Safety, efficacy and pharmacokinetics profile of antimalarial drugs in pregnancy: pharmacoepidemiology studies

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Dominic Mosha

aus

Kilimanjaro, Tanzania

Basel, 2014

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

Prof. Dr. Marcel Tanner, Prof. Dr. Blaise Genton und Prof. Dr. Clara Menendez.

Basel, 25. März 2014

Prof. Dr. Jörg Schibler

Dekan

Contents

List of abbreviations

ACT	Artemisinin-based combination therapy
ACPR	Adequate Clinical Parasitological Response
AIDS	Acquired Immunodeficiency Syndrome
ADR	Adverse Drug Reaction
AL	Artemether-Lumefantrine
ALIVE	Artemether-Lumefantrine In Vulnerable patients: Exploring health impact
AM	Artemether
ARV	Antiretroviral drug
BMC	Bugando Medical Centre
CI	Confidence Interval
CRESIB	Barcelona Centre for International Health Research
CSA	Chondroitin Sulphate A
DHA	Dihydroartemisinin
DLF	Debutyl-lumefantrine
DP	Dihydroartemisinin – piperaquine
EDCTP	European and Developing Countries Trial Partnership
ETF	Early Treatment Failure
HC	Health Centre
HDSS	Health Demographic Surveillance System
IHI	Ifakara Health Institute
HIV	Human Immunodeficiency Virus
INESS	INDEPTH Effectiveness and Safety Studies of Antimalarial Drugs in Africa
IPTp	Intermittent Preventive Treatment for malaria in pregnancy
IUGR	Intrauterine Growth Retardation
ITNS	Insecticide Treated Bed Nets
IST	Intermittent Screening and Treatment
KCMC	Kilimanjaro Christian Medical Centre

KCMUCo	Kilimanjaro Christian Medical University College
LBW	Low Birth Weight
LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
LCF	Late Clinical Failure
LF	Lumefantrine
LOQ	Lower limit quantification
LPF	Late Parasitological Failure
MIP	Malaria in Pregnancy
MIC	Minimum Inhibitory Concentration
MPC	Minimum Parasiticidal Concentration
MSP	Merozoite Surface Protein
NONMEM	Nonlinear Mixed Effect Modeling
NIMR	National Institute for Medical Research
NNT	Number needed to treat
OR	Odds ratio
PCR	Polymerase Chain Reaction
Pf	*Plasmodium falciparum*
PD	Pharmacodynamic
PK	Pharmacokinetic
PV	Pharmacovigilance
RCH	Reproductive and Child Health
RR	Relative risk
SP	Sulfadoxine-Pyrimethamine
Swiss TPH	Swiss Tropical and Public Health Institute
TFDA	Tanzania Food and Drug Authority
US FDA	United State Food and Drug Authority
VSA	Variant Surface Antigen

UMC	Uppsala Monitoring Centre
WHO	World Health Organization
WWARN	Worldwide Antimalarial Research Resistance Network

Acknowledgement

First and foremost thanks goes to my almighty God, the pathway through this PhD thesis would have never been possible without his grace and blessings – praise and honor to your holiness forever amen.

There are many people I would like to express my sincere gratitude for their great role in supporting this work. First of all my sincere gratitude goes to my supervisor Prof. Blaise Genton for his superb academic and scientific support. Despite of residing far from Basel, you have always been there for me to ensure my work is moving successfully in the field, during analysis of samples in Lausanne and Swiss TPH laboratories as well as in the process of write-up. You have made me a true clinical epidemiologist. For that I say *Merci beaucoup.*

I would also like to thank Prof. Marcel Tanner, the director of Swiss TPH for being my faculty representative and to ensure all my financial and academic matters are settled accordingly, both in Basel and in Tanzania. Special thanks go to Christine Mensch and Doris Stamm our student administrators for making my life easier in Basel, Margrit Slaoui and Dagmar Batra for your remarkable managerial assistance, Prof Ingrid Felger and Felista Mwingira for your laboratory expertise. Many thanks to all my academic lecturers during course work, fellow students and friends in Basel. You all made my studies and my stay in Basel a happier moment. To all I say *Vielen Dank*

I acknowledge the support and knowledge I acquired from University of Lausanne, departments of Pharmaceutical Science, and pharmacology and toxicology under the support of Dr. Chantal Csajka, Laurent Decosterd, Thomas Mercier and Monia Guidi. The knowledge gained on bioassay of drugs level and pharmacokinetic analysis will be a valuable asset in my future carrier. All I say *Merci beaucoup.*

Special thanks to the funders of this PhD study, European and Developing Countries Trial Partnership through Malaria in Pregnancy Preventive Alternative Drugs (EDCTP MiPPAD: IP.2007.31080.002) project. I also register my sincere appreciation to the personnel of Barcelona Centre for International Health Research (CRESIB) namely Prof. Clara Menendez, Dr. Esperanca Sevene and Dr Golbahar Pahlavan for their support and corporation. All I say *Muchas gracias.*

This PhD carrier would not have been possible without a good will of Dr. Salim Abdulla, the director of Ifakara Health Institute (IHI) who offered me the opportunity. He understood my eager and determination to undertake PhD. Through his push and support, thus made possible for me to finish this PhD in a shorter period - I confess to say that I will carry such a noble memory for the rest of my life. I would also like to thank the support of Drs Rose Nathan, Godfrey Mbaruku, Mwaka Athuman, Abdunoor

Mulokozi, Honorati Masanja and Mr barnabas Lugendo. Many thanks goes to all the people who supported my field activities in Rufiji and Kigoma namely, Advocatus Kakorozya, Richard Amaro, Jackson Thomas, Happy Mkali, Festo Mazuguni, Sigilbert Mrema, Kahema Irema, Tabu Kiyenzi, Julieth Likata, Kusudi Mapande, Athumani Mzuyu, Fadhili Mwakitete, Bakari Kissa, Ismail Sajidu, Evance Ntilla, Christian Membi, Amri Shamte, Warren Temba, Nathan Gakwandi, Yusuph Msagati, Essau Gharika and Matrida Lihenye. To all I say *Asanteni sana.*

To all my closest friends and relatives who supported me from far in this journey; Edward Furaha and his family, Jeremia Buluba, Deo Mosha both in Dar es Salaam, Dr. Jackline Mosha in Mwanza, Richard Mosha in USA, Sofia Urassa and Avelina in Rombo, Duma Nairo in Kongwa, Dr. Jaff Chilongola and Alutu Masokoto in Moshi, Dr. Rabi Ndesarua in Muhimbili National Hospital, and all staff members of Community Health Department in KCMC Teaching Hospital between 2007 and 2010 – your concern, love, care, support and respect made me a different person today. I have nothing to offer you back but *Mungu awabariki na Kuwazidishia*.

I am grateful to my beloved wife Tabitha for your warm support and taking care of the family alone during my distant stay in the field sites and Basel. I'm greatly indebted to my son Francis and Janine my daughter who used to complain persistently whenever I was away for studies, especially at times when they need me most. Special thanks and dedication to my parents, my dad Prof. Frank Mosha and Mary my mum for their struggle and sacrifice to raise me as a child and schooling from kindergarten to this level. All I say *Ruwa nawatarame*

Summary

Background: Malaria in pregnancy is an important public health problem in sub Saharan Africa. It is known to be the most common and preventable cause of harmful birth outcomes in malaria endemic areas. It is therefore important for a pregnant woman to be treated with safe and effective antimalarial medication. Drug safety in pregnancy is of a greater concern due to limited safety data available in this vulnerable group. This is because pregnant women are not involved in clinical trials related to drug development process due to safety reasons and hence, most of these medicines come to market with limit information available about their safety in pregnancy. Hence, establishing a drug safety monitoring mechanism would be important to generate safety data when a given medicine is already in the market, especially medications against tropical diseases.

Pregnant women are at increased risk of malaria infection and illness than non-pregnant individuals due to physiological, hormonal and immunological changes that occur in their body after conception. The changes are also responsible for various therapeutic challenges that face this vulnerable group. This explains the presence of significant alteration of antimalarial pharmacokinetic (PK) properties in pregnancy and hence lead to a reduced drug blood concentration, which will ultimately lower antimalarial cure rate. Another factor that affects antimalarial effectiveness in pregnancy is parasite resistance against sulfadoxine-pyrimethamine (SP), a drug that is used for intermittent preventive treatment of malaria in pregnancy (IPTp).

The objectives of the thesis were to assess the magnitude of drugs exposure during pregnancy in relation to pregnancy outcomes, to describe the feasibility of establishing active pharmacovigilance system in developing countries using Health Demographic Surveillance System (HDSS) platform, to determine safety of artemether-lumefantrine (AL) exposure in first trimester of pregnancy, to evaluate pharmacokinetics and pharmacodynamics properties of artemether-lumefantrine in pregnant and non-pregnant women, and to determine the effectiveness of IPTp-SP in prevention of placental malaria, maternal anaemia and low birth weight in areas with different malaria transmission intensity.

