is expected to augur well for adherence in pregnant women if it is approved for treatment of uncomplicated malaria in this population. The combination has been used to treat pregnant women for close to a decade in Papua New Guinea without evidence of excess events of adverse pregnancy outcomes (Poespoprodjo *et al.*, 2014) even though surveillance for these outcomes has been limited.

The predominance of *Pfcrt* K76 and *Pfmdr*1 N86 alleles suggest a possible early decline in *P.falciparum* susceptibility to AL which is currently used to treat uncomplicated malaria in the general population. This may not be evident in *in vivo* efficacy studies in the very near future but the Ghanaian NMCP/ Ministry of Health need to strengthen education on testing for presence of parasites before treatment to ensure proper of targeting of ACTs. This will help to reduce drug pressure and delay development of drug resistance. The NMCP and district health administrations could

run random checks on prescribers to ascertain adherence to this policy. Education is especially important in the context of pregnant women as many pregnancy-related complaints mimic malaria symptoms. In this wise therefore, the use of First Response® malaria RDTs ought to be encouraged more at ANC clinics in lower level facilities. First Response® RDT correctly identifies about 80% of parasitaemic pregnant women in light of declining parasite prevalence in pregnant women.

Parasite mutations known to underlie marked SP resistance were either absent or present in very low prevalence. These findings underscore the continued usefulness of SP for IPTp in Ghana and justify recent WHO recommendations to maintain SP for IPTp. Furthermore, the study provides an updated and comprehensive report of prevalence of molecular markers in pregnant women in Ghana which can be used as a baseline for future monitoring of drug resistance.

Though pregnant women and their relatives find blood draws in their homes acceptable, they would prefer sampling in a health facility as that appears to clear inherent doubts of the final destination and purpose of the blood sample drawn. Researchers planning studies that involve scheduled blood sampling in pregnant women may need to emphasize transportation to a health facility for the procedure to help increase confidence and trust in the research team.

The study also suggested the socioeconomic status of potential study participants is pertinent to packaging of study information and that there may be a need for differential subject emphasis. While it may be useful to emphasize altruism and/or contribution to science in a study involving highly educated pregnant women, this may not be convincing to pregnant women with low-level socioeconomic status. Focus should be on health benefits to both mother and foetus. In an era of increasing

acceptance that drug trials involving pregnant women are needed, the study contributes knowledge to help clinical researchers appreciate the socio-cultural aspects of research from the perspective of these pregnant women.

The implications of the study for further research are outlined as below;

- the artemisinin-combination treatment DHA-PPQ was shown to be safe and efficacious in second and third trimester pregnant women in spite of the limitations enumerated. However, there is still paucity of information regarding its long term effect on developmental milestones in babies born to mothers who received DHA-PPQ treatment and further studies are needed to address this gap in knowledge. Furthermore, large studies are needed to evaluate rarer AEs and the effects of repetitive treatments in mothers.
- again, having demonstrated its safety, DHA-PPQ may be explored for intermittent prevention of malaria in pregnancy. The long-acting partner drug PPQ has a terminal half-life comparable to SP and could replace it in IPTp. That DHA-PPQ has to be taken over 3 days may pose challenges for its use as IPTp but it is a question that needs to be addressed in an effectiveness and acceptability study of DHA-PPQ for IPTp.
- the prevalence of *Pfdhfr* in pregnant women appears to have stabilized in the 5-6 years preceding the study. Further research is required to ascertain how recent WHO recommendations for up to five doses of SP-IPTp will impact on selection of *Pfdhfr/ Pfdhps* mutations and how these will correlate with low birth weight, maternal anaemia and placental malaria.

REFERENCES

Abdul-Ghani R, Al-Mekhlafi AM, Karanis P (2012). Loop-mediated isothermal amplification (LAMP) for malarial parasites of humans: would it come to clinical reality as a point-of-care test? *Acta Trop.* 122(3), 233–240

Abruquah H, Bio F, Tay S, Lawson B (2010). Resistance-mediating polymorphisms of *Plasmodium falciparum* among isolates from children with severe malaria in Kumasi, Ghana**.** Ghana Medical Journal, 44:52–58

Abuaku BK, Koram KA, Binka FN (2004). Antimalarial drug use among caregivers in Ghana. Afr Health Sci 4: 171–177

Acheampong DO, Appiah MG, Boamponsem JN, Afoakwa R (2011). The efficacy of rapid diagnostic test in diagnosing Plasmodium falciparum malaria at some selected health facilities in the Cape Coast metropolis of Ghana. Advances in Applied Science Research 2 (4): 348-356

Achidi EA, Kuoh AJ, Minang JT, Ngum B, Achimbom BM, Motaze SC et al (2005). Malaria infection in pregnancy and its effect on haemoglobin levels in women from a malaria endemic area of Fako division, Southwest pronvince, Cameroon. J Obstet Gynaecol 25(3) 235-240.

Afoakwa R, Boampong JN, Egyir-Yawson A, Nwaefuna EK, Verner ON, Asare KK (2014). High prevalence of PfCRT K76T mutation in Plasmodium falciparum isolates in Ghana. Acta Trop 136: 32-6

Afrane YA, Klinkenberg E, Drechsel P, Owusu-Daaku K, Garms R, Kruppa T (2004). Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? Acta Trop. 2004 Jan; 89(2):125-34.

Agyei GO, Kurtzhals JAL, Rodrigues OP, Alifrangis M, Hoegberg LCG, Kitcher ED et al (2008). Amodiaquine-artesunate vs artemether-lumefantrine for uncomplicated malaria in Ghanaian children: a randomized efficacy and safety trial with one year follow-up. Malaria Journal 7: 127

Ahmed SM, Zerihun A (2010) Possession and usage of insecticidal bed nets among the people of Uganda: is BRAC Uganda Health Programme pursuing a pro-poor path? PLoS One 5(9): e12660 doi: 10.1371/journal.pone.0012660

Ahmed T, Sharma P, Gautam A, Varshney B, Kothari M, Ganguly S et al. (2008). Safety, Tolerability, and Single- and Multiple-Dose Pharmacokinetics of Piperaquine Phosphate in Healthy Subjects. J. Clin. Pharmacol 48; 166

Ahorlu CK, Dunyo S, Afari EA, Koram KA, Nkrumah FA (1997). Malaria-related beliefs and behaviour in southern Ghana; implications for treatment, prevention and control. Tropical Medicine and International Health 2(5) pp 488-499

Alam MT, de Souza KD,Vinayak S, Griffing SM, Poe AC, Duah NO et al (2011). Selective Sweeps and Genetic Lineages of *Plasmodium falciparum* Drug-Resistant Alleles in Ghana. Journal of Infectious Diseases 203 (2): pp 220-227

Alifrangis M, Enosse S, Pearce R, Drakeley C, Roper C, Khalil IF, Nkya WMMM, Ronn AM, Theander TG, Bygjerg IBC (2005). Simple, high-throughput method to detect *Plasmodium falciparum* single nucleotide polymorphisms in the dihydrofolate reductase, dihydropteroate synthase, and *P. falciparum* chloroquine resistance transporter genes using polymerase chain reaction- and enzyme-linked immunosorbent assay-based technology. Am J Trop Med Hyg. 72:155–162

Alifrangis M, Lusingu JP, Mmbando B, Dalgaard MB, Vestergaard LS, Ishengoma D, Khalil IF, Theander TG, Lemnge MM, Bygbjerg IC (2009). Five-year surveillance of molecular markers of *Plasmodium falciparum* antimalarial drug resistance in Korogwe District, Tanzania: accumulation of the 581G mutation in the *P. falciparum* dihydropteroate synthase gene. Am J Trop Med Hyg 80:523–527.

Allen SJ, Raiko A, O'Donnell A, Alexander ND, Clegg JB (1998). Causes of preterm delivery and intrauterine growth retardation in a malaria endemic region of Papua New Guinea. *Arch. Dis. Child. Fetal Neonatal Ed.* 79(2), F135–F140

Altman DG (1991). Practical statistics for medical research. Chapman and Hall. London.

Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N et al (2014). A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature 505 (7481) 50-55

Asante KP, AbokyI L, Zandoh Z et al (2010). Community perception of malaria treatment behaviour in a rural district of Ghana: implication for artemisinin combination therapy. BMC Public Health 10:409

Asare KK, Boampong JN, Afoakwa R, Ameyaw EO, Sehgal R, Quarshie NB (2014). Use of proscribed chloroquine is associated with an increased risk of pfcrt t76 mutation in some parts of Ghana. Malaria Journal 13: 246 doi: 10.1186/1475-2875-13-246

Ashley EA, Krudswood S, Phaiphun L, Srivilairit S, McGready R, Leowattana W et al. (2004). Randomized controlled dose-optimization studies of dihydroartemisinin-piperaquine for the treatment of uncomplicated multidrug resistant falciparum malaria in Thailand. J. Infect. Dis. 190, 1773-1782

Ashley EA, McGready R, Hutagalung R, Phaiphun L, Slight T, Proux S et al. (2005). A randomized controlled study of a simple once-daily regimen of dihydroartemisinin-piperaquine for the treatment of uncomplicated multidrug-resistant falciparum malaria. Clin. Infect. Dis 41,425-432

Ayeh-Kumi PF, Akalifa BG, Nkrumah NO, Asmah PH, Dayie NTKD (2011). Performance of rapid Diamed Optimal-IT malaria test in an endemic Ghanaian setting. J Parasit Dis 35(2): 129-133

Baiden F, Webster J, Tivura M, Delimi R, Berko Y, Amengo-Etego S et al (2012). Accuracy of rapid tests for malaria and treatment outcomes for malaria and non-malaria cases among under-five children in rural Ghana. PLOS ONE 7 (4):e34073 doi 10.1371

Baker J, Gatton M, Peters J, Mei-Fong H, McCarthy JS, Cheng Q (2011). Transcription and Expression of Plasmodium falciparum Histidine –Rich Proteins in different stages and strains: Implications for Rapid diagnostic tests. PLOS ONE 6(7). e22593.

Bam Victoria (2009). Control of pregnancy-associated malaria through community involvement in rural Ghana. PhD Thesis, Kwame Nkrumah University of Science and Technology.

Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, Menendez C et al (2009). Dihydroartemisinin-Piperaquine and Artemether-Lumefantrine for treating uncomplicated malaria in African children: A Randomized, Non-inferiority Trial. PLOS One 4(11): e7871

Bekwai Municipality Health Directorate Annual Report (2012)

Belay M, Deressa W (2008). Use of insecticide-treated bednets by pregnant women and associated factors in a predominantly rural population of northern Ethiopia. Trop Med Int Health 13:1303-1313.

Berlin JA, Ellenberg SS (2009). Inclusion of women in clinical trials. BMC Medicine 7:56

Bertin G, Briand V, Bonaventure D, Carrie A, Massougbodji A, Cot M and Deloron P (2011). Molecular markers of resistance to sulphadoxine-pyrimethamine during intermittent preventive treatment of pregnant women in Benin. Malaria Journal 10:196

Bhaskar R (1978). A Realist Theory Of Science. Hassocks, West Sussex. Harvester Press

Binka FN, Adongo P (1997) Acceptability and use of insecticide impregnated bednets in northern Ghana. Trop Med Int Health 2: 499–507

Birku Y, Welday D, Ayele D, Shepherd A (1999). Rapid diagnosis of severe malaria based on the detection of pf-HRP-2 antigen. Ethiop Med J; 173-179

Bissofi Z, Sirima SB, Menten J, Pattaro C, Angheben A, Gobbi F et al (2010). Accuracy of a rapid diagnostic test on the diagnosis of malaria infection and of malaria-attributable fever during low and high transmission season in Burkina Faso. Malaria Journal 9:192

Boahen O, Owusu-Agyei S, Febir LG, Tawiah C, Tawiah T, Afari S, Newton S (2013). Community perception and beliefs about blood draw for clinical research in Ghana. Trans R Soc Trop Med Hyg 107(4):261-5.

Boyou-Akotet MK, Kombila M, Kremsner PG, Mavoungou E (2004).Cytokine profiles in peripheral, placental and cord blood in pregnant women from an area endemic for *Plasmodium falciparum*. Eur Cytokine Netw. 2004 Apr-Jun;15(2):120-5

Brabin BJ (1983). An analysis of malaria in pregnancy in Africa. Bulletin of the World Health Organization 61; 1005-1016

Brabin BJ, Romagosa C, Abdelgalil S et al (2004). The sick placenta-the role of malaria. Placenta 25: 359-378

Brice BK, William Y, Lacina O, Félix Y, Hugues A, Léonardo B et al (2010). In vitro susceptibility of Plasmodium falciparum isolates from Abidjan, Côte d'Ivoire, to artemisinin, chloroquine, dihydroartemisinin and pyronaridine. Tanzan J Health Res. 12(1):73-9.

Browne ENL, Maude GH and Binka FN (2001). The impact of insecticide-treated bednets on malaria and anaemia in pregnancy in Kassena-Nankana district, Ghana; a randomized controlled trial. Tropical Medicine and International Health 6: 667-676

Buabeng KO, Duweija M, Dodoo ANO, Matowe L, Enlund H (2007). Self-reported use of antimalarial drugs and health facility management of malaria in Ghana. Malaria Journal 6:85

Buchbinder SP, Metch B, Holte SE, Scheer S, Coletti A, et al. (1999) Determinants of enrollment in a preventive HIV vaccine trial: hypothetical versus actual willingness and barriers to participation. Journal of Acquired Immune Deficiency Syndrome 36: 604–612.

Catania C, De Pas T, Goldhirsch A, Radice D, Adamoli L, et al. (2008) Participation in clinical trials as viewed by the patient: understanding cultural and emotional aspects which influence choice. Oncology 74: 177–187.

Chandra S, Tripathi AK, Mishra S, Amzarul M and Vaish AK (2012). Physiological changes in Hematological parameters during pregnancy. Indian J Hematol Blood Transfus 28(3) 144-146

Changxiong L, Lin X, Huang ZS, Chen YS, Guo RN (1989). In-vivo sensitivity of Plasmodium falciparum to piperaquine phosphate assayed in Linshui and Baisha counties, Hainan Province. Chinese Journal of Parasitology and Parasitic Diseases. 7, 163-165

Chen LJ, Wang MY, SunWK, Lui MZ (1984). Embryotoxicity and teratogenicity studies on artemether in mice, rats and rabbits. Acta Pharmacol Sin 5: 118-22

Chotivanich K, Udomsangpetch R, Suwanarusk R *et al (2012*. *Plasmodium vivax* adherence to placental glycosaminoglycans. *PLoS ONE* 7(4): e34509

Clark RL, White TEK, Clode S et al (2004). Developmental toxicity of artesunate and an artesunate combination in the rat and rabbit. Birth Defects Res B Dev Reproduc Toxicol 71:380-94

Clerk CA, Bruce J, Affipunguh KP, Mensah N, Hodgson A, Greenwood B and Chandramohan D (2008). A randomized controlled trial of intermittent preventive treatment with sulphadoxine-pyrimethamine, amodiaquine or the combination in pregnant women in Ghana. J Infect Dis 198 (8) 1202-1211

Conroy AL, McDonald CR, Kain KC (2012). Malaria in Pregnancy. Diagnosing Infection and Identifying Fetal Risk. Expert Rev Anti Infect Ther. 10 (11):1331-1342

D'Alessandro U, Langerock P, Francis N, Greenwood BM (1996) The impact of a national impregnated bednet programme on the outcome of pregnancy in Gambian primigravidae. Transactions of Royal Society of Tropical Medicine and Hygiene 90 **,** 487–492.

Danquah I, Coulibaly B, Meissner P, Petruschke I, Muller O, Mockenhaupt FP (2010). Selection of pfmdr1 and pfcrt alleles in amodiaquine treatment failure in north-western Burkina Faso. Acta Trop 114(1): 63-6 doi:10.1016/ jactatropica 2009.12.008

Davis TM, Hung TY, Sim IK, Karunajeewa HA, Ilett KF (2005). Piperaquine; a resurgent antimalarial drug. Drugs 65, 75-87

Dawes M and Chowienczyk PJ (2001). Drugs in Pregnancy. Pharmacokinetics in pregnancy. Best Pract. Clin. Obstet. Gynaecol 15: 819-826

De Beaudrap P, Turyakira E, White LJ, Nabasumba C, Tumwebaze B, Muehlenbachs A et al (2013). Impact of malaria during pregnancy outcomes in a Ugandan prospective cohort with intensive malaria screening and prompt treatment. Malaria Journal 12:139

Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO (2010). Quantifying the number of pregnancies at risk of malaria in 2007: A demographic study. PLOS Med 7(1): e1000221 doi10.1371/journal.pmed 1000221

Denis MB, Davis TM, Hewitt S, Incardona S, Nimol K, Frandeur T et al (2002). Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian Children and adults with uncomplicated falciparum malaria. Clin. Infect. Dis. 35, 1469-1476

Desai M, Gutman J, L'lanziva A, Otieno K, Juma E, Kariuki S et al (2015). Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: An open-label, three-group, randomised controlled superiority trial. Lancet 2015 Sep 28; [e-pub].(http://dx.doi.org/10.1016/S0140-6736(15) 00310-4)

Desai M, O ter Kuile F, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD (2007). Epidemiology and burden of malaria in pregnancy. Lancet Infectious Disease, 7:93-104

Djimde A, Doumbo OK, Cortese JF, Kayentao K, Doumbo S, Diourte Y, Dicko A, Su X-Z, Nomura T, Fidock D, Wellems TE, Plowe CV (2001). A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med. 344:257–263

Djimde AA, Doumbo OK, Traore O, Guindo AB, Kayentao K, Diourte Y et al *(*2003): Clearance of drug-resistant parasites as a model for protective immunity in *Plasmodium falciparum* malaria. *Am J Trop Med Hyg*, 69**:**558-563.

Dolan G, Ter Kuile FO, Jacoutot V et al. (1993) Bednets for the prevention of malaria and anaemia in pregnancy. Transactions of the Royal Society of Tropical Medicine and Hygiene 87**,** 620–626.

Dondorp AM, Nosten F, Yi P et al (2009) . Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 2009 Jul30; 361 (5): 455-67

Dondorp AM, Yeung S, White L, et al (2010). Artemisinin resistance: current status and scenarios for containment. Nat Rev Microbiol 2010 Apr; 8 (4): 272-80

Duah NO, Matrevi SA, de Souza KD, Binnah DD, Tamakloe MM, Opoku VS et al (2013). Increased *Pfmdr1* gene copy number and the decline in *Pfcrt* and *Pfmdr1* resistance alleles in Ghanaian *Plasmodium falciparum* isolates after the change of antimalarial drug treatment policy. Malaria Journal 12: 377

Duah NO, Quarshie NB, Abuaku BK, Sebeny PJ, Kronman KC, Koram KA (2012). Surveillance of molecular markers of *Plasmodium falciparum* resistance to Sulphadoxine-Pyrimethamine 5 Years after the change of Malaria Treatment Policy in Ghana. Am J Trop Med Hyg 87(6) pp 996-1003.

Duah NO, Wilson MD, Ghansah A, Abuaku B, Edoh D, Quarshie NB and Koram KA (2007). Mutations in Plasmodium falciparum Chloroquine Resistance Transporter and Multidrug Resistance Genes, and Treatment Outcomes in Ghanaian Children with Uncomplicated Malaria. J Trop Paediatr 53(1); 27-31 doi: 10.1093/tropej/fm1076

Duraisingh MT, Roper C, Walliker D, Warhurst DC (2000). Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the pfmdr1 gene of *Plasmodium falciparum*. Mol Microbiol. 36:955–961.

Dyer ME, Tjitra E, Currie BJ, Anstey NM (2000). Failure of the "pan malarial" antibody of the ICT Malaria P.f/P.v immunochromatographic tests to detect symptomatic Plasmodium malariae infection. Trans R Soc Trop Med Hyg 94: 518-22

Eckstein-Ludwig U, Webb RJ, van Goethem IDA, et al (2003). Artemisinins target the SERCA of Plasmodium falciparum. Nature 2003 Aug 21; 424 (6951): 957-61

Egan TJ, Kaschula CH (2007). Strategies to reverse drug resistance in malaria. Curr Opin Infect Dis 20(6): 598-604

Erhardt S, Eggelte TA, Kaiser S, Adjei L, Burchard GD, Anemana S et al (2007). Large-scale surveillance of *Plasmodium falciparum crt* (K76T) in Northern Ghana. Antimicrobial Agents and Chemotherapy 51(9): pp 3407-3409

Espino F, Manderson L, Acuin C, Domingo F, Ventura E (1997). Perceptions of malaria in a low endemic area in the Philippines: transmission and prevention of the disease. Acta Trop 63: 221-39

Espinoza E, Hidalgo I, Chedraui P (2005). The effect of malarial infection on maternal-fetal outcome in Ecuador. J Matern Fetal Neonatal Medicine 18: 101-05

Evans JA, May J, Tominski D, Eggelte T, Marks F, Abruquah HH et al (2005). Pre-treatment with chloroquine and parasite chloroquine resistance in Ghanaian children with severe malaria. Q.J.Med 98: 789-796

Ezzet F, Mull R, Karbwang J (1998). The population pharmacokinetics of CGP56697 and its effect on the therapeutic response in malaria patients. British Journal of Clinical Pharmacology 46: 553-61

Ezzet F,van Vugt M, Nosten F, Looareesuwan, White NJ (2000). Pharmacokinetics and Pharmacodynamics of Lumefantrine (Benflumetol) in Acute falciparum malaria. Antimicrobial Agents Chemother 44(3): 697-704

Fade S (2004). Using Interpretative phenomenological analysis for public health nutrition and dietetic research; a practical guide. Proceedings of the Nutritional Society 63:647-653

Fairhead J, Leach M, Small M (2006). Where techno-science meets poverty: Medical research and the economy of blood in the Gambia, West Africa. Social Science and Medicine 63(4) 1109-1120

Falade CO, Tongo OO, Ogunkole OO, Orimadegun AE (2010). Effects of malaria in pregnancy on newborn anthropometry. J Infect Dev Ctries 4(7) 448-453

Faye B, Offianan AT, Ndiaye JL, Tine RC et al (2010). Efficacy and tolerability of artesunate-amodiaquine (Camoquin plus®) *versus* artemether-lumefantrine (Coartem®) against uncomplicated *Plasmodium falciparum* malaria: multisite trial in Senegal and Ivory Coast. Tropical Medicine & International Health 15(5) 608-613

Febir GL, Asante KP, Dzorgbo SDB, Senah KA, Letsa TS, Owusu-Agyei S (2013). Community perceptions of a malaria vaccine in the Kintampo districts of Ghana. Malaria Journal 12: 156

Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT et al (2000). Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell. 6:861–871.

Finaurini S, Ronzoni L, Colancecco A (2010). Selective toxicity of dihydroartemisinin on human CD34+ erythroid cell differentiation. Toxicology 276: 128-134

Fiske ST and Taylor SE (1991). Social Cognition, 2nd edition. New York: McGraw Hill

Folarin OA, Bustamante C, Gbotosho GO, Sowunmi A, Zalis MG, Oduala AMJ, Happi CT (2011). Invitro amodiaquine resistance and its association with mutation in pfcrt and pfmdr1 genes of P.falciparum isolates from Nigeria. Acta Trop 120: 224-230

Foley M, Tilley L (1998). Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. Pharmacol. Ther. 79: pp. 55–87

Fried M and Duffy PE (1996). Adherence of Plasmodium falciparum to chondroitin sulphate A in the human placenta. Science 272(5627): 1502-1504

Fried M, Avri M, Chaturvedi R, Fernandez P, Narum D et al (2013). Preclinical evaluation of pregnancy malaria vaccine immunogens: a multi laboratory approach. Infect Immun 81:487-95

Fried M, Muehlenbachs A, Duffy PE (2012). Diagnosing Malaria in Pregnancy: An Update. Expert Rev Anti Infect Ther. 10(10):1177-1187.

Froberg G, Jornhagen L, Morris U, Shakely D, Msellem M et al (2012). Decreased prevalence of *Plasmodium falciparum* markers to amodiaquine despite its wide scale use as ACT partner drug in Zanzibar. Malaria Journal 11:321

Gadalla N, Elzaki SE, Mukhtar E, Warhurst D, Badria El-Sayed, Sutherland CJ (2010). Dynamics of pfcrt alleles CVMNK and CVIET in chloroquine-treated Sudanese patients infected with *Plasmodium falciparum*. Malar J. 9:74

Gailbraith RM, Faulk WP, Gailbraith GM, Holbrook TW, Bray RS (1980). The human maternofetal relationship in malaria I: Identification of pigment and parasites in the placenta. Trans. R. Soc. Trop. Med. Hyg. 74: 52-60

Gale NK, Heath G, Cameron E, Rashid S and Redwood S (2013). Using the framework method for the analysis of qualitative data in multidisciplinary health research. BMC Medical Research Methodology 13: 117

Gamble C, Ekwaru PJ, Garner P, ter Kuile FO (2007) Insecticide-Treated Nets for the Prevention of Malaria in Pregnancy: A Systematic Review of Randomised Controlled Trials. PLoS Med 4(3): e107.

Gatny HH, Axinn WG (2011). Willingness to participate in research during pregnancy: race, experience and motivation. Field Methods 24: 135

Gerstl S, Dunkley S, Mukhtar A, Maes P, De Smet M, et al. (2010) Long-lasting insecticide-treated net usage in eastern Sierra Leone – the success of free distribution. Trop Med Int Health 15: 480–488

Ghana Demographic and Health Survey Report (2008)

Ghana Urban Malaria Study Report (2013)

GHS (2005). Ghana Health Service Annual Report. Accra

Giao PT, de Vries PJ, Hung LQ, Binh TQ, Nam NV, Kager PA (2004). CV8; a new combination of dihydroartemisinin-piperaquine, trimethoprim and primaquine, compared with atovaquone-proguanil against falciparum malaria in Vietnam. Trop. Med .Int. Health 9,209-216

Gillet P, Mori M, Van EM, Van den Ende J, Jacobs J (2009). Assessment of the prozone effect in malaria rapid diagnostic tests. Malaria Journal 8:271

Glover-Amengo M, Owusu WB, Akanmori BD (2005). Determinants of anaemia in pregnancy in Sekyere West District, Ghana. Ghana Med J 39: 102-107.