Method: Three different study designs were used independently to respond to different specific objectives of this thesis; (i) a longitudinal follow up study was conducted to generate artemether/lumefantrine (AL) safety data in first trimester secondary to its inadvertent exposure in two Health Demographic Surveillance System (HDSS) areas in Tanzania. Pregnant women with gestational age ≤ 20 weeks were enrolled and followed up on monthly bases until delivery. Drugs exposures during the entire pregnancy period were also recorded. The latter was used to document the feasibility of

establishing active pharmacovigilance system using HDSS platform in one of the studied HDSS area. (ii) To determine AL PK, a prospective study involving pregnant in second and third trimester and non-pregnant women, both with uncomplicated *P falciparum* malaria. Plasma samples were collected at pre-defined dates for bioassay to determine drug level. Participants were followed up on pre-defined schedule visits until day 42. Inter- and intra-individual variability was assessed and covariated effects quantified using a nonlinear mixed-effect modeling approach (NONMEM®). (iii) Another prospective study enrolling pregnant women to assess the effectiveness of IPTp in two areas with different malaria transmission intensity. Pregnant women were recruited in the labor ward and structured questionnaire was used for interview. Placental parasitaemia was screened by using both light microscope and real-time quantitative PCR.

Findings

Pharmacovigilance system

91% (994 of 1089) of pregnant women who were piloted to assess feasibility of establishing active PV system completed the follow up until delivery. 98% of pregnant women reported to have taken at least one medication during pregnancy, mainly drugs provided in the antenatal program. Other most reported drugs were analgesics (24%), antibiotics (17%) and antimalarials (15%), excluding IPTp. Iron and folate supplementations were associated with decreased risk of miscarriage/stillbirth (OR 0.1; 0.08 – 0.3).

AL safety

82% (1783 of 2167) of pregnant women who used and not used antimalarial drugs in first trimester were followed until delivery and recorded their pregnancy outcome. 319 (17.9%) used antimalarial drugs in first trimester and AL was the most frequent antimalarial used [53.9% (172 of 319)]. Others were 24.4 % quinine, 20.7% SP and 3.4% amodiaquine. Quinine exposure in first trimester was associated with increased risk of miscarriage/stillbirth (OR 2.5; 1.3 – 5.1) and premature birth (OR 2.6; 1.3 – 5.3). AL, SP and amodiaquine exposure were found not to be harmful.

PK analysis

33 pregnant women and 22 non-pregnant women with malaria were treated with AL (80/480mg) twice daily for 3 days. Lumefantrine (LF) bioavailability and metabolism rate into desmethyl-lumefantrine were respectively 34% lower and 78% higher in pregnant than in non-pregnant patients. Overall PCR uncorrected therapeutic failure was 18% in pregnant and 5% in non-pregnant women (OR 4.0; p value

0.22). A higher median day 7 LF concentration was associated with adequate clinical and parasitological response.

Effectiveness of IPTp

350 pregnant women were recruited and screened for placental parasitaemia (175 each from high and low malaria transmission areas). Prevalence of placenta parasitaemia was 16.6% in high transmission area and 2.3% in low transmission area. One or more doses of IPTp in high transmission area had 80% impact against placental malaria (OR 0.2; CI 0.06 – 0.7; p=0.015) and 60% in low transmission (OR 0.4; CI 0.04 – 4.5; p=0.478). Primigravida and residing in high transmission area were significant risk factors for placental malaria (OR 2.4; CI 1.1 – 5.0) and (OR 9.4; CI 3.2 – 27.7), respectively. The numbers needed to treat (NNT) was 4 (CI 2 – 4) women in high transmission area and 33 (CI 20 – 50) low transmission area to prevent one placental malaria. IPTp use was not statistically significant associated with decreased risk of maternal anaemia or low birth weight, regardless are of transmission intensity.

Conclusion:

Overall medicine use in pregnancy period is very high, including AL exposure in first trimester albeit this drug is not the first line treatment for malaria in early pregnancy. AL use in first trimester was safer as opposed to quinine, the first line drug which was associated with adverse pregnancy outcomes. We therefore recommend to consider other options than quinine for standard antimalarial drug in first trimester, and AL could be the best one.

HDSS platforms represent a reliable and feasible support to build on a pharmacovigilance system to assess safety of drugs in pregnancy since it has proved to be feasible. We recommend that pharmaceutical companies and other global financial bodies should invest more on the establishment of active pharmacovigilance system in pregnancy in tropical developing countries. The latter will boost safety data pool of newly marketed medicines and anti-infective agents for treating different illnesses in pregnancy.

LF bioavailability is significantly lowered in pregnant women due to altered PK properties as opposed to non-pregnant women in the same area. This may be responsible for therapeutic failure among pregnant women secondary to the observed low post-treatment prophylaxis. We recommend to evaluate a modified treatment regimen of malaria in pregnancy.

Muhtasari

Utangulizi: Ugonjwa wa malaria kwa mama mjamzito ni tatizo kuu kwenye afya ya jamii hasa Africa kusini mwa jangwa la Sahara. Malaria ni miongoni mwa magonjwa yanayoweza kuzuilika. Ugonjwa huu unasababisha mazara makubwa sana kwa mtoto mchanga tokea akiwa tumboni kwa mama yake hasa sehemu zenye malaria kwa kiwango cha juu. Hivyo basi ni vema mama mjamzito atibiwe na dawa salama na zenye uwezo mkubwa wa kuangamiza vidudu vya malaria. Usalama wa dawa kwa mama mjamzito ni kitu chenye changamoto kubwa kutokana na uhaba wa takwimu muhimu za usalama wa dawa za malaria kwa wajawazito. Sababu kuu inatokana na mama wajawazito kutohusishwa kwenye majaribio ya dawa kipindi cha za mwanzoni pale ambapo dawa husika bado hazijapewa kibali cha kuingia sokoni kwa sababu ya kuhofia usalama wa kiafya hasa kwa mtoto aliyopo tumboni. Hilo linapelekea kwa dawa nyingi kuingia sokoni zikiwa na upungufu wa taarifa muhimu juu ya usalama wake kwa mama mjamzito. Kwa sababu hiyo, ni muhimu kuwa na mfumo wa kipekee wa kumfuatilia mama mjamzito pale atakapotumia dawa ambazo zipo tayari sokoni ili kuboresha taarifa za kiusalama kiafya kutokana na matumizi yake kipindi cha ujauzito.

Mama mjamzito anahatari kubwa ya kuambukizwa ugonjwa wa malaria pamoja na kuuguwa kuliko mama ambaye hana ujauzito. Hili linatokana na mabadiliko kipindi cha ujauzito ambayo yanasababishwa na kupunguwa kwa kinga ya mwili na mabadiliko ya homoni mwilini mwake. Mabadiliko haya yanachangia pia kuathiri ufanisi wa dawa mwilini kwake kupambana na vijidudu vya malaria na hivyo kupunguza uwezo wa uponyaji. Usugu wa dawa dhidi ya vijidudu vya malaria, kwa mfano dawa ya SP huchangia pia kuathiri uwezo wa kumponya mgonjwa wa malaria.

Dhumini kuu la utafiti huu ni (i) kujuwa wingi wa dawa anazotumia mama mjamzito ukilinganisha na matokeo ya mimba yake, (ii) kuonyesha uwezekano wa kuwa na mfumo pekee wa kudhibitisha matumizi ya dawa ambao utaweza kufuatilia usalama na matumizi ya dawa kwa ujumla kwa mama mjamzito, kwenye nchi inayoendelea kwa kutumia mfumo wa HDSS (Health Demographic Surveillance System), (iii) kuhakiki usalama wa matumizi ya dawa mseto (ALU) ya malaria kipindi cha mimba changa, (iv) kutathimini unyambulisho wa dawa ya mseto mwilini mwa mgonjwa sambamba na kulinganisha ufanisi wake wa kuangamiza vijidudu vya malaria, na (v) kutathimini ufanisi wa dawa ya SP ambayo mama mjamzito anapatiwa kliniki kama inasaidia kuangamiza vijidudu vya malari kwenye kondo la uzazi, kuzuia upungufu wa damu kwa mama na mtoto kutozaliwa na kilo pungufu kwenye maeneo yenye viwango tofauti vya maambukizo ya malaria.