Goldberg DE, Slater AF, Cerami A, Henderson GB (1990). Hemoglobin degradation in the malaria parasite *Plasmodium falciparum*: an ordered process in a unique organelle.Proc. Natl. Acad. Sci. U.S.A. 87: 2931–2935

Green MD, van Eijk AM, Ter Kuile FO, Ayisi JG, Parise ME, Kager PE et al. (2007). Pharmacokinetics of Sulphadoxine-pyrimethamine in HIV-infected and uninfected pregnant women in Western Kenya. J Infect Dis 196(9) 1403-8

Gregson A and Plowe CV (2005). Mechanisms of resistance of malaria parasites to Antifolates. Pharmacol Review 57: 117-145

Grietans KP, Ribera JM, Erhadt A, Hoibak S, Ravinetto R, Gryseels C et al (2014). Doctors and Vampires in Sub-Saharan Africa: Ethical Challenges in Clinical Trial Research. Am J Trop Med Hyg 19(2): 213-215

Guyatt HL, Snow RW (2004). Impact of malaria during pregnancy on low birthweight in sub-Saharan Africa. Clin Microbiol Rev, 17:760-69

Harrington WE, Mutabingwa TK, Muehlenbachs A, Sorensen B, Bolla MC, Fried M, Duffy PE (2009). Competitive facilitation of drug-resistant Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci USA 106 (22); 9027-32

Hasugian AR, Purba HL, Kenangalem E, Muwung RM, Ebsworth EP, Maristela P (2007). Dihydroartemisinin-piperaquine versus artesunate-amodiaquine: Superior efficacy and post-treatment prophylaxis against multidrug-resistant Plasmodium falciparum and Plasmodium vivax malaria. Clin. Infect Dis 44(8): 1067-74

Hatton CSR, Peto TEA, Bunch C et al. (1986). Frequency of severe neutropenia associated with amodiaquine prophylaxis against malaria. Lancet 1:411-414

Hayward RE, Sullivan DJ, Day KP (2000). Plasmodium falciparum histidine-rich protein 2 is expressed during gametocyte development. Exp Parasitol 96: 139-146

Heggenhougen KH, Hackethal V, Vivek P (2003). The behavioural and social aspects of malaria and its control. An introduction and annotated bibliography. Social, Economic and Behavioural Research, UNDP/ World Bank/ WHO/TDR.

Hien TT, Davis TM, Choung LV, Illet KF, Sinh DX, Phu NH et al. (2004a). Comparative pharmacokinetics of intramuscular artesunate and arthemeter in patients with severe falciparum malaria. Antimicrob Agents Chemother 48(11):4234-4239

Hien TT, Dolecek C, Mai PP et al (2004b). Dihydroartemisinin-piperaquine against multidrug-resistant Plasmodium falciparum malaria in Vietnam: randomised clinical trial. Lancet 363, 18-22

Hill J, D'Mello-Guyett L, Hoyt J, van Eijk AM, ter Kuile FO, Webster J (2014). Women's Access and Provider practices for the case management of malaria during pregnancy: A systematic review and meta-analysis. PLoS Med 11(8) e1001688

Hoglund RM, Adam I, Hanpithakpong W, Ashton M, Lindegardh N, Day NPJ et al.(2012). A population pharmacokinetic model of piperaquine in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* malaria in Sudan. Malaria Journal 11(398)

Holloway, I., & Wheeler, S. (1999). Ethical issues in qualitative nursing. *Nursing Ethics, 2*, 223-232

Holmgren G, Gil JP, Ferreira PM, Veiga MI, Obonyo CO and Bjorkman A (2006). Amodiaquine resistant Plasmodium falciparum malaria in vivo is associated with selection of Pfcrt 76T and Pfmdr1 86Y. Infection Genetics Evolution 6, 309-314

Houzé S, Hubert V, Le Pessec G, Le Bras J, Clain J (2011). Combined deletions of *pfhrp2* and *pfhrp3* genes results in *Plasmodium falciparum* malaria false-negative rapid diagnostic test. J Clin Microbiol, 49:2694–2696.

Humar A, Ohrt C, Harrington MA, Pillai D, Kain KC (1997). Parasight F test compared with the polymerase chain reaction and microscopy for the diagnosis of Plasmodium falciparum malaria in travellers. Am J Trop Med Hyg 56, 44-48

Humphreys GS, Merinopoulous I, Ahmed J, Whitty CJM, Mutabingwa TK, Sutherland CJ, Hallet RL (2007). Amodiaquine and Artemether-Lumefantrine select distinct alleles of the Plasmodium falciparum mdr1 gene in Tanzanian children treated for uncomplicated malaria. Antimicrobial Agents and Chemotherapy 51, 991-997

Hung TY, Davis TM, Ilett KF et al (2004). Population pharmacokinetics of piperaquine in adults and children with falciparum or vivax malaria. British Journal of Clinical Pharmacology 57, 253-262

Hussain-Gambles M (2004) South Asian patients' views and experiences of clinical trial participation. Family Practice 21(6): 636–642

Hviid L (2005). Naturally acquired immunity to Plasmodium falciparum malaria in Africa. Acta Tropica 95(3): 270-5

Hyde JE (1990). The dihydrofolate reductase-thymidylate synthetase gene in the drug resistance of malaria parasites. Pharmacol Ther 48: 45-49

ICH (International Conference on Harmonization) Steering Committee (1998). Statistical Principle of Clinical Trials (E9). Geneva, Switzerland: Available at www.ich.org/LOB/media/media485.pdf

Idczak SE (2007). I am a nurse: Nursing students learn the art and science of nursing. Nurs Educ Perspect 28:66-71

INESS (2011). INESS Report. Cohort Event Monitoring With Artesunate-Amodiaquine at Kintampo HDSS: a unique platform for collection of rigorous safety data on medicines. Presentation in Maputo 2011. www. Indepth-network.org/ISC 2011/presentations/Tuesday. Accessed 30/05/13

Ismail MR, Ordi J, Menendez C, Ventura PJ, Apponte JJ, Kahigwa E et al (2000). Placental pathology in malaria: a histological, immunohistochemical and quantitative study. Human Pathology 31: 85-93

Jagoe G (2009). Monitoring and Improving the flow of ACTs in the Public Sector: Medicines for Malaria Venture: In 2nd Global Health Supply Chain Summit. Zaragoza, Spain

Jambou R, Legrand E, Niang M et al (2005). Resistance of *Plasmodium falciparum* field isolates to in-vitro artemether and point mutations of the SERCA-type PfATPase6. Lancet Dec 3; 366 (9501): 1960-3

Jones B, Jarvis P, Lewis PA et al (1996). Trials to assess equivalence: the importance of rigorous methods. BMJ 313; 36-39

Jones JM, Nyhof-Young J, Moric J, Friedman A, Wells W, et al. (2006) Identifying motivations and barriers to patient participation in clinical trials. Journal of Cancer Education: 21: 237–242.

Kamya MR, Yeka A, Bukirwa H, Lugemwa M, Rwakimari JB, et al. (2007) Artemether-lumefantrine versus dihydroartemisinin-piperaquine for treatment of malaria: a randomized trial. PLoS Clin Trials 2: e20

Karbwang J, Tassanor O, Kanda T, Wattanagoon Y, Ibrahim M, Na-Bangchang K et al (1996). Parasight-F test for the detection of treatment failure in multidrug resistant *Plasmodium falciparum* malaria. Trans. R. Soc. Trop Med Hyg 90: 513-515

Karbwang J, White NJ (1990). Clinical Pharmacokinetics of Mefloquine. Clinical Pharmacokinetics 19(4): 264-79

Karema C, Fanello CI, Van Overmeir C, Van Geertruyden JP, van Doren W, et al. (2006) Safety and efficacy of dihydroartemisinin/piperaquine (Artekin((R))) for the treatment of uncomplicated Plasmodium falciparum malaria in Rwandan children. Trans R Soc Trop Med Hyg 100: 1105–1111

Karnieli-Miller O, Strier R and Pessach L (2009). Power Relations in Qualitative Research. Qualitative Health Research 19; 279

Karunajeewa H, Lin C, Hung TY et al (2004). Safety evaluation of fixed combination piperaquine plus dihydroartemisinin (Artekin) in Cambodian children and adults with malaria. Br.J. Clin. Pharmacol, 57: 93-99

Katrak S, Gasasira A, Arinaitwe E, Kakuru A, Wanzira H, Bigira V et al (2009). Safety and tolerability of artesunate-lumefantrine versus dihydroartemisinin-piperaquine for malaria in young HIV-infected and uninfected children. Malaria Journal 8: 272

Kattenberg JH, Ochodo EA, Boer K, Schally HDFH, Mens PF, Leeflang MMG (2011). Systematic review and meta-analysis: rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women. Malaria Journal 10:321

Kattenberg JH, Tahita CM, Versteeg IAJ, Tinto H, Traore-Coulibaly M, Schalig HD, Mens PF (2012). Antigen persistence of rapid diagnostic tests in pregnant women in Nanoro, Burkina Faso and the implications for the diagnosis of malaria in pregnancy. Trop Med Int Health 17: 550-557

Kayentao K, Garner P, van Eijk AM, Naidoo I, Roper C, Mulokozi A et al. (2013). Intermittent preventive therapy for malaria during pregnancy using 2 vrs 3 or more doses of sulphadoxine-pyrimethamine and risk of low birth weights in Africa. JAMA Feb 13; 309(6) 594-604.

Kennedy DL, Uhl K, Kweder SL (2004). Pregnancy Exposure Registries. Drug Saf 27: 215-228

Koita OA, Doumbo OK, Ouattara A, Tall LK, Konaré A, Diakité M et al (2012). False-negative rapid diagnosis tests for malaria and deletion of the histidine-rich repeat region of the *hrp2* gene. Am J Trop Med Hyg 86:194–198.

Koram KA, Abuaku B, Duah N, Quashie N (2005**).** Comparative efficacy of antimalarial drugs including ACTs in the treatment of uncomplicated malaria among children under 5 years in Ghana. *Acta Trop* 95:194-203

Krishna S, White NJ (1996). Pharmacokinetics of quinine, chloroquine and amodiaquine: clinical implications. Clin. Pharmacokinet 30 (4) 263-99.

Kublin JG, Cortese JF, Njunju EM, RA GM, Wirima JJ, Kazembe PN et al (2003). Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *J Infect Dis* 187: 1870-1875.

Kublin JG, Dzinjalamala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM et al (2002). Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. J Infect Dis 185: 380-8

Kuermmele A, Dodoo ANO, Olsson S, Van Erps J, Burri C, Lalvani PS (2011). Assessment of global reporting of adverse drug reactions for anti-malarials, including artemisinin-based combination therapy, to the WHO Programme for International Drug Monitoring. Malaria Journal 10:57

Kumar N, Pande V, Bhatt RM, Shah NK, Mishra N, Srivastava B, Valecha N, Anvikar AR (2013). Genetic deletion of HRP2 and HRP3 in Indian *P. falciparum* population and false negative malaria rapid diagnostic test. Acta Trop 125:119–121.

Kwansa-Bentum B, Ayi I, Suzuki T, Otchere J, Kumagai T, Anyan WK, Osei JH, Ashi H, Ofori MF, Akao N, Wilson MD, Boakye DA, Ohta N (2011). *Plasmodium falciparum* isolates from southern Ghana exhibit polymorphisms in the SERCA-type PfATPase6 though sensitive to artesunate in vitro**.** Malaria Journal, **10:**187.

Lee N, Baker J, Andrews KT, Gatton M, Bell D, Cheng Q, McCarthy J (2006). Effect of sequence variation in Plasmodium falciparum histidine-rich protein 2 on binding of specific monoclonal antibodies: Implications for Rapid Diagnostic Tests for Malaria. J Clin Microbiol. 44 (8) 2775-2778

Li Q and Weina PJ (2010). Severe embryotoxicity or artemisinin derivatives in experimental animals but possibly safe in pregnant women. Molecules 15, 40-57

Li XQ, Bjorkman A, Anderson TB, Ridderstrom M, Masirembwe CM (2002). Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new affinity and turnover enzyme specific probe substrate. J Pharmacol Exp Ther 300(2) 399-407

Limkakeng A, Phadtare A, Shah J, Vaghasia M, Wei DY, Shah A, Pietrobon R (2013). Willingness to participate in clinical trials among patients of Chinese heritage: a meta-synthesis. PLoS One.8(1):e51328.

Longo M, Zanoncelli S, Manera D, Brughera M, Colombo P, Lansen J et al (2006). Effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos in vitro. Reproductive Toxicology 21, pp 83-93.

Lucchi NW, Peterson DS, Moore JM (2008). Immunologic activation of human syncytiotrophoblast by Plasmodium falciparum Mal J, **7**:42 (29 February 2008)

Ly O, Gueye PE, Deme AB, Dieng T, Badiane AS, Ahouidi AD et al (2012). Evolution of the Pfct T76 and Pfmdr1 Y86 markers and chloroquine susceptibility 8 years after cessation of chloroquine use in Pikine, Senegal. Parasitol Res 111(4): 1541-6

Lyerly AD, Namey EE, Gray B, Geeta S, Faden RR (2012). Women's views about participating in research while pregnant. IRB: Ethics and Human Research 34 (4) 1-8

Macklin R (2010). Perspectives. The art of medicine: Enrolling pregnant women in biomedical research. The Lancet 375:632-3

Malaria NJ (2012). Counter Perspective: Artemisinin Resistance: Facts, Fears and Fables. Am J Trop Med Hyg 87(5): 785

Malhotra I, Dent A, Mungai P, Muchiri E, King CL (2005). Real-time quantitative PCR for determining the burden of *Plasmodium falciparum* parasites during pregnancy and infancy. J Clin Microbiol. 43:3630–3635.

Malmberg M, Ngasala B, Ferreira PE, Larsson E, Jovel I, Hjalmarsson A, Petzold M, Premji Z, Gil JP, Bjorkman A, Martensson A (2013). Temporal trends of molecular markers associated with artemether-lumefantrine tolerance/resistance in Bagamoyo district, Tanzania. Malar J. 12:103.

Maltha J, Gamboa D, Bendezu J, Sanchez L, Cnops L, Gillet P, Jacobs J (2012). Rapid diagnostic tests for malaria diagnosis in the Peruvian Amazon: Impact of *pfhrp2* gene deletions and cross-reactions. PLoS One, **7**:e43094

Manyando C, Mkandawire R, Lwipa P, Sinkala M, Mpabalwani E, Njunju E (2010). Safety of arthemeter-lumefantrin in pregnant women with malaria: results of a prospective cohort study in Zambia. Malaria Journal 9:249

Marks F, Evans J, Meyer CG, Browne EN, Flessner C, von Kalckreuth V et al (2005). High Prevalence of markers for Sulfadoxine and Pyrimethamine resistance in Plasmodium falciparum in the absence of drug pressure in the Ashanti Region of Ghana. Antimicrobial Agents Chemother 49(3):1101-1105.

Masiye F, Kass N, Hyder A, Mfutsu-Bengo J (2008). Why mothers choose to enrol their children in malaria clinical studies and the involvement of relatives in decision making: evidence from Malawi. Malawi Med J 20(2); 50-56

Matangila JR, Lufuluabo J, Ibalanky AL, da Luz RAI, Lutumba P and Van Geertruyden JP (2014). Asymptomatic Plasmodium falciparum infection is associated with anaemia in pregnancy and can be more cost-effectively detected by rapid diagnostic test than by microscopy in Kinshasa, Democratic Republic of Congo. Malaria Journal 13:132

Mayxay M, Keomang S, Khanthavong M, Souvannasing P, Stepniewska K, Khonthilath T et al (2010). A Phase III, Randomised, Non-inferiority to assess the efficacy and safety of Dihydroartemisinin-piperaquine in comparison with Artesunate-Mefloquine in patients with uncomplicated Plasmodium falciparum malaria in Southern Laos. Am J Trop Med Hyg 83(6): 1221-1229

Mayxay M, Thongpraseuth V, Khanthavong M, Lindegardh N, Barends M, Keola S et al (2006). An open randomized comparison of artesunate plus mefloquine vs. Dihydroartemisinin-piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria in the Lao People's Democratic Republic (Laos). Trop. Med. Int. Health 8, 1157-1165

Mbaye A, Richardson K, Balayo B, Dunyo S, Schulman C, Milligan P et al (2006). A randomized, placebo-controlled trial of IPT with SP in Gambian multigravidae. Trop Med Int Health 2006, 11, 992-1002

Mbonye AK, Neema S, Magnussen P (2006) Preventing malaria in pregnancy: a study of perceptions and policy implications in Mukono district, Uganda. Health Policy Plan 21: 17–26.

McGready R, Lee SJ, Wiladphaingern J, Ashley EA, Rijken MJ et al (2012). Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population based study. Lancet Infect Dis 12(5): 388–396.

McGready R, Stepniewska K, Edstein MD, Cho T, Gilverag G, Looareesuwan S, White NJ, Nosten F (2003). The pharmacokinetics of atovaquone and proguanil in pregnant women with acute falciparum malaria. Eur J. Clin Pharmacol 59(7): 545-52

McGready R, Stepniewska K, Lindegardh N, Ashley EA, La Y, Singhasivanon P et al (2006). Pharmacokinetics of Arthemeter-lumefantrine in pregnant women with uncomplicated falciparum malaria. Eur J. Clin Pharmacol 62(12) 1021-31

McMorrow ML, Masanja MI, Abdulla SMK, Kahigwa E, and Kachur SP (2008). Challenges in routine implementation and quality control of rapid diagnostic tests for malaria—Rufiji District, Tanzania. Am J Trop Med Hyg 79(3): 385-390

Menendez C, Ordi J, Ismail JR, Ventura PJ, Aponte JJ, Kaghiwa E et al (2000). The impact of placental malaria on gestational age and birth weight. J Infect Dis 181: 1740-1745

Meshnick S (2012). Perspective: Artemisinin-resistant malaria and the Wolf. Am J Trop Med Hyg 87(5): 783-784

MICS (2011). Ghana Multiple Indicator Cluster Survey 2011 Draft Report.

Minja DTR, Schmiegelow C, Mmbando B, Bostrom S, Oesteholt M, Magistrado P et al (2013). Plasmodium falciparum mutant haplotype infection during pregnancy associated with reduced birthweight, Tanzania. Emerging Infectious Diseases vol 19 no 9.

Minja DTR, Schmiegelow C, Oesteholt M, Magistrado PA, Bostrom S, John D et al (2012). Reliability of rapid diagnostic tests in diagnosing pregnancy-associated malaria in north-eastern Tanzania. Malaria Journal 11: 211

Minja H, Schellenberg JA, Mukasa O, Nathan R, Abdualla S, Mponda H et al. (2001). Introducing insecticide-treated nets in the Kilombero Valley, Tanzania: the relevance of local knowledge and practice for an Information, Education and Communication (IEC) campaign. Trop. Med. Int. Health 6: 614-623

Mockenhaupt FP, Bedu-Addo G, Eggelte TA, Homerich L, Holmberg V, von Oertzen C, Bienzle U (2008). Rapid increase in the prevalence of Sulphadoxine-pyrimethamine resistance among Plasmodium falciparum isolated from pregnant women in Ghana. J Infect Dis 198 (10) 1545-1549

Mockenhaupt FP, Bedu-Addo G, Lunge C, Hommerich L et al (2007). Markers of Sulphadoxine-pyrimethamine resistant Plasmodium falciparum malaria in placenta and circulation of pregnant women. Antimicrobial Agents Chemother 51(1) 332-334

Mockenhaupt FP, Eggelte TA, Till H and Bienzle U (2001). Plasmodium falciparum pfcrt and pfmdr1 polymorphisms are associated with the pfdhfr N108 pyrimethamine-resistance mutation in isolates from Ghana. Tropical Medicine and International Health 6(10); 749-755

MOH (2008). Draft Guidelines for Case Management of Malaria in Ghana. Ministry of Health/ Ghana Health Service/ National Malaria Control Programme. Accra.

MOH (2009). Antimalarial Drug Policy for Ghana, 2nd revised version. Ghana Health Service, Accra

MOH (2014). Presentation at Ministry of Health Training of Trainers Workshop, Accra.

Mohammed A, Ndaro A, Kalinga A, Manjurano A, Mosha JF, Mosha DF et al (2013). Trends in chloroquine resistance marker *Pfcrt*-K76T mutation ten years after chloroquine withdrawal in Tanzania. Malaria Journal 12: 415

Mohanna K (1998). Giving and withholding consent to participate in clinical trials: Decisions of pregnant women. Primary and community care small projects scheme. Final report. Available at www.warwick.ac.uk/ftp/wmro/finalreport. Accessed June 2012

Moody A (2002). Rapid diagnostic tests for malaria parasites. Clin. Microbiol Rev 15: 66-78

Mount AM, Mwapasa V, Elliot SR, Beeson JG, Tadesse E, Lema VM et al (2004). Impairment of humoral immunity to *Plasmodium falciparum* malaria in pregnancy by HIV infection. Lancet 363 (9424): 1860-7

Mousiliou A, De Tove YS, Doritchamou J, Luty AJF, Massougbodji A, Alifrangis M, Deloron P and Ndam NT (2013). High rates of parasite recrudescence following intermittent preventive treatment with sulphadoxine-pyrimethamine during pregnancy in Benin. Malaria Journal 12: 195

Msellem MI, Martensson A, Rotlland G, Bhattarai A, Stromberg J et al (2009). Influence of rapid malaria diagnostic tests on treatment and health outcomes in fever patients, Zanzibar: a cross-over validation study. PLOS Med 6(4): e1000070 doi: 10.1371/journal.pmed.1000070

Muehlenbachs A, Mutabingwa TK, Edmonds S, Fried M, Duffy PE (2006). Hypertension and maternal-fetal conflict during placental malaria. PLOS Med 3(11):e446

Mukhtar EA, Gadalla NB, El-Zaki SE, Mukhtar I, Mansour FA, Babiker A, El-Sayed BB (2007). A comparative study on the efficacy of artesunate plus sulphadoxine/pyrimethamine versus artemether-lumefantrine in eastern Sudan. Malar J 6:92

Mungthin M, Khositnithikul R, Sitthichot N, Suwandittakul N, Wattanaveeradej V, Ward SA and Na-Bangchang K (2010). Association between the *pfmdr1* gene and in vitro artemether and lumefantrine sensitivity in Thai isolates of *Plasmodium falciparum.* Am J Trop Med Hyg 83(5): 1005-1009

Murphy SC, Breman JG (2001). Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anaemia, respiratory distress, hypoglycaemia, and complication of pregnancy. Am J Trop Med Hyg 64 (suppl 1-2) pp 57-67

Murray CK, Gasser RA Jr, Magill AJ, Miller SR (2008). Update on rapid diagnostic testing for malaria. Clin Microbiol Rev. January 21(1): 97-110

Mutabingwa TK, Anthony D, Heller A, Hallet R, Ahmed J, Drakeley C et al (2005). Amodiaquine alone, amodiaquine+sulfadoxine-pyrimethamine, amodiaquine+artesunate, artemether-lumefantrine for outpatient treatment of malaria in Tanzanian children: a four-arm randomised effectiveness trial. Lancet 365: 1474-1480

Mutabingwa TK, Muze K, Ord R, Briceño M, Greenwood BM, et al. (2009) Randomized Trial of Artesunate+Amodiaquine, Sulfadoxine-Pyrimethamine+Amodiaquine, Chlorproguanal-Dapsone and SP for Malaria in Pregnancy in Tanzania. PLoS ONE 4(4): e5138.

Mutabingwa TK, Nzila A, Mberu E, Nduati E, Winstanley P, Hills E, Watkins W (2001). Chlorproguanil-dapsone for treatment of drug-resistant falciparum malaria in Tanzania. Lancet 358: 1218-23

Mwai L, Ochong E, Abdirahman A, Kiara SM, Ward S, Kokwaro G et al (2009). Chloroquine resistance before and after its withdrawal in Kenya. Malar J 8:106.

Myint HY, Ashley EA, Daya NPJ, Nosten F, White NJ (2007). Efficacy and safety of Dihydroartemisinin-piperaquine. Transactions of the Royal Society of Tropical Medicine and Hygiene. 101, 858-866

Naing C, Mark JW, Aung K, Wong JY (2013). Efficacy and safety of dihydroartemisinin-piperaquine for treatment of uncomplicated *Plasmodium falciparum* malaria in endemic countries: a meta-analysis of randomised controlled studies. Transactions of the Royal Society of Tropical Medicine and Hygiene 107(2): 65-73

Ndiaye M , Faye B, Tine R, Ndiaye JL, Lo A, Abiola A et al (2012). Assessment of the Molecular Marker of *Plasmodium falciparum* Chloroquine Resistance (*Pfcrt*) in Senegal after Several Years of Chloroquine Withdrawal. Am J Trop Med Hyg. 3; 87(4): 640–645.

Ndjinga J, Minakawa N (2010). The importance of education to increase use of bednets in villages outside of Kinshasa, Democratic Republic of Congo. Malar J 9:279

Nechuta S, Mudd LM, Biery L, Elliot MR, Lepkowski JM, Paneth N and the Michigan alliance for the National Children's Study (2009). Attitudes of pregnant women towards participation in perinatal epidemiological research. Paediatric and Perinatal Epidemiology 23: 424-30

Neftel KA, Woodtly W, Schmid M, Frick PG, Ferh J (1986). Amodiaquine induced agranulocytosis and liver damage. Br. Med. J 297:721

Newman R. D., Parise M. E., Slutsker L., Nahlen B. and Steketee R. W. (2003). "Safety, efficacy and determinants of effectiveness of antimalarial drugs during pregnancy: Implications for prevention programmes in Plasmodium falciparum-endemic sub-Saharan Africa." Tropical Medicine & International Health 8: 488-506.

Newton PN, Van Vugt M, Teja-Isavadharm P et al (2002). A comparison of oral artesunate and dihydroartemisinin antimalarial bioavailability in acute falciparum malaria. Antimicrobial Agents and Chemotherapy 46,125-127

Newton S, Doku V, Geissler W, Asante KP and Cousens S (2009). Drawing blood from young children: lessons learned from a trial in Ghana. Trans R Soc Trop Med Hyg 103(5): 497499.

Ngasala BE, Malmberg M, Carlsson AM, Ferreira PE, Petzold MG, Blessborn G et al (2011). Effectiveness of artemether-lumefantrine provided by community health workers in under-five children with uncomplicated malaria in rural Tanzania: an open label prospective study. Malar J 10:64

NHIS (2013). National Health Insurance Scheme Tariffs for CHAG Primary Care Hospitals.

Nijhawan LP, Janodla MD, Muddukrishna BS, Bhat KM, Balry KL, Udupa N and Musmade PB (2013). Informed Consent: Issues and Challenges. Journal of Advanced Pharmaceutical Technological Research, 4(3): 134-140.