Methodolojia: Njia tatu tofauti zilitumika kupata majibu husika ya malengo ya utafiti huu; (i) Kufuatilia mama wajawazito tokea kipindi cha mwanzo cha ujauzito wao hadi wanapojifungua na kurekodi taarifa za matumizi ya dawa (ikiwemo dawa mseto) na matokeo ya ujauzito. Zoezi hili lilifanyika kwenye vituo vya HDSS huko Rufiji na Kigoma mjini. (ii) Unyambulisho wa ufanisi wa dawa mseto uliwahusisha wanawake ambao ni wajawazito (wenye umri wa mimba kuanzia wiki 13 na kuendelea) na wale wasio wajawazito lakini wote wakiwa wametambulika hawana malaria kali. Walipewa dawa mseto na kutolewa damu kwa kipindi tofauti tofauti ndani ya siku 42 za kuwafuatilia ili kupima kiwango cha dawa kwenye damu na kuhakiki vijidudu vya malaria vinavyo angamia. (iii) Kuhakiki ufanisi wa SP kama kinga ya malaria kwa mama mjamzito (IPTp) ilihusisha kuwatambua akina mama wajawazito wakiwa kwenye hospitali mbili tofauti ambazo zipo kwenye maeneo yanye viwango tofauti vya uambukizaji wa malaria. Utambuzi wa akinamama hawa ulikuwa muda mfupi kabla hawajajifungua na ulihusisha kukusanya damu toka kwenye kondo la uzazi mara tu baada ya kujifungua na kupima kama kuna maambukizi ya vijidudu vya malaria.

Matokea: (i) Mfumo wa ukusanyaji taarifa ya matumizi ya dawa kipindi chote cha ujauzito. Asilimia 90 (994/1089) ya mama wajawazito waliweza kufuatiliwa mpaka walipo jifunguwa. Jumla ya 98% waliripoti kutumia walau aina moja ya dawa kipindi cha ujauzito, hasa zikiwa dawa zinazotolewa kwenye mpango maalumu wa mama na mtoto. Dawa nyingi zikiwa ni dawa za kuzuia maumivu (24%), antibayotiki (17%) na dawa za kutibu malaria (15%). Imeonekana dawa za kuongeza wingi wa dama zinahusiana na kupunguza hatari ya mimba kuharibika na mtoto kuzaliwa njiti.

(ii) Usalama wa dawa mseto: Jumla ya mama wajawazito 1783 kati ya 2167 (82%) waliyotumia na ambao hawajatumia dawa za malaria kipindi cha miezi mitatu ya mwanzo ya ujauzito walifuatiliwa na kurekodi matokeo yao ya ujauzito wao. 319 (17.9%) walitumia dawa za malaria kipindi hicho cha mwanzo cha ujauzito na kati ya hawa 53.9% walitumia dawa mseto. Wengine walitumia quinine (24.4%), SP (20.7%) na amodiaquine (3.4%). Matumizi ya quinine kipindi cha miezi mitatu ya mwanzo ya mimba yalihusishwa na kuharibika kwa mimba na kuzaa mtoto njiti. Dawa ya mseto, SP na amodiaquine zilionyesha kutokuwa na mathara yeyote.

(iii) Unyambulisho wa ufanisi ya dawa mseto: Utafiti huu ulihusisha wajawazito 33 na wanawake wasio wajawazito 22 waliyo na malaria na kutibiwa na dozi kamili ya dawa mseto mara mbili kutwa kwa siku 3. Sehemu ya dawa ya mseto ilionekana kuwa pungufu kwa wajawazito ukilinganisha na wale wasiyo wajawazito. Kwenye kipindi cha kuwafuatiliya wagonjwa (ndani ya siku 42), 18% ya wajawazito na 5% ya

wasiyo wajawazito waligundulika kuwa bado wana vijidudu vya malaria. Kuwa na kiwango kikubwa cha dawa ya mseto kwenye mzunguko wa damu ulihusishwa na kupona malaria kwa ufasaha.

(iv) Ufanisi wa SP kama kinga ya malaria kwa mama mjamzito. Jumla ya mama wajawazito 350 walihusiswa kwenye utafiti huu, 175 toka kila sehemu yenye malaria ya kwa kiwango cha juu na pia toka kwenye sehemu ya malaria kwa kiwango cha chini. Maambukizo ya malaria kwenye kondo la uzazi ilikuwa 16.6% kwenye eneo la malaria cha kiwango cha juu na 2.3% kwenye eneo lenye malaria kwa kiwango cha chini. Matumizi ya SP yalionyesha uwezekano wa kuzuia maambukizi ya kondo la uzazi hasa eneo lenye malaria ya juu. Kuwa na ujauzito wa kwanza na kuishi eneo lenye malaria ya juu ni kiambata hatarishi cha kupata maambukizo ya kondo la uzazi

Hitimisho: Kwa ujumla matumizi ya dawa kipindi cha ujauzito yapo kwenye kiwangu cha juu, ikiwemo matumizi ya dawa mseto kwenye kipindi cha mimba changa, japokuwa dawa hii siyo chaguo la kwanza kwenye tiba ya malaria kwenye kipindi hichi. Dawa mseto imeonekana kuwa salama zaidi kuliko quinine hivyo ni bora kuanza kufikiria jinsi itakavyoweza kupendekezwa kwa matumizi kipindi cha mimba changa.

Kupitia HDSS imeonyesha inaweza kusaidia kuwa na mfumo wa uhakika na kuaminika wa kukusanya taarifa muhimu za matumizi ya dawa kwa mama mjamzito kwenye nchi masikini. Hivyo ni bora makampuni ya dawa, wafadhili kwa kushirikiana na taasisi za afya ndani na nje ya nchi wafikirie jinsi ya kufadhili mfumo huu ili kusaidia kuboresha takwimu za usalama wa dawa kwa mama wajawazito.

Imethibitika kuwa dawa mseto inapunguwa kwa kiasi kikubwa mwilini mwa mwanamke mjamzito ukilinganisha na mwanamke asiyo mjamzito. Hili huenda ikapelekea mama mjamzito kutopona kwa ufasaa na kupungukiwa uwezekano wa kukabiliyana na maambukizo mapya ya malaria kipindi cha usoni hasa baada ya kumaliza dozi ya malaria. Hivyo tunapendekeza kupitiwa upya dozi ya malaria inayotumika sasa na mama mjamzito na kushauri upatikanaji wa dozi mpya kwa hili kundi la wajawazito.

PART I: BACKGROUND

Chapter 1: Introduction

1.0 Burden of malaria in pregnancy

Globally, about 125 million pregnancies are at risk of malaria every year and 32 million are in Africa [1], a continent which bears 90% of the world's burden of malaria [2]. It is estimated that sub-Saharan Africa has as many as 10,000 malaria related deaths which occur every year in pregnant women and mainly secondary to maternal anaemia [3]. Other substantial direct risks of malaria in pregnancy (MIP) include severe maternal anaemia and puerperal sepsis, and those affecting the baby are intra-uterine growth retardation, intrauterine death, stillbirth, premature delivery, low birth-weight, perinatal and neonatal morbidity and mortality [2]. The latter suggest that malaria has severe consequences to both mother and fetus. *Plasmodium falciparum* is the predominant malaria specie in sub-Sahara and carries the biggest burden compared to any other malaria specie [4]. Despite of malaria being one of the most common and preventable cause for adverse pregnancy and birth outcomes, it continues to be a major public health problem in malaria endemic countries [5].

Pregnant women have a higher risk of malaria compared to non-pregnant adults due to physiological, hormonal and immunological changes during pregnancy [6]. Hormonal change is explained by elevation of cortisol levels which is associated with increased risk of malaria in pregnant women [7]. Increased attractiveness of a mosquito to pregnant woman is explained by both physiological and behavioral changes. Increased abdominal temperature and exhaled breathing may explain why pregnant women are more easily detected by mosquitoes compared to non-pregnant adults. Physiological changes during pregnancy are the reason why pregnant women urinate twice as frequent as non-pregnant women. The latter exposes more pregnant women to mosquitoes bites at night when they leave their bed nets to the toilet [8, 9], and it is even of a greater concern in most of rural areas whereby toilets are usually located outside the main house building. Parasite densities have been reported to be higher in pregnant women than in non-pregnant adults and this may suggest that the ability to suppress parasite replication is impaired in pregnancy [10].

There are maternal factors which are associated with the risk of malaria in pregnancy and include maternal age, parity and gestational age. Younger women particularly adolescents are at higher malaria risk than older women, regardless of their gestational age or parity. Primigravidae are at higher risk than multigravidae and it is more obvious in high malaria transmission areas. Immunological differences explain the mentioned malaria risks [11, 12]. Malaria risk increases with the increase of gestational age

and reports shows that the risk starts to peak from second trimester. However, risk for malaria infection in relation to a given gestational age is still questionable because of limitations for collecting maternal related information in first trimester which is explained by most pregnant women tend to start antenatal clinic from second trimester [13]. HIV infection, one of the infections which carry the biggest burden in sub-Sahara Africa in terms of morbidity and mortality [14], is associated with increased risk of malaria in pregnancy. Regardless of the parity or gestational age, HIV infected pregnant women have a higher and severe risk for malaria infection than non-pregnant women [15, 16]. HIV infection suppresses the ability of a pregnant woman to control *P falciparum* infection [17].