Nji AM, Innocent MA, Moyeh MN, Ngongang E-O, Ekollo AM, Chedjou J-P et al (2015). Randomized non-inferiority and safety trial of dihydroartemisinin-piperaquine and artesunate-amodiaquine versus artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Cameroonian children. Malaria Journal 14: 27

Nkhoma S, Nair S, Mukaka M, Molyneux ME, Ward SA, Anderson TJC (2009). Parasites bearing a single copy of the multi-drug resistance gene (Pfmdr1) with wild type SNPs predominate amongst Plasmodium falciparum isolates from Malaria. Acta Trop 111(1): 78-81

Noedl H, Se Y, Schaecher K, et al (2008). Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med 11; 359 (24): 2619-20

Nosten F, McGready R, Mutabingwa T (2007). Case management of malaria in Pregnancy. Lancet Infect Dis. 7:118-125

Nosten F, McGready R, Simpson AJ et al (1999). Effects of Plasmodium vivax malaria in pregnancy. Lancet 354: 546-9

Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, Brabin B (2004). Malaria in pregnancy and the endemicity spectrum: what can we learn? Trends Parasitol 20:425-32

Nosten F, ter Kuile FO, Maelankirri L, Decludt B, White NJ (1991). Malaria during pregnancy in an area of unstable endemicity. Trans R Soc Trop Med Hyg 85;424-29.

Nosten F, White NJ (2007). Artemisinin-based combination treatment of falciparum malaria. Am J.Trop Med. Hyg. 77: 181-192

Nyut MM, Adam I, Kayentao K, van Dijk J, Thuma P, Mauff K et al (2010). Pharmacokinetics of sulphadoxine and pyrimethamine in intermittent preventive treatment of malaria in pregnancy. Clin Pharmacol Ther 87(2) 226-34

O'Meara WP, Mangeni JN, Steketee R, Greenwood B (2010). Changes in the burden of malaria in sub-Saharan Africa. Lancet Infect Dis 10: 545-55

Ofori MF, Ansah E, Agyepong I, Ofori-Adjei D, Hviid L, Akanmori BD (2009). Pregnancy-associated malaria in a rural community of Ghana. Ghana Med J 43(1), 13-18

Ogutu BR, Onyango KO, Koskei N, Omondi EK, Ongecha JM, Otieno GA et al (2014). Efficacy and safety of artemether-lumefantrine and dihydroartemisinin-piperaquine in the treatment of uncomplicated Plasmodium falciparum malaria in Kenyan children aged less than five years: results of an open-label, randomized, single-centre study. Malaria Journal 13:33

Okoko BJ, Enwere G, Ota MOC (2003). The epidemiology and consequences of maternal malaria: a review of immunological basis. Acta Trop 87(2): 193-205.

Okyere R (2011). Assessing the coverage and consistent use of insecticide-treated bednets in the prevention of malaria among pregnant women in Nkoranza South district in the Brong Ahafo Region of Ghana. Masters In Public Health Thesis. Accessed from www.dspace.knust.edu on 07/ 03/ 2013.

Olsson S, Pal SN, Stergachis A, Couper M (2010). Pharmacovigilance activities in 55 low- and middle-income countries: a questionnaire- based analysis. Drug Saf 33: 689-703

Omar SA, Adagu IS, and Warhurst DC (2001) Can pretreatment screening for *dhps* and *dhfr* point mutations in Plasmodium falciparum infections be used to predict sulfadoxine-pyrimethamine treatment failure? Trans R Soc Trop Med Hyg 95:315–319.

Orish VN, Onyeabor OS, Boampong JN, Aforakwah R, Nwaefuna E, Iriemenam NC (2012) Adolescent pregnancy and the risk of *Plasmodium falciparum* malaria and anaemia—A pilot study from Sekondi-Takoradi metropolis, Ghana. Acta Tropica 123 (2012) 244– 248.

Osei-Kwakye K, Asante KP, Mahama E, Apanga S, Owusu R et al. (2013) The Benefits or Otherwise of Managing Malaria Cases with or without Laboratory Diagnosis: The Experience in a District Hospital in Ghana. PLoS ONE 8(3): e58107. doi:10.1371/journal.pone.0058107

Owusu-Agyei S, Asante KP, Adjuik M, Adjei G, Awini E, Adams M et al (2009). Epidemiology of Malaria in the forest savannah transition zone of Ghana. Malar J 8:220

Paradis C, Phelan MP, Brinich M (2010) A pilot study to examine research subjects' perception of participating in research in the emergency department. Journal of Medical Ethics 36(10): 580–7.

Parise M. E., Ayisi J. G., Nahlen B. L., Schultz L. J., Roberts J. M., Misore A., Muga R., Oloo A. J. and Steketee R. W. (1998). "Efficacy of sulfadoxine-pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection." Am J Trop Med Hyg 59: 813-822.

Pearce RJ, Drakeley C, Chandramohan D, Mosha F, Roper C (2003). Molecular determination of point mutation haplotypes in the dihydrofolate reductase and dihydropteroate synthase of Plasmodium falciparum in three districts of northern Tanzania. Antimicrob Agents Chemother. 47:1347–1354.

Phillips-Howard PA, West LJ (1990). Serious adverse reactions to pyrimethamine-sulphadoxine, pyrimethamine-dapsone and to amodiaquine in Britain. J. Royal Soc. Med. 83: 82-85

Pillai DR, Lau R, Khairnar K, Lepore R, Via A et al (2012).Artemether resistance in vitro is linked to mutation in PfATP6 that also interact with mutations in PfMDR1 in travellers returning with Plasmodium falciparum. Malar J 11:131

Pirmohamed M, Atuah KN, Dodoo AN, Winstanley P (2007). Pharmacovigilance in developing countries. BMJ 335:462

Plowe CV, Roper C, Barnwell JW, Happi CT, Joshi H, Mbacham W, Meshnick SR et al (2007). World Antimalarial Resistance Network (WARN) 111: Molecular markers for drug resistant malaria. Malaria Journal 6:121

Pocock SJ. (2003). The pros and cons of noninferiority trials. Fundamentals and Clinical Pharmacology 17 (2003) 483-490

Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Sugiarto P, et al. (2014) Dihydroartemisinin-Piperaquine Treatment of Multidrug Resistant Falciparum and Vivax Malaria in Pregnancy. PLoS ONE 9(1): e84976

Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Warikar N, Seal A, McGready R.(2008) Adverse pregnancy outcomes in an area where multidrug-resistant plasmodium vivax and *Plasmodium falciparum* infections are endemic. Clin Infect Dis 46(9):1374-81.

Pradines B, Tall A, Parzy D, Spiegel A, Fusai T, Hienne R et al (1998). In vitro activity of Pyronaridine and amodiaquine against African isolates (Senegal) of *Plasmodium falciparum* against standard antimalarial agents. Journal of Antimicrobial Chemotherapy 42: 333-339

Ratcliff A, Siswantoro H, Kenangelem E, Maristela R, Wuwung RM, Laihad F et al (2007). Two fixed-dose combinations for drug-resistant falciparum and vivax malaria in Papua, Indonesia: an open-label randomised comparison. Lancet 369(9563): 757-765 doi:10.1016/S0140-6736(07)60160-3

Raynes K (1999). Bisquinoline antimalarials: their role in malaria chemotherapy**.** Int. J. Parasitol., 29 pp. 367–379

RBM (2006). Roll Back Malaria. Malaria in pregnancy. www.rbm.who.int/cmc. Accessed 07/11/09

Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF (2000). Pgh1 modulates sensitivity and resistance to multiple antimalar ials in *Plasmodium falciparum.* Nature 403: 906-909.

Reynolds J, Mangesho P, Lemnge MM, Vestergaard LS and Chandler CIR (2013). '…..in the project they really care for us': Meaning and experiences of participating in a clinical study of first-line treatment for malaria and HIV in Tanzanian adults. Global Public Health: An International Journal of Research, Policy and Practice, 8:6, 670-684

Rijken MJ, McGready R, Boel ME, Barends M, Proux S, Mupawjay P et al (2008).Dihydroartemisinin-Piperaquine Rescue Treatment of Multidrug resistant *Plasmodium falciparum* Malaria in Pregnancy: A Preliminary Report. Am. J. Trop Med Hyg. 78(4), 543-545

Rijken MJ, McGready R, Boel ME, Poespoprodjo R, Singh N et al (2012). Malaria in pregnancy in the Asia-Pacific region. Lancet Dis 12: 75-88

Rijken MJ, McGready R, Julien V, Tarning J, Lindegardh N, Phyo AP et al (2011b). Pharmacokinetics of amodiaquine and desethylamodiaquine in pregnant and post-partum women with *Plasmodium vivax* malaria. Antimicrob Agent Chemother 55(9) 4338-42

Rijken MJ, McGready R, Phyo AP, Lindegardh N, Tarning J, Laochan N et al (2011a). The pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and non-pregnant women with uncomplicated falciparum malaria. Antimicrobial Agents Chemother. Doi 10.1128/AAC. 05067-11. Published online on 26 September 2011. Accessed November 2011

Rodger MA, Makropoulos D, Walker M, Keely E, Karovitch A, Well PS (2003). Participation of pregnant women in clinical trials: will they participate and why? American Journal of Perinatology 20(2) pp 69-76

Rogerson S J., Chaluluka E., Kanjala M., Mkundika P., Mhango C. and Molyneux M. E. (2000). "Intermittent sulfadoxine-pyrimethamine in pregnancy: effectiveness against malaria morbidity in Blantyre, Malawi, in 1997-99." Transactions of the Royal Society of Tropical Medicine & Hygiene 94: 549-553.

Rogerson SJ, Boeuf P (2007). New approaches to pathogenesis of malaria in pregnancy. Parasitology, 134 (2007), pp. 1883–1893

Rogerson SJ, Hviid L, Duffy PE, Leke RFG, Taylor WD (2007). Malaria in Pregnancy: Pathogenesis and immunity. Lancet Infect Dis 7: 105-17.

Rogerson SJ, Pollina E, Getachew A, Tadesse E, Lema VM, Molyneux M (2003). Placental monocyte infiltrates in response to *Plasmodium falciparum* infection and their association with adverse pregnancy outcomes. Am. J. Trop. Med. Hyg. 68 (1) pp 115-119.

Rowe JA, Kyes SA (2004). The role of Plasmodium falciparum var genes in malaria in pregnancy. Molecular Microbiology 53: 1011-1019

Sa JM, Twu O, Hayton K, Reyes S, Fay MP, Ringwald P, Wellems TE (2009). Geographic patterns of *Plasmodium falciparum* drug resistance distinguished by differential responses to amodiaquine and chloroquine. Proc Natl Acad Sci 106: 18883-18889.

Salanti A, Staalsoe T, Lavsten T, Jensen AT, Sowa MP, Arnot DE et al (2003). Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. Mol Microbiol 49: 179-91

Sarr O, Ahouidi AD, Ly O, Daily JP, Ndiaye D, Ndir O et al (2008). Mutations in PFCRT K76T do not correlate with sulfadoxine-pyrimethamine-amodiaquine failure in Pikine, Senegal. Parasitol Res 103(4): 765-769 doi:10.1007/S00436-008-1038-9

Schantz-Dunn J, Nour NM (2009). Malaria and pregnancy: a global health perspective. Rev Obstet Gynecol 2: 186-192

Scifres CM, Nelson DM (2009). Intrauterine growth restriction, human placental development and trophoblast cell death. J. Physiol., 587 pp. 3453–3458

Shah JY, Phadtare A, Rajgor D, Vaghasia M, Pradhan S, et al. (2010).What leads Indians to participate in clinical trials? A meta-analysis of qualitative studies. PloS One 5(5): e10730.

Shiff CJ, Premji Z, Minjas JN (1993). The rapid manual ParaSight-F test. A new diagnostic tool for *Plasmodium falciparum* infection. Trans R Soc Trop Med Hyg 87:646-648

Shulman C. E., Dorman E. K., Cutts F., Kawuondo K., Bulmer J. N., Peshu N. and Marsh K. (1999). "Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial." Lancet 353: 632-636.

Shulman CE, Dormon EK, Talisuna AO et al. (1998) A community randomized controlled trial of insecticide-treated bednets for the prevention of malaria and anaemia among primigravid women on the Kenyan coast. Tropical Medicine and International Health 3**,** 197–204.

Shulman CE, Marshall T, Dorman EK, Bulmer JN, Cutts F, Peshu N, Marsh K (2001). Malaria in pregnancy: adverse effects on haemoglobin levels and birthweight in primigravida and multigravida. Trop Med Int Health 6 (10):770-8

Sidhu AB, Valderramos SG, Fidock DA (2005). *Pfmdr*1 mutations contribute to quinine resistance and enhance mefloquine and artemisinin sensitivity in *Plasmodium falciparum*. Mol. Microbiol. 57 pp. 913–926

Silver KL, Zhong K, Leke RG, Taylor DW, Kain K (2010). Dysregulation of angiopoietins is associated with placental malaria and low birth weight. PLOS ONE; Mar 1 5(3) e9481

Singh N, Shukla MM, Sharma VP (1999). Epidemiology of malaria in pregnancy in central India. Bulletin of the World Health Organization 77(7): 567-72

Sisowath C, Petersen I, Veiga MI, Mårtensson A, Premji Z, Björkman A, Fidock DA, Gil JP (2009). In vivo selection of *Plasmodium falciparum* parasites carrying the chloroquine-susceptible *Pfcrt* K76 allele after treatment with artemether-lumefantrine in Africa. J Infect Dis 199:750–755.

Smith JA, Jarman M, Osborn M (1999). Doing interpretative phenomenological analysis. In Qualitative Health Psychology: Theories and Methods; pp 218-240. (M Murray and K Chamberlain, editors). London: Sage Publications

Smith LA, Jones C, Adjei RO, Antwi GD, Afrah NA, Greenwood B et al (2010). Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: user acceptability. Malaria Journal 9:18

Smith NC (1996). An immunological hypothesis to explain the enhanced susceptibility to malaria during pregnancy. Parasitology Today 12(1): pp 4-6

Smithuis F, Kyaw MK, Phe O, Aye KZ, Htet L, Barends M et al (2006). Efficacy and effectiveness of dihydroartemisinin-piperaquine versus artesunate-mefloquine in falciparum malaria: an open label randomised comparison. Lancet 367, 2075-2085

Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo AP et al (2010). Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. Lancet Infect Dis 2010 10:673–81.

Snounou G, Zhu X, Siripoon N, Jarra W, Thiathong S, Brown KN, Viriyakosol S (1999). Biased distribution of msp1 and msp2 allelic variants in Plasmodium falciparum populations in Thailand. Trans R Soc Trop Med Hyg 93(4) 369-74

Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature 434(7030): 214-7

Steketee RW, Nahlen BL, Parise ME, Menendez C (2001). The Burden of Malaria in Pregnancy in malaria-endemic areas. Am J Trop Med Hyg 64(1): 28-35

Suguitan Jr. AL, Leke RGF, Fouda G, Zhou A, Thuita L, Metenou S et al (2003). Changes in the Levels of Chemokines and Cytokines in the Placentas of Women with "Plasmodium falciparum" Malaria. Journal of Infectious Diseases Vol. 188, No. 7 pp. 1074-1082

Tagbor H, Antwi G, Dogbe J (2014). Safety of antimalarial drug exposure during early pregnancy. Research and Reports in Tropical Medicine 5: 23-33

Tagbor H, Bruce J, Agbo M, Greenwood B and Chandramohan D (2010). Intermittent Screening and Treatment versus Intermittent Preventive Treatment of Malaria in Pregnancy: A Randomised Controlled Non-inferiority Trial. PLOS ONE 5(12):e14425. doi:10.1371

Tagbor H, Bruce J, Browne E, Greenwood B and Chandramohan D (2008a). Malaria in pregnancy in an area of stable and intense transmission; is it really asymptomatic? Journal of Tropical Medicine and International Health 13(8);pp 1016-1021

Tagbor H, Bruce J, Browne E, Greenwood B and Chandramohan D (2008b). Performance of the Optimal dipstick in the diagnosis of malaria infection in pregnancy. Ther Clin.Risk. Manag June 4(3) 631-636

Tagbor H, Bruce J, Browne E, Randal A, Greenwood B, Chandramohan D (2006). Efficacy, safety and tolerability of amodiaquine plus sulphadoxine-pyrimethamine used alone or in combinations for malaria treatment in pregnancy: a randomised trial. Lancet 368: 1349-56.

Tako EA, Zhou A, Lohoue J, Leke R, Teylor DW, Leke RF (2005). Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. Am J Trop Med Hyg 75:236-242

Tarning J, Ashley A, Lindegardh N, et al (2008). Population pharmacokinetics of piperaquine after two different treatment regimens with dihydroartemisinin-piperaquine in patients with Plasmodium falciparum malaria in Thailand. Antimicrobial Agents and Chemotherapy 52: 1052-1061

Tarning J, Chotsiri P, Julien V, Rijken M, Bergstrand M, Cammas M et al (2012a). Population pharmacokinetic and pharmacodynamics modelling of amodiaquine and desethylamodiaquine in women with *Plasmodium vivax* malaria during and after pregnancy. Antimicrobial Agents Chemother 56 (11): 5764-73

Tarning J, Lindegardh N, Annerberg A, Singtoroj T et al. (2005). Pitfalls in estimating piperaquine elimination. Antimicrobial Agents and Chemotherapy 49, 5127-5128

Tarning J, Rijken MJ, McGready R, Phyo AP, Hanpithakpong W, Day NPJ et al (2012b). Population pharmacokinetics of Dihydroartemisinin and Piperaquine in pregnant and non-pregnant women with uncomplicated malaria. Antimicrobial Agents Chemother 56(4):1997-2007.

Tay SCK, Agboli E, Abruquah HH, Walana W (2013). Malaria and Anaemia in pregnant and non-pregnant women of child-bearing age the University Hospital, Kumasi, Ghana. Open Journal of Medical Microbiology 3: 193-200

ter Kuile FO and Rogerson SJ (2008). Plasmodium vivax infection during pregnancy: An Important Problem in Need of New Solutions. Clin Infect Dis 46(9): 1382-1384

ter Kuile FO, Parise ME, Verheoff FH et al. (2004). The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. Am J Trop Med Hyg 71 (2 Suppl): 41-54

ter Kuile FO, Terlouw J, Phillips-Howard PA, Hawley WA, Friedmam JF, Kariuki SK et al (2003). Reduction of malaria during pregnancy by permethrin-based bednets in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg 68(Suppl 4); 50-60

Thanh NX, Trung TN, Phong NC, Thien NX, Dai B, Shanks GD et al (2009). Open label randomized comparison of dihydroartemisinin-piperaquine and artesunateamodiaquine for the treatment of uncomplicated Plasmodium falciparum malaria in central Vietnam. Trop Med Int Health 14:504–11.

Thomsen TT, Ishengoma DS, Mmbando BP, Lusingu JP, Vestergaard LS, Theander TG, Lemnge MM, Bygbjerg IC, Alifrangis M (2011). Prevalence of single nucleotide polymorphisms in the *Plasmodium falciparum* multidrug resistance gene (Pfmdr-1) in Korogwe District in Tanzania before and after introduction of artemisinin-based combination therapy. Am J Trop Med Hyg. 12:979–983.

Thomsen TT, Madsen LB, Hansson HH, Tomas EV, Charlwood D, Bygbjerg IC, Alifrangis M (2013). Rapid selection of *Plasmodium falciparum* chloroquine resistance transporter gene and multidrug resistance gene-1 haplotypes associated with past chloroquine and present artemether-lumefantrine use in Inhambane District, southern Mozambique. Am J Trop Med Hyg 3; 12: 536–541.

Thwing JI, Odero CO, Odhiambo FO, Otieno KO, Kariuki S, Ord R, Roper C et al (2009). In-vivo efficacy of amodiaquine-artesunate in children with uncomplicated Plasmodium falciparum malaria in western Kenya. Trop Med Int Health 14: 294-300

Trung NT, Bo TAN, Phuc DV, Jian-ping S (2009). A randomized controlled trial of Artemisinin-piperaquine vs Dihydroartemisinin-piperaquine phosphate in treatment of falciparum malaria. Chin. J. Integr. Med 15(3): 189-192

Tutu OE, Larbi J, Lawson B, Brown C, Browne E (2011). Genetic diversity of Plasmodium falciparum in pregnant women in an IPTp setting in the Offinso District of Ghana. Journal of Parasitology and Vector Biology 3(1): 12-18

Umbers AJ, Aitken HE and Rogerson SJ (2011) .Malaria in pregnancy: small babies, big problem. Trends in Parasitology vol 27, Iss 4 pp 168-175

UNICEF (2007). Malaria Fact Sheet on Ghana. www.unicef.org /wcaro/ WCARO_ Ghana_Factsheet_malaria.pdf Accessed on 06/05/2014

Valecha N, Phyo PA, Mayxay M, Newton PN, Krudsood S, Keomany S et al. (2010). An open-label, randomised study of dihydroartemisinin-piperaquine versus artesunate-mefloquine for falciparum malaria in Asia. PLOS ONE 5(7): e11880

Van Eijk AM, Terlouw DJ (2011). Azithromycin for treating uncomplicated malaria. Cochrane Database Syst. Review. Doi 10.1002/14651858.CD006688.pub2

Venkatesan M, Gadalla NB, Stepniewska K, Dahal P, Nsanzabana C, Moriera C, Price RN et al (2014). Polymorphisms in *Plasmodium falciparum* Chloroquine Resistance Transporter and Multidrug Resistance 1 Genes: Parasite Risk Factors that Affect Treatment Outcomes for *P.falciparum* Malaria after Artemether-Lumefantrine and Artesunate-Amodiaquine. Am J Trop Med Hyg 91(4): 833-843

Verhoeff FH, Brabin B J, Chimsuku L, Kazembe P, Russell WB and Broadhead R L (1998). "An evaluation of the effects of intermittent sulfadoxine-pyrimethamine treatment in pregnancy on parasite clearance and risk of low birthweight in rural Malawi." Annals of Tropical Medicine & Parasitology 92: 141-150.

Viebig NK, Gamain B, Scheidig C, Lepolard C, Przyborski J, Lanzer M et al (2005). A single member of the Plasmodium falciparum var multigene family determines cytoadhesion to the placental receptor chondroitin sulphate A. EMBO Reports 6 (8): 775-781

Villegas L, McGready R, Htwang M, Paw MK, Pimanpanarak M, Arunjerda R et al (2007). Chloroquine prophylaxis against vivax malaria in pregnancy: a randomized, double-blind, placebo-controlled trial. Trop Med Int Health 12 (2): 209-218

Vittinghoff E, Glidden DV, Shiboski SC, McCulloch CE (2004). Regression Methods in Biostatistics: Linear, Logistic, Survival, and Repeated Measures Models. Second Edition. Springer, New York

Walker PGT, ter Kuile FO, Garske T, Menendez C, Ghani AC (2014). Estimated risk of plancental infection and low birthweight attributable to Plasmodium falciparum malaria in Africa in 2010: a modelling study. The Lancet Global Health 2(8) pp e460-e467

Walter PR, Garin Y, Blot P (1982). Placental pathologic changes in malaria. A histologic and ultrastructural study. American Journal of Pathology 109: 330-342

Wang P, Read M, Sims PF, Hyde JE (1997). Sulfadoxine resistance in the human malaria parasite Plasmodium falciparum is determined by mutations in dihydropteroate synthase and an additional factor associated with folate utilization. Molecular Microbiology 23, 979-986

Ward SA, Sevene EPJ, Hastings AM, Nosten F, McGready R (2007). Antimalarial Drugs and Pregnancy: Safety, Pharmacokinetics and Pharmacovigilance. Lancet Infectious Diseases 7: 136-144

White N (1999). Antimalarial drug resistance and combination chemotherapy. Philo Trans R Soc Lond B Biol Sci 354: 739-749.

White NJ (2006). Malaria--time to act. N. Engl. J. Med 355: 1956-1957

WHO (2006). Guidelines for the treatment of malaria. World Health Organization, Geneva. http://www.who.int/malaria/treatment guidelines. Accessed 11/12/09.

WHO (2007). Assessment of safety of artemisinin compounds in pregnancy. http://who.int/tdr/svc/publications. Accessed 02/06/10

WHO (2010a). Guidelines for the treatment of malaria 2010. http://www. who. int/malaria/publications . Accessed 02/06/10

WHO (2010b). Parasitological Confirmation of malaria diagnosis. Report of a WHO Technical Consultation. Geneva. World Health Organization, 2010

WHO (2011). World Malaria Report, 2011.

WHO (2012a). World Malaria Report, 2012.

WHO (2012b). Evidence Review Group Meeting Report. Intermittent Preventive Treatment of malaria in pregnancy(IPTp) with Sulfadoxine Pyrimethamine (SP). WHO Headquarters, Geneva, 9-11 July 2012

WHO (2012c). T3—Test. Treat. Track, World Health Organization (www.who.int /malaria/publications/atoz/test_treat_track_brochure.pdf). Accessed 25/04/12.

WHO (2013). World Malaria Report, 2013.

WHO/FIND/CDC (2013). Malaria Rapid Diagnostic Test Performance. Summary of results of WHO product testing of malaria RDTs, round 1-5 (2008-2013). www. finddiagnostics.org. accessed 06/08/2014

Wilson NO, Ceesay FK, Obed SA, Adjei AA, Gyasi RK, Rodney P et al. (2011). Intermittent preventive treatment with Sulphadoxine-pyrimethamine against malaria in pregnant women. Am J Trop Med Hyg 85(1) 12-21.

Wolfe MJ, Cordero JF (1985). Safety of chloroquine in chemosuppression of malaria during pregnancy. BMJ (Clin Res Ed) 290 (6480): 1466-7

Wong ML, Chia KS, Wee S, Chia SE, Lee J, et al. (2004) Concerns over participation in genetic research among Malay-Muslims, Chinese and Indians in Singapore: a focus group study. Public Health Genomics 7: 44–54.

Wooden J, Kyes S, Sibley CH (1993). PCR and strain identification in *Plasmodium falciparum*. Parasitol Today. 9:303–305.