1.1 Malaria pathophysiology and immunology in pregnancy

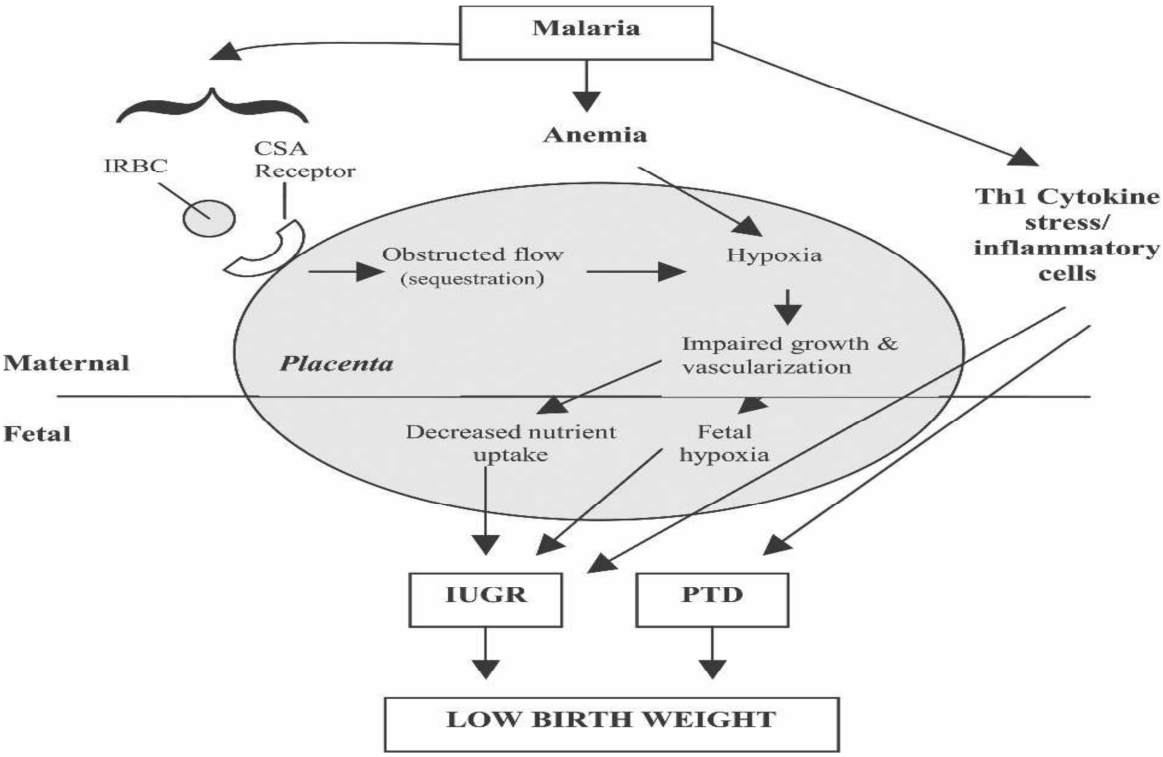
Adults including pregnant women in high and moderate malaria transmission areas acquire immunity against malaria. The acquired immunity enables them to control malaria infection but not to clear it, regardless the immunological maternal variations which are observed in pregnancy [6]. The level of acquired immunity which is determined by the degree of transmission intensity to which an individual is residing also has an impact on malaria presentation (signs and symptoms) and pregnancy outcome [18]. The latter explains why pregnant women in stable transmission areas often have asymptomatic malaria infection, lower prevalence of peripheral parasitaemia, higher prevalence of placenta parasitaemia and higher prevalence of maternal anaemia as opposed to those in unstable transmission areas [18]. Hence, immunological variation which is challenged in pregnancy can better explain differences in malaria presentation patterns and its related adverse outcomes.

Immunological change induced by pregnancy is one of the most important factors that justifies why pregnant women are at increased risk for malaria than non-pregnant women. Infected erythrocytes express a unique variant surface antigen (VSA) which mediate adhere to chondroitin sulphate A (CSA) receptor in the placenta endothelia and sequester in the intervillous spaces. This unique VSA rarely binds to the two commonly described receptors (CD36 and intercellular adhesion molecule [ICAM-1]) in non-pregnant individuals. Sequestration leads to obstructed blood flow and hence placenta insufficient which is due to inflammatory reaction to the infected erythrocytes. The reaction causes vasoconstriction and vascular damage which jeopardize haemodynamics of placenta [10, 19]. The expressed VSAs in pregnancy are different from those expressed in non-pregnant individuals and are not recognized by the immunity system in stable malaria transmission settings. The binding of the VSA with CSA (VAR2CSA) has been associated with *P falciparum* in pregnancy [20]. Anti-VAR2CSA specific IgGs are only found in women and its levels increases with parity. The latter suggest why primigradividae are more at risk for

malaria than multigravidae [21]. Anti-VAR2CSA are associated with favourable pregnancy outcome and explain why malaria risk decreases with increase of parity [20, 21]. There are cytokines to respond to VSA in pregnant women with malaria and include Th1, Th2, TNF, IFN gamma, interleukins and monocytes [6, 22, 23].

Intrauterine growth retardation (IUGR) and preterm delivery are important cause of low birth weight (LBW) secondary to malaria in pregnancy. LBW due to IUGR is associated with maternal anaemia and elevation of cytokines levels. This is explained by the observed reduced foetal circulation and placenta insufficient secondary to chronic malaria infection [24]. Preterm delivery have been associated with acute infection and high parasitaemia which may explain the presence of fever, anaemia and elevation of TNF alpha and interleukin 10 [25, 26]. *Figure 1.1* by Rogerson SJ *et al.,* [10] summarizes potential pathological mechanisms in which malaria affects placenta function and leads to preterm delivery and IUGR.

Figure1.1: Potential pathological mechanism associates malaria in pregnancy and low birth weight



IRBC = Infected red blood cell; CSA = Chondroitin sulfate A; IUGR = Intrauterine growth retardation; PTD = preterm delivery

1.2 Malaria control in pregnancy

Malaria in pregnancy should be control by effective gears because it is responsible for a wider range of adverse effects to pregnant woman, foetus and a newborn. World Health Organization (WHO) currently recommends a package of malaria control interventions during pregnancy and including use of insecticide-treated nets (ITNs), intermittent preventive treatment (IPT) and effective case management of malaria illness and anaemia [27].

1.2.1 Insecticide-treated nets (ITNs)

ITNs prevent malaria by reducing physical human-vector contact through excluding mosquito vector from feeding on human blood and may even be killed if it lands on an insecticide treated ITN [28, 29]. The ITNs use in pregnancy is associated with 23% reduction of placental malaria, 33% reduction of miscarriage or stillbirth and 23% reduction of low birth weight prevalence, according to a systematic review of ITNs randomized controlled trials [30]. It is important to consider that ITNs use in pregnancy is now into policy in 47 sub-Saharan countries including Tanzania [31].

1.2.2 Intermittent preventive treatment for malaria in pregnancy (IPTp)

Using an effective antimalarial for IPTp is considered by the WHO as the most effective preventive approach for malaria in pregnancy in areas with stable *P. falciparum* transmission. Sulfadoxine-pyrimethamine (SP) is still recommended to date as an effective and safer antimalarial for IPTp [27]. Recent WHO recommendations regarding IPTp-SP suggests the following: "the first IPTp-SP dose should be administered as early as possible during 2nd trimester of gestation; each SP dose should be given at least 1 month apart from the other and up to the time of delivery; the last dose of IPTp-SP can be administered late (after 36 weeks) in the 3rd trimester of gestation without safety concerns; IPTp should be administered as direct observed therapy (DOT); SP can be given on an empty stomach; folic acid at a daily dose equal or above 5 mg should not be given concomitantly with SP as this counteracts its efficacy as an antimalarial; and SP is contraindicated in women receiving cotrimoxazole prophylaxis" [32].

There is a growing concern with the increase *P falciparum* resistant to SP which might further jeopardize importance of IPTp-SP. A study in Tanzania in an area with widespread SP resistant of more than 50% have associated IPTp-SP with exacerbation of malaria infection, and it was also observed not to improve an overall pregnancy outcome [33]. However, there are strong evidence which still support the benefits of IPTp-SP even in areas with high SP resistance because it is reported that effectiveness of SP increases with the increase of IPTp-SP doses i.e. two, three or more doses [34]. This has raise awareness that

there is a need to find a new antimalarial which is safer and more efficacious than SP for IPTp. The drug should have a feasible regimen to be administered in antenatal care services as it is the case in SP.

1.2.3 Effective case management of malaria illness

Malaria case management is an essential component of malaria control in pregnancy and aiming at complete clearing of the infection. Any level of malaria parasites may have an effect on mother and foetus. However, effective case management should be preceded by proper diagnosis of malaria (including parasitological confirmation) to reduce unnecessary antimalarial exposure to the mother and her developing foetus [27]. Treating malaria in pregnancy is a challenging issue when comes to selection of an appropriate drug that would ensure safety of both mother and the foetus.