Worrall E, Morel C, Yeung C, Borghi J, Webster J, Hill J et al (2007). The economics of malaria in pregnancy—a review of the evidence and research priorities. Lancet Infect Dis 7(2): 156-168

Wurtz N, Fall B, Bui K, Pascual A, Fall M, Camara C et al. (2013). Pfhrp2 and pfhrp3 polymorphisms in Plasmodium falciparum isolates from Dakar, Senegal: impact on rapid malaria diagnostic tests. Malaria Journal, 12:34

Yamada M, Steketee R, Abramowsky C et al. (1989). *Plasmodium falciparum* associated placental histology: a light and electron microscopic and immunohistologic study. Am. J. Trop. Med. Hyg. 41; 161-168

Zani B, Gathu M, Donegan S, Olliaro PL, Sinclair D (2014). Dihydroartemisinin piperaquine for treating uncomplicated *Plasmodium falciparum* malaria. Cochrane Database of Systematic Reviews, Issue 1, CD010927

Zongo I, Dorsey G, Rouamba N, Dokomajilar C, Sere Y, et al. (2007) Randomized comparison of amodiaquine plus sulfadoxine-pyrimethamine, artemether-lumefantrine, and dihydroartemisinin-piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria in Burkina Faso. Clin Infect Dis 45: 1453–1461.

Zwang J, Ashley EA, Karema C, D'Alessandro U, Smithius F, Dorsey G et al. (2009). Safety and Efficacy of Dihydroartemisinin-Piperaquine in Falciparum Malaria: A Prospective Multi-Centre Individual Patient Data Analysis. PLOS ONE 4(7): e6358.

APPENDICES

APPENDIX 1: CONSENT FORM

We are studying the efficacy, safety and tolerability of dihydroartemisinin-piperaquine (DHA-PPQ) in pregnancy. This drug is being taken as an alternative to artesunate-amodiaquine (ASAQ) for the treatment of uncomplicated malaria in pregnant women. Malaria during pregnancy can have negative consequences like low birth weights and maternal anaemia and thus must be treated very well. Similar negative consequences can still result if a pregnant woman has the parasite without obvious illness. This study seeks to validate the use of DHA-PPQ for the purpose stated.

If you agree to participate in the study, you will be screened for malaria parasites. If positive, you will be allocated to receive either DHA-PPQ or ASAQ over three (3) days. You will be required to take the first day's dose today in our presence. The other days' dose will be given to you to take home with clear instructions on how to take them. We will be visiting your home to find out how you are doing, whether you have any complaints with the study drugs, to take blood samples for checking malaria parasites and your haemoglobin and also filter paper blots. Specifically, we will be visiting you at home on days 1, 2, 3, 7, 14, 28 and 42 after the start of medication. In the event of marked complaints, reactions or adverse events, we will take necessary action to safeguard your health as it is of great importance to us.

The blood samples will be taken through both finger-pricking and venous sampling (about 5ml) just as are done when you normally go to the laboratory in any hospital.

We encourage you also to come and deliver here in this hospital when you are due. At delivery, we will also take blood samples and small pieces of the placenta for more studies all for the purposes already explained above. We will follow you and your baby for six (6) weeks more to look for anomalies and jaundice in the baby.

You will be provided with free insecticide-treated nets to protect you from mosquito bites and you will have access to the study team all the time whenever you have concerns or questions about the study and your participation in it. You will also be making significant contribution to knowledge in this field. We assure you that your identity and the information you give us will be kept in very strict confidence. We also assure you that we will **NOT** use your blood/ placenta samples for purposes other than those described.

We want to remind you that participation in this study is completely voluntary and nobody can punish you or refuse to give you medical care for refusing to participate in it. You are free to withdraw from the study anytime you wish to do so without any recourse or whatsoever to access to medical care. If you want further information, you may call the leader of the study team on **0244562908**.

I understand the nature and requirements of this study as presented above or as has been explained to me in a language I understand and would like to participate in it. I accept the risks involved.

NAME OF PARTICIPANT ……..... NAME OF WITNESS……

DATE…… SIGNATURE/ THUMBPRINT ……..

SIGNATURE/THUMBPRINT…….

IDENTIFICATION NUMBER……..

APPENDIX 2: In-Depth Interview Guides

GROUP 1 (TRIAL PARTICIPANTS)

Demographic data	**Code:** Day: Length of the interview: Place of the interview: Community she/he is living (rural): Community she/he is from: Age: Occupation: Level of Education: Marital status: Number of children: Religion, denomination: Ethnic group: Number of people in house: Bednets ownership and use: How did you find her/him: Delivered since:
1. To explore their reasons for agreeing to participate in the trial	**Questions** Why do you visit the ANC clinic What happens when you visit the ANC ? What were you told by the research team? Did you believe what you were told by the research team? Why and why not i) How did you feel about the invitation to participate in our study? ii) How did you take what you were told about the possible complications of having malaria parasites in your blood? iii) What reasons made you agree to join the study?
2. To explore their experiences and thoughts about the trial	Which kind of medicine were you given at the ANC clinic? How do you feel after taking these medicines ? Which kind of medicine were you given by the research

	team? i) How did you feel about taking malaria medicine when you did not feel sick at all? ii) How did you feel after taking the medicine iii) What were your experiences after taking the medicine Eg vomiting, …. iv) How do you feel about the field workers who come to visit you in the house (or who used to come and visit you)? Do their / Did their visits draw attention from your relatives and neighbours? What were the feelings of other family members, about fieldworkers who came to your home to visit you? What happened whenever the field workers came home v) What are your views about taking your blood samples when the field workers visited you in your home? What are some of your concerns about your blood being taken especially in the house? What did you like or dislike about taking your blood at your house What did you like or dislike about your blood being taken in the clinic? Which one will you prefer?
3. To assess willingness to participate in future trials	vi) Do you think you should have been compensated for all your troubles during the study? What sort of compensation would you suggest?? vii) Do you think you benefitted from joining the study? How? viii) Will you join a similar study in the future if you are invited (whether you are pregnant or not)? What will be your reasons for joining or refusing to join ix) How do you feel about joining a similar trial so that other people can benefit from the trial findings

GROUP 2 (PREGNANT WOMEN AT ANC)

Demographic data	**Code:** Day: Length of the interview: Place of the interview: Community she/he is living (rural): Community she/he is from: Age: Occupation: Level of Education: Marital status: Number of children: Religion, denomination: Ethnic group: Number of people in house: Bednets ownership and use: How did you find her/him: Gestation period:
To explore their reasons\motivations for visiting the ANC clinic	- Why do you come for ANC visits at the hospital? - What happens when you visit the ANC clinic? - What are some of the common illness that affects pregnant women in your community? Which of this\these do you consider as most severe? Why? - What illnesses most affect you? And why are you most vulnerable to this? - What causes malaria in pregnancy? Have you ever had malaria whilst pregnant? - What are the effect of malaria on the pregnant woman and unborn child? - How do you treat malaria in when pregnant? (Isolate points on known anti malarial commonly used. If woman cannot remember the name, let her describe the colour)
To explore their knowledge about malaria and malaria *treatment* methods in pregnancy. -use of ant malarial - bed nets -repellents etc	*(Read and explain this information to the woman)* There is an on going study which seeks to explore the efficacy, safety and tolerability of an antimalarial drug called DHPQ and ASAQ. We have chosen this district and health facility to conduct the study. ***AIM***: The aim of this research is to find out whether DHPQ (show samples of the medicine to the woman) is not inferior to ASAQ(show samples of the medicine to the woman) in terms of clearing malaria parasites. Also it intends to investigate the safety of either medicine in

To examine their perception about the about the trial -aim -procedure -risk -benefit	terms of birth outcomes such as miscarriages, stillbirths, premature births, birth defects among others and the potential discomfort associated with their use. - Given this aim, will you partake in the study? If yes, why? If no, why not? - If, no, what conditions should be changed concerning the aim, to encourage you to participate in the research? ***PROCEDURE:*** These are the procedures you have to go through if you accept to be part of this study • Although not feeling sick, you will be physically examined • Your blood samples will be taken through the veins and finger tips to test for the presence of malaria parasites at the clinic • You will be given either of these anti malarial medicine (*show medicines to her)* if the test proves positive • The field staff will follow you up at home and gather information concerning complains regarding the medication you took, they will also take blood samples via finger pricks and blood drawn from the veins to check for malaria parasites - Given these *procedures* would you partake in the study? If yes what will motivate you to partake? If no, why will you not partake in the study? *(try to identify which of the procedures the woman feels less comfortable with)* - If, no, what conditions should be changed to encourage you to participate in the research? - What are your perceptions about the taking of blood samples at the clinic? - How would you feel about been given an anti malarial at the clinic when not feeling sick? - What would be your perception about field workers coming to your home to visit and take blood samples and your concern regarding the medicine you took? *(Refer back to the demographics to identify the number of people living in the house)* - How would you perceive the feelings of other family members (husband, mother, siblings etc) about fieldworkers coming to your home to take blood samples? And can this potentially influence your decision to partake or not to partake in this study? **RISK**: We anticipate some discomfort with both DHPQ and ASAQ, including vomiting, abdominal discomfort, diarrhoea, nausea, general weakness and dizziness. There is also the risk of pain and discomfort from the needle pricks, but no known reports of association between DHPQ and miscarriages, stillbirths or birth defects.

	- Given this associated risk, will you partake in the study? If yes, why? If no, why? - If, no, what conditions should be changed to encourage you to participate in the research? **BENEFITS:** You will be given a long-lasting insecticide-treated bed net to protect you against mosquitoes during sleep so that you can reduce your chances of getting malaria. By participating in the study you will be indirectly contributing to the generation of new knowledge on the effectiveness, safety and tolerability of DHPQ and ASAQ for the treatment of malaria in pregnancy. - Given this expected humanitarian\altruism benefits, will you partake in the study? If yes, why? If no, why? **MONETARY BENEFITS:** This research does not provide direct money benefit to you. - Given the absence of direct monetary benefits, will you partake in the study? If yes why? - If no, how much monetary benefit can motivate you to voluntarily partake in the study? - Which of the following issues discussed above will make you most willing to partake in this study? - In future researches, what should be included\changed to enhance your chances of been involved in such a research. - Who usually takes decisions regarding the treatment of malaria whenever you are pregnant? *Besides the pregnant woman and the medical health personnel* - How can the decisions of this one influence your willingness to partake in this study?
Decision making regarding the treatment of Malaria in Pregnancy	

GROUP 3 (HUSBANDS)

Demographic data	**Code:** Day: Length of the interview: Place of the interview: Community he is living (rural): Community he is from: Age: Occupation: Level of Education: Marital status: Number of children: Religion, denomination: Ethnic group: Number of people in house: How did you find him:
To explore their general knowledge about malaria and malaria *treatment* methods in pregnancy.	- What are some of the common illness that affects pregnant women in your community? Which of this\these do you consider as most severe? Why? - What causes malaria in pregnancy? Did your wife ever have malaria whilst pregnant? If yes, what were the common symptoms that helped you know that she (wife) has malaria. **Note to fieldworker**: If the answer to the above question is NO, please try to identify his general knowledge about the symptoms of malaria. What do you think are the effects of malaria on a pregnant woman and the unborn child? - What advice or what do you normally tell your wife to do whenever she has malaria? **Note to fieldworker:** If wife has never had malaria, try to find out the kind of advice he will give to the wife, if ever she gets malaria. - How does your wife treat malaria in pregnancy? *(Isolate points on known anti malarials commonly used. If husband cannot remember the name, let him describe the colour)* (Remind husbands of the clinical trial that involved their wives before asking the following questions) - What is your perception about the taking of blood samples from your wife , during the study, to examine if she has the malaria parasites at the clinic ? - Was your wife sick with malaria (or did she show any signs of malaria) when she was recruited to be part of the study. What is your perception about the giving of an anti-malarial to your wife when she did or did not know that she was sick with

	malaria? - What were some of the common complaints given by your wife after taking the drug? - If any, can these complaints make you potentially prevent her from engaging in this study? *(explore the reasons for any answer given)*
To explore their experiences and perception about the drug trial process at the clinic and at home	- How do you feel about the field workers who come to visit your wife in the house (or who used to come to visit your wife in the house)? - What happened whenever the field workers came home? **Note to fieldworker:** If he does not know remember\know, ask what the wife tells him about fieldworkers who came to visit her at home.
To explore their experiences about the schedule visits (follow up visits) at their individual homes	- What are your views about the taking of the blood samples of your wife by field workers at home? *(explore their likes and dislikes)* - Where do you most likely want the blood samples of your wife to be taken, at home or in the clinic? *(explore the one they prefer, and why)* - How have your wife and the family benefited from joining the study. If any? - Will you encourage your wife to join a similar study in the future if she is invited? Why or Why Not? - How do you feel about letting her join so that other people can benefit from the trial findings? *(emphasis on the humanitarian benefits)*
To explore the benefits of the study to the family	- Do you think your wife should have being compensated for all the troubles she went through (taking of trial medicine with its associated discomfort, pricking for blood samples etc) during the study? - What form of compensation (monetary, items etc) do you consider reasonable to be given your wife in order for you to encourage her to partake in future studies? - In future researches, which of the above should be maintained\excluded\changed to enhance the chances of encouraging your wife to be involved in such a research.
To assess willingness to allow their wives participate in a future study.	