There is limited drug safety information in pregnancy which is due to lack of evidence-based data. The latter is because pregnant women are not involved in clinical trials related to product development and hence safety information comes from animal studies or from inadvertent exposed pregnancies following product approval [35]. The scarcity of drug safety information in pregnancy is more serious for antimalarials and drugs for treating tropical diseases because these diseases occur mostly in developing countries where there is poor medical record system and drug exposure registry system in pregnant women does not exist [36].

When treating malaria during pregnancy, it is essential to know the gestational age of the woman to determine appropriate antimalarial of choice and not just rely on severity as it is the case in non-pregnant adults. Most antimalarials are not recommended during first trimester due to safety reasons as it is when organogenesis mainly takes place in a developing foetus and hence makes it the most at risk period for teratogenicity. The safety concern in first trimester is due to insufficient safety information of most antimalarial drugs in this early pregnancy stage. Antimalarial which are considered to be safe in first trimester namely, quinine, chloroquine, clindermycin and proguanil [37].

WHO recommends that pregnant women with uncomplicated falciparum malaria in first trimester should be treated with quinine plus clindamycin for seven days or quinine monotherapy if clindamycin is not available. If the latter fails then artesunate plus clindamycin for seven days is indicated. ACTs are indicated if this is the only treatment immediately available, or if treatment with seven days quinine plus clindamycin fails, or if there is uncertainty about patient compliance with a seven days treatment. However, artemisinins are considered safe in second and third trimester and hence ACTs are the treatment of choice from second trimester of pregnancy [37].

Clindamycin is mostly not available for treating malaria in most of sub-Saharan countries including Tanzania due to its high cost. Hence, quinine monotherapy is frequently being prescribed as the drug of choice for treating malaria in first trimester [38]. There is growing evidence from several observational studies to support artemisinins safety in first trimester [39, 40]. Furthermore, there is strong evidence showing effectiveness and tolerability of AL in pregnancy [41] which might outweigh the advantages of recommended quinine use for treating uncomplicated malaria in first trimester. Below are some of the important reasons which might suggest artemisinins compounds to be considered as first drug of choice in early pregnancy for treating uncomplicated malaria and not quinine:

- Although quinine is believed to be safe in pregnancy, the three-times daily 7-day regimen is not ideal because of the adverse events or dosage lead to poor compliance [41, 42]. ACTs such as artemether-lumefantrine (AL) has a shorter regimen of two-times daily for three days with less to no adverse effects.
- The taste of most ACTs is preferred compared to the bitter taste of oral quinine. This increases tolerability and acceptability of ACTs among the users.
- Quinine, particularly parenteral administration, is associated with hypoglacaemia by stimulating insulin secretion [43].Quinine is associated with premature uterine contraction (commonly seen in drug overdose) and hence may lead to premature labour or abortion [44, 45]. Premature labour secondary to artemisinin overdose has not been reported.
- Quinine is a monotherapy and like many other antimalarial monotherapy drugs, it is more susceptible to develop resistance against *Plasmodium* species as it is the case in South-Eastern Asia countries [46, 47]. Quinine should therefore be restricted for treating severe cases of malaria only.

Pregnant women as an important vulnerable group for malaria infection need to be treated with an effective antimalarial. Unfortunately, pregnancy has a tendency of affecting the efficacy of most of the antimalarial drugs by reducing drug absorption, more rapid drug clearance and larger body fluid volume for a drug to distribute following physiological changes in pregnancy [48]. It thus, explain thesignificant alteration in pharmacokinetic (PK) properties of most of antimalarial drugs during pregnancy which leads to reduced drug blood concentrations and lower cure rates in pregnancy, especially in advanced pregnancy. Population PK study conducted in Thai pregnant women treated with AL reported that 40% of 103 women in the study had low lumefantrine capillary plasma concentration at day 7 which had been associated with an increased risk of therapeutic failure in non-pregnant patients [49]. Recent

population PK study of artesunate and dihydroartemisinin (DHA) in pregnant and non-pregnant women with malaria reported that pregnant women have accelerated DHA clearance compared to non-pregnant women receiving orally administered artesunate [50]. Low drug blood concentration in pregnancy explains why pregnant women tend to have higher treatment failure rates compared to non-pregnant adults living in the same area [51]. Therefore, there is a need to assess PK properties of antimalarial drugs during pregnancy, particularly AL and to determine its optimum dose regimen in pregnancy.

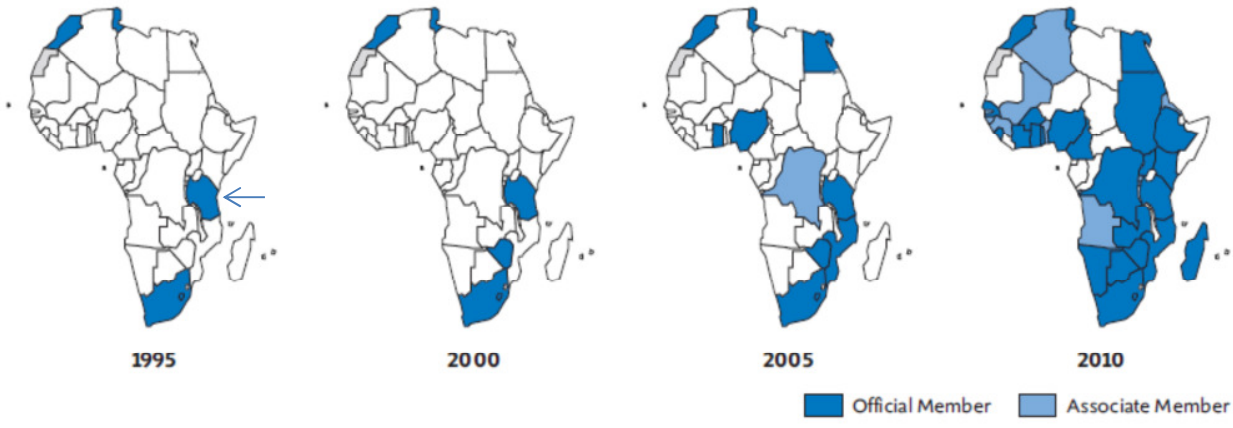
1.3 Pharmacovigilance and antimalarial

Pharmacovigilance (PV) is defined as the "science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problem". It aims at insuring client or patient receives safe medicines. There are two major systems in PV which are (i) passive PV or spontaneous reporting and (ii) active PV or cohort event monitoring. Passive PV means there is no active measures taken to look for adverse drug effects other than encouragement of health care professionals to report safety concerns. It is the most common method of surveillance which is easy to establish and cheap to run. The reporting rate is very low and subjected to strong biases. There is no database of all users or information on utilization of a drug and therefore is subjected to lack of accuracy in risk assessment. Active safety surveillance means that active measures are taken to detect adverse events through active follow-up after treatment and the events are detected by asking directly to the patients or screening patient's records. It is sometimes very descriptive with more reliable information. It has minimal biases as opposed to passive PV but may be expensive to operate [52].

Since the disaster of thalidomide teratogenicity reported in 1961, there has been a continuous system developmentfor detecting previously unknown or poorly understood adverse effects of medicines [53]. Currently, WHO program for International Drug Monitoring has been established whereby systems are in place in WHO member states for collecting individual case safety reports for evaluation. The reports are sent to national drug regulatory authories and forwarded to Uppsala Monitoring Centre (UMC) in Sweden, a WHO collaborating centre for international drug monitoring. Until the end of 2010, there were 136 countries participating in this program including Tanzania. Unfortunately, less than 27% of low- and middle-income countries have national pharmacovigilance systems registered with the WHO program compared with 96% of the high income countries [54]. It is therefore important to address challenges such as lack of resources, infrastructures and expertise which have been reported to be the obstacles for having safety drug monitoring system in developing countries [55].

In Africa, the number of countries with good PV capacity has increased from 5 in 2000 to 23 by the end of 2010 [*Figure 1.2*]. The increased capacity of PV in Africa is due to different reasons to meet specific targets. For example, focus on HIV/AIDS, malaria and tuberculosis has influenced the growth of PV in African countries due to demand for greater transparency, accountability and accessibility of the information [54]. Several countries in this region have taken advantage of having high disease burdens and co-morbidities to establish the frameworks for systematic capture, evaluating and reacting to PV information. For example, Senegal and South Africa have tried to report PV of antimalarials in a specific geographical area from the established cohort studies. These studies managed to report adverse effects of different antimalarial in the population and were supported by their National Malaria Control Program [56-58]. Other studies have gone further in PV by trying to assess safety of antimalarial exposure in first trimester of pregnancy, particularly artemether-lumefantrine (AL). Zambia conducted cohort event monitoring and Senegal analyzed medical database in the health facility [40, 59]. Both of the two studies aim at extending marginal safety of ACTs in this vulnerable group. Antimalarial clinical trials have also been conducted in different sub Saharan countries with successful outcomes. All these justifies that sub Saharan Africa have an important role in monitoring drug safety, particularly newly antimalarial drugs because of the greater exposure group in this region and hence, it is important to enhance their capacity to carry effectively and successively PV studies at facility and national level.

Figure 1.2*:* Growth of pharmacovigilance in Africa between 1995 and 2010



The arrow in the first left map of Africa points to Tanzania in shadow

Source: The world medicines situation 2011: WHO report [54]

1.3.1 Pharmacovigilance in Tanzania

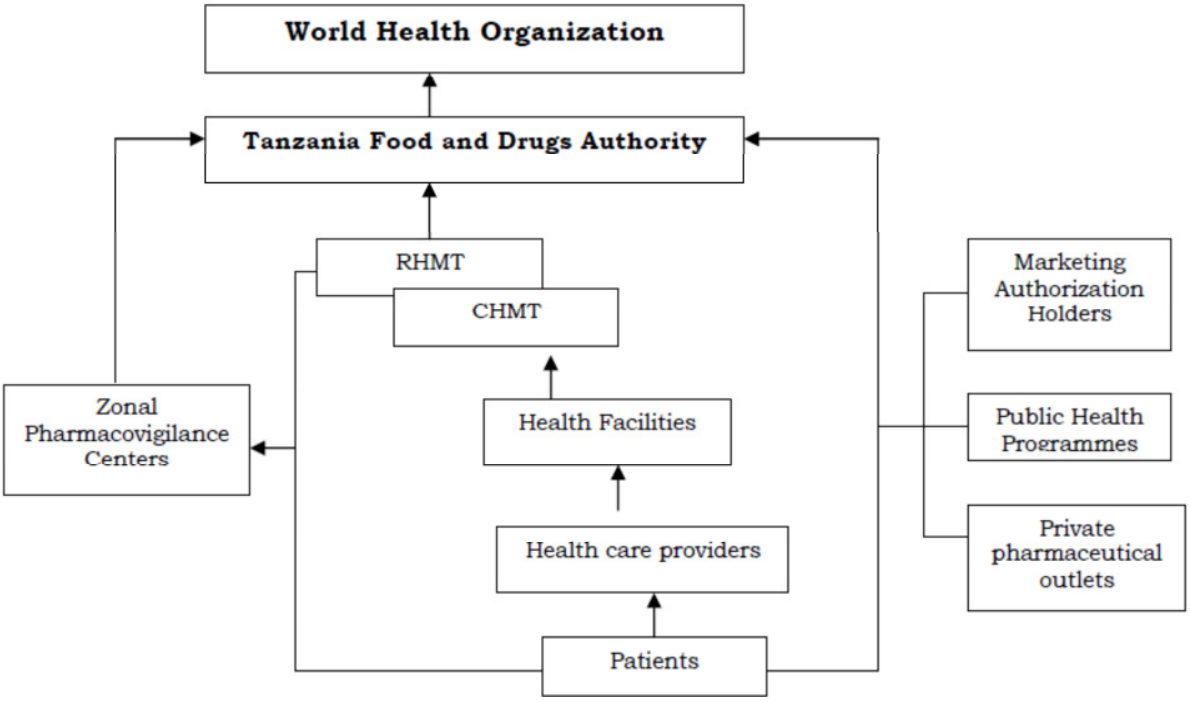
Tanzania has a national health policy which emphasize the need for raising awareness on reporting adverse drug reaction (ADR) at all levels of services delivery [*Figure 1.3*]. PV activities in Tanzania started way back in 1989 and the country joined WHO Program for International Drug Monitoring in 1993 and making Tanzania one of the earliest countries in Africa joining this program [*Figure 1.2*]. PV system is being coordinated and strengthened by the Tanzania Food and Drugs Authority (TFDA). TFDA operates in four zonal PV centers which are geographical distributed in the country namely, Kilimanjaro Christian Medical Centre (KCMC) in Kilimanjaro, Muhimbili National Hospital (MNH) in Dar es Salaam, Bugando Medical Centre (BMC) in Mwanza and Mbeya Medical Centre (MMC) in Mbeya. The spontaneous reporting system has mainly used specifically designed forms (Yellow forms) to collect adverse events data from patients. However, TFDA acknowledges that very few reports have been received to its office since the inception of the PV system in the country [60].

Reports on ADR of antimalarial drugs have been collected and received by the TFDA. In 2009, TFDA analyzed antimalarial ADR reports collected from 2006 to 2008 involving two medicines i.e. AL and sulpha containing antimalarial agents (SP). Although the general reporting rate was very low, there were a total of 18 cases of ADR reported which included 12 to AL (Coartem), 2 to Metakelfin (Sulfamethoxazole-pyrimethamine), and 4 Fansidar(Sulfadoxine-pyrimethamine) [61]. The latter suggests that there may be many more antimalarial ADR which are either not captured or not reported to TFDA which is the biggest challenge of spontaneous reporting system. Tanzania like many other resources-limited setting countries has limited access to healthcare facilities, high illiteracy rates, high drugs prescription from informal market, poor medical record keeping, shortage of qualified health personnel and lack of awareness among health healthcare professional on the importance of reporting suspected ADR. All these are important responsible factors that limit successful implementation PV system in the country [62].

ALIVE (Artemether-Lumefantrine In Vulnerable patients: Exploring health impact) project was one of the biggest antimalarial cohort event monitoring conducted in the country for about three years duration. The study was carried in rural Health Demographic Surveillance System (HDSS) area and testified that medicine safety monitoring and reporting is possible in settings with weak health infrastructure by regular and appropriate training of healthcare providers [62]. In 2009, INDEPTH Effectiveness and Safety Studies of Antimalarial Drugs in Africa (INESS) project started to operate in Tanzania and Ghana. An important goal of the project was to enable African researchers to carry out phase IV malaria drug

studies. This project contributed substantially on national and global safety information of ACT use. Therefore, these are all potential opportunities and possibilities to reinforce PV systems in Tanzania with great focus in vulnerable groups, especially in pregnant women who are always excluded in clinical trials involving unlicensed products.

Figure 1.3: Flow chart of adverse drug reaction information in Tanzania



RHMT = Regional Health Management Team; CHMT = Council Health Management Team

Source: National Guideline for Monitoring Medicine Safety – TFDA: Ministry of Health [60]

Chapter 2: Rationale

Malaria treatment in pregnancy has special challenges related to safety and efficacy issues which do not commonly present in non-pregnant individuals. This might be explained by limited drug safety and efficacy information in pregnancy because pregnant women are always excluded in clinical trials. In most cases, drugs are commercialized with little knowledge available regarding their safety in pregnancy which makes evidence based risk-benefit decision another important issue in this vulnerable group.

There is a growing concern of increased levels of drug use in most sub Saharan countries where over the counter prescription is commonly practiced. Most of these drugs are poorly regulated and become a big problem when contraindicated drugs are administered to a pregnant women or women of child bearing age. For this reason, it is important to know the actual prevalence, type and safety of drug use in pregnancy since there is no active mechanism of monitoring pregnancy exposure in relation to pregnancy outcome in almost all developing countries. This is the objective of chapter five where the magnitude of drug used during pregnancy is assessed and the feasibility to establish active PV system for monitoring drug exposure among pregnant women in resource-limited setting is described, piloting HDSS platform as a potential model.

Although ACTs are not being recommended in first trimester because of associated embryo-foetal toxicity in animals, there are at least 600 reported exposures to artemisinins in pregnant women during first trimester with no reported maternal or birth adverse outcomes. The number of women exposed to artemisinins in first trimester in published safety studies is not sufficient enough to reassure about their safety. The current unprecedented ACT roll-out at an affordable price makes inadvertent exposure to ACT, particularly AL, inevitable in early pregnancy. Furthermore, most women use antimalarials without knowing that they are pregnant. Acquiring medicines from drug venders without formal physician consultation and screening for illnesses is indeed a common practice in most African countries. It is therefore important to take advantage of the above experiences to increase the safety data pool information of ACTs in first trimester. In chapter six, the magnitude of inadvertent AL exposure in first trimester is evaluated in comparison to other groups of antimalarial drugs, including quinine which is the only antimalarial drug regarded to be safe in first trimester. Safety of AL and other groups of antimalarial exposure in first trimester are also described in relation to pregnancy outcomes.

Efficacy of antimalarial in pregnancy is challenged by the altered physiological changes which occur after a woman had conceived. These changes have been associated with altered PK properties of medicines, including antimalarial and hence, affect the therapeutic outcome. The latter have raised questions of

whether there is a need to adopt a new AL regimen for treating malaria in pregnancy. Chapter seven described PK of AL used to treat pregnant and non-pregnant women with uncomplicated *P falciparum* malaria. The evaluated drug levels in the two study groups are compared with therapeutic outcome.

Malaria in pregnancy is known to have deleterious consequences on the placenta, which leads to multiple adverse maternal and newborns outcomes. IPTp-SP has universally been implemented in malaria endemic countries so as to overcome this burden. However, the effectiveness of IPTp regimen is jeopardized by SP resistance and its advantage is not well documented in areas with low malaria transmission. This is evaluated in chapter eight based on findings from two areas with different transmission intensity.