APPENDIX 3: PCR conditions and primers for amplification

Table A. PCR conditions and primers for *msp2*, *glurp* and *Pfcrt* amplification

	Primers	Primer Sequence (5'-3')	PCR conditions
msp2 outer	M2-OF (fwd) M2-OR (rev)	ATG AAG GTA ATT AAA ACA TTG TCT ATT ATA CTT TGT TAC CAT CGG TAC ATT CTT	94°C,15' followed by 30X (94°C, 1';58°C, 2';72°C, 2'); 58°C,2'; 72°C, 5'
msp2 nest	M2-FCF M2-FCR M2-ICF M2-ICR	AAT ACT AAG AGT GTA GGT GCA RAT GCT CCA TTT TAT TTG GTG CAT TGC CAG AAC TTG AAC AGA AGT ATG GCA GAA AGT AAK CCT YCT ACT GAT TGT AAT TCG GGG GAT TCA GTT TGT TCG	94°C,15' followed by 30X (94°C,30"; 61°C,2'; 72°C, 1'); 58°C,2'; 72°C, 5'
glurp outer	G4-Out G5-Out	ACA TGC AAG TGT TGA TCC GAT GGT TTG GGA GTA ACG	Same for *msp2* outer
glurp nest	G1 G3	TGA ATT CGA AGA TGT TCA CAC TGA AC TGT AGG TAC CAC GGG TTC TTG TTG	94°C,15' followed by 30X (94°C,30"; 58°C,1'; 72°C,1'); 58°C,2'; 72°C, 5'
crt outer	CRTP1-OUT (fwd) CRTP2-OUT (rev)	CCGTTAATAATAAATACACGCAG CGGATGTTACAAAACTATAGTTACC	94°C,15' followed by 45X (94°C,30";56°C, 30"; 72°C,1'); 72°C,10'
crt nest	CRTD1-NEST CRTD2-NEST	TGTGCTCATGTGTTTAAACTT CAAAACTATAGTTACCAATTTTG (**Biotinylated in 5'-end**)	94°C,15' followed by 30X (94°C,30";48°C, 30"; 72°C,1'); 72°C,10'

Table B. PCR conditions and primers for amplification at *Pfmdr1* gene

***Pfmdr1* codons**	**Primer**	**Primer Sequence***	**PCR condition**
OUTER PCR			
86/184	FN1/1	AGGTTGAAAAAGAGTTGAAC (FW)	95°C,15'; 30X (95°C,30";55°C,60";72°C,90"); 72°C,5'
	Rev/C1	ATGACACCACAAACATAAAT (RV)	
1246	Out-1246mdr1-FW	GTGTATTTGCTGTAAGAGCT (FW)	Similar conditions to nest PCR for codons 86/184
	Out-1246mdr1-RV	GACATATTAAATAACATGGGTTC (RV)	
NEST PCR			
86	Mdr2/1	ACAAAAAGAGTACCGCTGAAT (FW)	95°C,15' followed by similar conditions for outer 86/184 but with 35 cycles
	Newrev1	AAACGCAAGTAATACATAAAGTC (RV)	
184	184-FW-RFLP	CGTTTAAATGTTTACCTGCACAACATA (FW)	Same as for codon 86
	184-REV-RFLP	GTCAAACGTGCATTTTTTATTAATGACCATTTA (RV)	
1246	Nest-1246mdr1-FW	GAATTTTCAAACCAATCTGGA (FW)	94°C,15';30X (94°C, 30";60°C,30";72°C,60"); 72°C,5'
	Nest-1246mdr1-RV	TAAATAACATGGGTTCTTGACT (RV)	

*Primer sequences adapted from Thompsen *et al* (2011)

Table C. PCR conditions and primers for *Pfdhfr/Pfdhps* amplification

dhfr/dhps	**Primer**	**PCR condition**
Outer *dhfr/ dhps*	M1/M7 N1/N2	94°C, 15'; 40X(94°C,1'; 52°C, 2'; 72°C, 1'); 72°C, 10'
Nest *dhfr*	M3b/M9	94°C, 15'; 5X(94°C,1'; 44°C, 2'; 72°C, 1'); 35X(94°C,1'; 44°C, 2'; 72°C, 1') 72°C, 10'
Nest *dhps*	R2/R	94°C, 15'; 40X(94°C,1'; 51°C, 2'; 72°C, 1'); 72°C, 10'

Appendix 4: Study Drug Analysis Report

Drug samples (ARSUAMOON and P-ALAXIN) submitted by Mr Joseph Osarfo to test for quality.

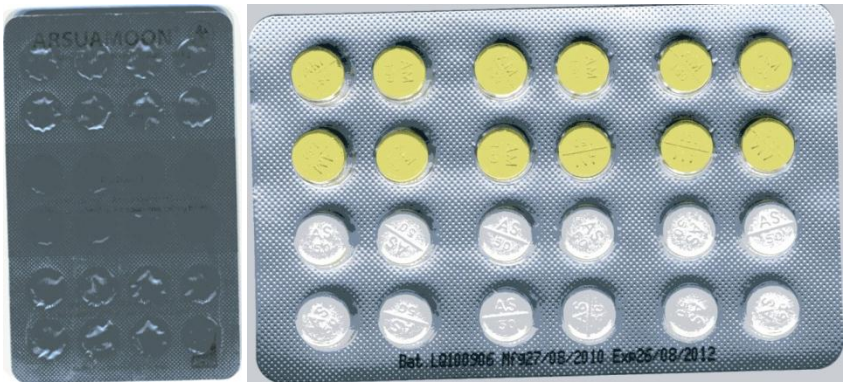
A) Arsuamoon (artesunate and amodiaquine)

The process followed to determine the quality of the tablets was as follows:

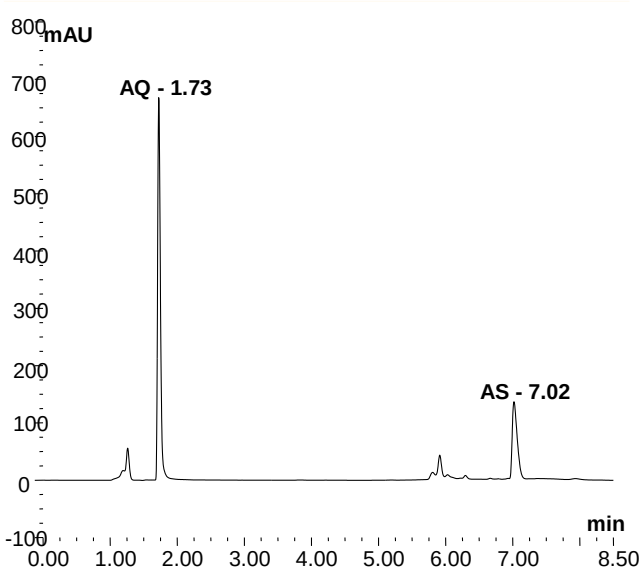
1. The submitted packet and blister of the tablets were scanned.
2. Two tablets from the blister pack were then pulverised separately and dissolved in solvent. The resulting mixture was then analysed using high performance liquid chromatography (HPLC) for amount of the active ingredients (Artesunate 20 mg and Amodiaquine 150 mg) in the tablets.
3. The active pharmaceutical ingredient (API) was expressed as a percentage of the expected amount, which should be within 90 -110% according to the United States Pharmacopeia (USP).

Results:

1. The scanned pictures of the packet [manufacturing date: 27/08/2010; expiry date: 26/08/2012; Batch No: LQ100906 A] and blisters contained within are shown below.



2. The HPLC chromatogram achieved is shown below where AQ is amodiaquine (1.73 min) and AS is artesunate (7.02 min).



3. The HPLC analysis gave the expected amount of active ingredients:
   Amodiaquine – API = 100%
   Artesunate – API = 93% which is within the 10% tolerance limits as accepted by USP.

B) P-Alaxin (dihydroartemisinin plus piperaquine)

The process followed to determine the quality of the tablets was as follows:

1. The submitted packet and blister of the tablets were scanned.
2. Two tablets from the blister pack were then pulverised separately and dissolved in solvent. The resulting mixture was then analysed using high performance liquid

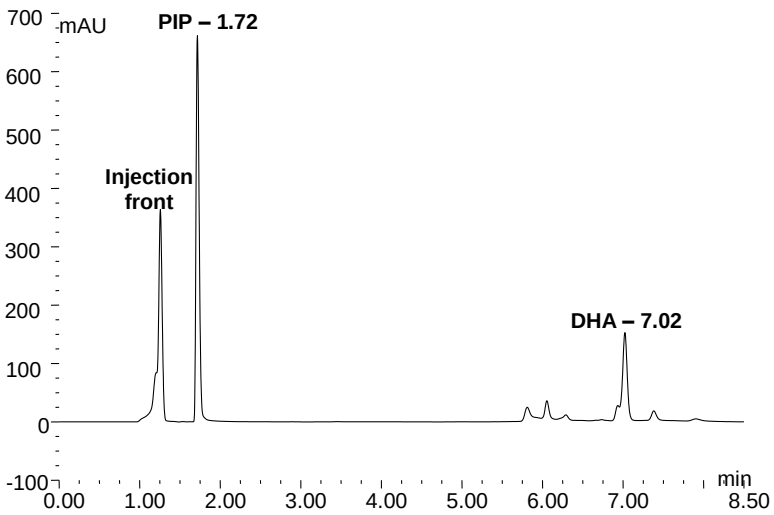
chromatography (HPLC) for amount of the active ingredients (dihydroartemisinin 40 mg + piperaquine 320 mg) in each tablet.

3. The active pharmaceutical ingredient (API) was expressed as a percentage of the expected amount, which should be within 90 -110% according to the United States Pharmacopiea (USP).

Results:

1. The scanned picture of the packet [manufacturing date: 22/01/2011; expiry date: 21/01/2014; Batch No: PX - 101] and blisters contained within are shown below.

2. The HPLC chromatogram achieved is shown below where PIP is piperaquine (1.72 min) and DHA is dihydroartemisinin (7.02 min).



3. The HPLC analysis gave the expected amount of active ingredients:
   Piperaquine – API =100%
   Dihydroartemisinin – API= 91.5 % which is within the 10% tolerance limits as accepted by USP and as this is an unstable ingredient and degrades soon after dissolution.

REPORT (second batch)

Drug samples (ARSUAMOON and P-ALAXIN) submitted by Mr Joseph Osarfo to test for quality.

C) Arsuamoon (artesunate and amodiaquine)

The process followed to determine the quality of the tablets was as follows:

4. The submitted packet and blister of the tablets were scanned.
5. Two tablets from the blister pack were then pulverised separately and dissolved in solvent. The resulting mixture was then analysed using high performance liquid chromatography (HPLC) for amount of the active ingredients (Artesunate 20 mg and Amodiaquine 150 mg) in the tablets.
6. The active pharmaceutical ingredient (API) was expressed as a percentage of the expected amount, which should be within 90 -110% according to the United States Pharmacopeia (USP).

Scans:

The scanned pictures of the packet [manufacturing date: 12/07/2011; expiry date: 11/07/2013; Batch No: LQ110715 A] and blisters contained within are shown below.

Results:

The HPLC analysis gave the expected amount of active pharmaceutical ingredients:
Amodiaquine – API = 100%
Artesunate – API = 103% which is within the acceptable limits by USP.

C) P-Alaxin (dihydroartemisinin plus piperaquine)

The process followed to determine the quality of the tablets was as follows:

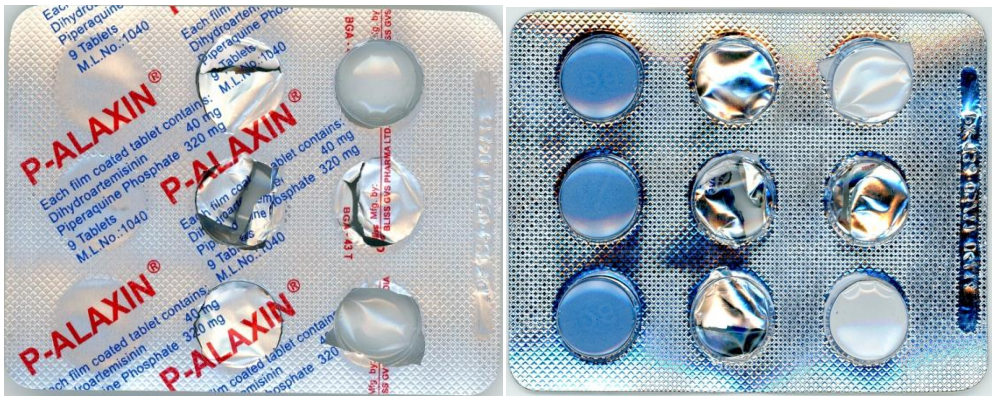
-100

4. The submitted packet and blister of the tablets were scanned.
5. Two tablets from the blister pack were then pulverised separately and dissolved in solvent. The resulting mixture was then analysed using high performance liquid chromatography (HPLC) for amount of the active ingredients (dihydroartemisinin 40 mg + piperaquine 320 mg) in each tablet.

6. The active pharmaceutical ingredient (API) was expressed as a percentage of the expected amount, which should be within 90 -110% according to the United States Pharmacopiea (USP).

Scans:

The scanned picture of the packet [manufacturing date: 05/2011; expiry date: 04/2014; Batch No: PX - 134] and blisters contained within are shown below.



Results:

The HPLC analysis gave the expected amount of active ingredients:

Piperaquine – API = 100%

Dihydroartemisinin – API= 104 % which is within the acceptable limits by USP.

General conclusion

The tablets of both Arsuamoon (artesunate and amodiaquine) and P-Alaxin (dihydroartemisinin plus piperaquine) contain the stated amounts of the active pharmaceutical ingredient and hence are of good quality.

June 2012