PART II: OBJECTIVES AND METHODOLOGY

Chapter 3: Goal and objectives

Goals

To upsurge safety and efficacy information of antimalarial drugs used in pregnancy

General objective

To determine the safety, efficacy and pharmacokinetic profile of antimalarial drugs used for treating and preventing malaria in pregnancy

Specific objectives

i. To assess the magnitude of medicines exposure during pregnancy in relation to pregnancy outcome *[Chapter 5]*
ii. To described the feasibility of establishing active pharmacovigilance registry system in developing country using Health Demographic Surveillance System (HDSS) platform [*Chapter 5*]
iii. To determine the safety of artemether-lumefantrine exposure in first trimester of pregnancy *[Chapter 6]*
iv. To evaluate pharmacokinetics and pharmacodynamics properties of artemether-lumefantrine in pregnant and non-pregnant women *[Chapter 7]*
v. To determine risk factors associated with placental malaria, maternal anaemia and low birth weight in areas with different malaria transmission intensity *[Chapter 8]*
vi. To determine the effectiveness of IPTp-SP in preventing placental malaria, maternal anaemia and low birth weight in areas with different malaria transmission intensity *[Chapter 8]*

CHAPTER 4: Methods

4.1 Thesis structure

The thesis highlights the measures for malaria control in pregnancy in two main areas: (i) ensuring effective case management and (ii) preventing deleterious consequences of malaria on maternal and baby outcomes with intermittent preventive treatment.

Data used in this thesis came from two main research projects. The first project with the title 'safety, efficacy and PK profile of antimalarial drugs in pregnancy' was carried in Rufiji and Kigoma urban district in Tanzania mainland. The project started in April 2012 and enrolled prospectively pregnant women who did use and not use antimalarial in first trimester, with active follow-up until delivery. The project is still ongoing collecting developmental milestone information of the newborns delivered by the enrolled study women. The newborn follow up until the age of 12 months is expected to be completed by the end of October 2014 and will be an adjunct to this thesis. AL PK bioassays were part of an ancillary study conducted in Rufiji involving two groups, pregnant women and non-pregnant women as a comparative group. The second project titled 'effectiveness of IPTp against placental malaria' was conducted in two areas which have different malaria transmission intensity (Moshi urban with low transmission and Rufiji rural with moderate to high transmission), beginning in July 2012 and ended up in September 2012. When the latter study was conducted, the suggested recommendations of monthly IPTp-SP regimen from early second trimester [32] was not implemented. Figure 4.1 and 4.2 shows study area and malaria transmission intensity in Tanzania.

4.2 Study design and population

4.2.1 Monitoring of antimalarial safety in pregnancy

Antimalarial safety was assessed in pregnant women having gestational age below 20 weeks, between April 2012 and March 2013. The study was conducted in two areas (i) Rufiji HDSS in Rufiji district and (ii) Kigoma HDSS in Kigoma urban district. Women were followed until delivery to determine pregnancy outcome. Following newborns until the age of 12 months is still ongoing. The primary target of the study was to assess safety of different antimalarial exposure in first trimester in relation to pregnancy and baby outcomes. Ancillary study was also conducted in Rufiji to evaluate the feasibility of establishing PV system in resource-limited setting by piloting a pregnancy exposure registry in Rufiji HDSS as an example. Drug exposure history was gathered by interviewing pregnant women under surveillance and the given information was verified by reviewing medical registries, patient's RCH cards and personal patient's medical log. All women were followed up on monthly basis at Reproductive and Child Health

(RCH) clinic and at home through house visits. Delivered babies were carefully screened for any physical presence of congenital anomalies. Assessment details are presented in chapter five and six. Primary endpoints included pregnancy outcome (miscarriage, stillbirth or live birth) and baby outcome (birth weight and maturity status).

4.2.2 Population pharmacokinetics and pharmacodynamics of artemether-lumefantrine

PK and PD were assessed in pregnant and non-pregnant malaria patients recruited in Kibiti Health Centre within Rufiji HDSS between April and September 2012. Main inclusion criteria included adults above 18 years, all females patients diagnosed to have uncomplicated *P falciparum*, second and third trimester for pregnant women and no history of chronic illness. Enrolled patients received four tablets of AL (Coartem® Novartis Pharma AG, Basel; 20 mg AM and 120 mg LF) for 3 days at 0, 8, 24, 36, 48 and 60 hour under direct observation. To determine AL plasma levels and their metabolites, 2 ml of venous blood sample was drawn from the patient for bioassay at random times between 8 and 11 am on day 0, 1, 2, 3 and 7. To estimate the parasite density and clearance rate, capillary blood from a finger prick was taken at day 0, 3, 7, 14, 28 and 42 for microscopic screening and DNA genotyping. In chapter seven, PK and therapeutic outcome evaluation are described in detail.

4.2.3 Effectiveness of IPTp on placental malaria and pregnancy-associated malaria morbidity

Prevalence of placental malaria and associated malaria morbidities in pregnancy were assessed in two areas with different malaria transmission intensities i.e Moshi urban having low transmission and Rufiji having high to moderate transmission intensity. The study used a prospective design with enrolment of pregnant women who came for delivery in these facilities, between July and October 2012. Important obstetrics history was recorded including episodes of malarial illness and use of SP for IPTp during the current pregnancy. Information recorded was verified and supplemented by reviewing patient's medical registries and RCH card. Placental blood sample was collected on a blood slide and onto filter paper within one hour post-delivery. Collected samples were screened for presence of malaria parasite using light microscope and real-time quantitative Polymerase Chain Reaction (PCR). Parameters assessed included placental malaria, maternal age, parity, maternal anaemia, residency, history of malaria illness in pregnancy, ITN use, haematemics use, deworming and birth outcome (live birth or stillbirth). Details of this evaluation are provided in chapter 8.

4.2 Statistical methods

The study findings are mainly analytical, describing odds ratio and confidence interval of the associated adverse pregnancy outcome in relation to drug exposure. Magnitudes of various parameters of

therapeutic outcomes are presented in percentage, mean and median. STATA® 12.0 (Stata Corporation, College Station, Texas, USA) was used for data analysis. For PK study, Inter- and intra-individual variability was assessed and covariates effects quantified using a nonlinear mixed-effect modeling approach (NONMEM®). Details of specific statistical analytical methods applied in the thesis are presented in their respective chapters (chapter 5, 6, 7 and 8).

4.3 Ethical consideration

The studies were performed according to the WHO guidelines for (i) assessing exposure to antimalarial drugs in clinical field studies, and (ii) methods for surveillance of antimalarial drug efficacy [63, 64]. The study protocol was approved by the review board of Swiss TPH, the Ifakara Health Institute (IHI) ethical internal review board and ethical committee of National Institute of Medical Research (NIMR) in Tanzania. IPTp protocol was approved by Kilimanjaro Christian Medical University College (KCMUCo) research ethics committee which is recognized by NIMR. All participants signed voluntarily an informed consent prior to enrolment after reading and explained the procedures of the study.

Figure 4.1: Map of Tanzania showing the study sites

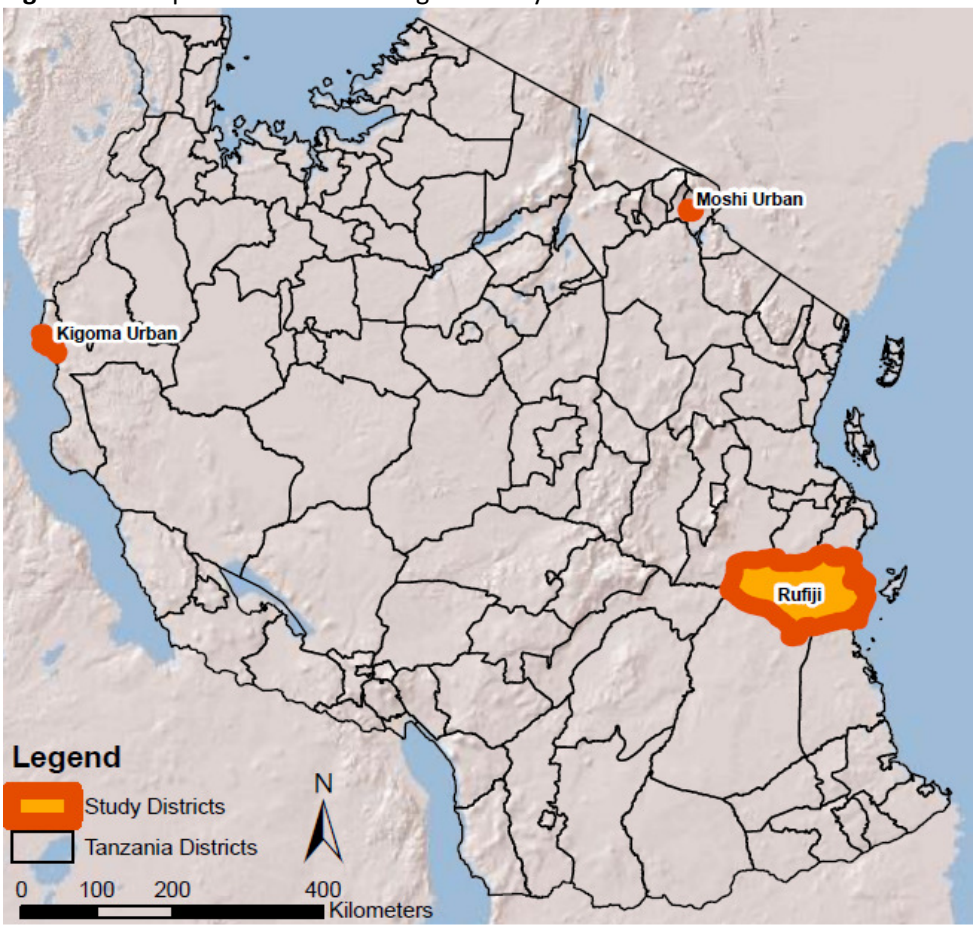
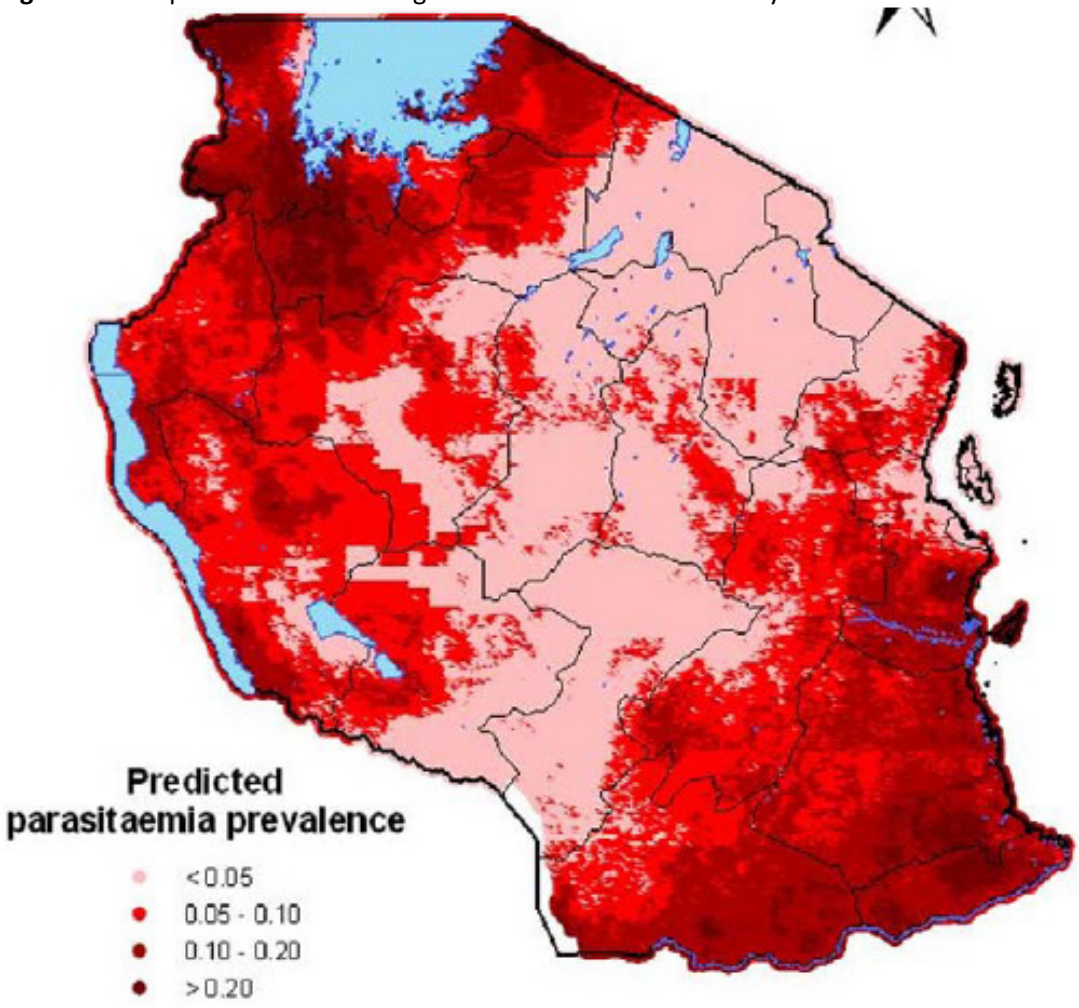


Figure 4.2: Map of Tanzania showing malaria transmission intensity in different areas of the country



PART III: PHARMACOVIGILANCE OF ANTIMALARIAL DRUGS IN PREGNANCY

Chapter 5: Medication exposure during pregnancy: a pilot pharmacovigilance system using health demographic surveillance system platform

Dominic Mosha[1,2*], Festo Mazuguni[1], Kahema Irema[1], Sigilbert Mrema[1], Salim Abdulla[1], Blaise Genton[2,3]

[1]Ifakara Health Institute, Rufiji HDSS, Tanzania

[2]Swiss Tropical and Public Health Institute, University of Basel, Switzerland

[3]Department of Ambulatory Care and Community Medicine & Division of Infectious Diseases, University Hospital, Lausanne, Switzerland

Under review in *BMC pregnancy and Childbirth Journal*

Abstract

Background: There is limited safety information on most drugs used during pregnancy. This is especially true for medication against tropical diseases because pharmacovigilance systems are not much developed in these settings. The aim of the present study was to assess medication exposure during pregnancy, and its relation to pregnancy outcome using a Health Demographic Surveillance System (HDSS) platform.

Methods: Pregnant women with gestational age below 20 weeks were recruited from Reproductive and Child Health (RCH) clinics or from monthly house visits carried out for the HDSS. A structured questionnaire was used to interview pregnant women. Participants were followed on monthly basis to record any new drug used as well as pregnancy outcome.

Results: 1089 pregnant women were recruited; 994 (91.3%) completed the follow-up until delivery. 98% women reported to have taken at least one medication during pregnancy, mainly those used in antenatal programmes. Other most reported drugs were analgesics (24%), antibiotics (17%), and antimalarial (15%), excluding IPTp. Artemether- lumefantrine (AL) was the most used antimalarial for treating illness by nearly 3/4 compared to other groups of malaria drugs. Overall, antimalarial and antibiotic exposures in pregnancy were not significantly associated with adverse pregnancy outcome. Iron and folic acid supplementations were associated with decreased risk of miscarriage/stillbirth (OR 0.1; 0.08 – 0.3).

Conclusion: Almost all women were exposed to medication during pregnancy. Exposure to iron and folic acid had a beneficial effect on pregnancy outcome. HDSS proved to be a useful platform to establish a reliable pharmacovigilance system in resource-limited countries. Widening drug safety information is essential to facilitate evidence based risk-benefit decision making for treatment during pregnancy, a major challenge with newly marketed medicines.

Keywords: Medication, Pregnancy, Pharmacovigilance

Background

Access to different therapeutic drugs such as antibiotics, antimalarial and antiretroviral (ARVs) have improved in recent years in most African countries, including Tanzania, thanks to the efforts facilitated by government, private sector and donor agencies [65, 66]. Safety of some of these therapies is unknown during pregnancy because pregnant women are not involved in clinical trials during the drug development process and hence, most pharmaceutical products come to market with little human data available regarding safety in pregnancy. Studies from animal models have been used to provide safety information during pregnancy at the time the new drug is approved. However, such findings are not easily translated into human risk. In most cases, information regarding safety of product or drug use during pregnancy is collected post product approval [35, 67]. Sufficient and valid data on safety of drug use during pregnancy is of high public health importance so as to facilitate evidence based risk-benefit decision making among health providers.

Drug exposure during pregnancy in Western Europe and US is reported to have increased in the past 10 years [68, 69]. In most developing countries, where proper drug monitoring system during pregnancy does not exist, it is difficult to know the magnitude of drug exposure in pregnancy. There are few studies in sub-Saharan Africa which have attempted to assess prevalence of drug use in pregnancy and its relation to pregnancy outcome. A study in Mozambique reported that antibiotics agents were the most common drugs used (41%), followed by antimalarial drugs (24%); Drug exposure in general was associated with a two fold increase risk of stillbirth [36].

First trimester of pregnancy is the most harmful period for teratogenic exposure because it is when organogenesis takes place albeit, some teratogens may have effect in later stage of pregnancy and may even cause miscarriage [70, 71]. Common medicines such as tetracycline, metronidazole, albendazole, mebendazole, efavirenz (EFV), sulphadoxine-pyrimethamine (SP) and artemisinin-based combination therapy (ACT) are some of therapeutic drugs which are not recommended during first trimester due to fear of embryo-toxicity [37, 72]. All these reported teratogenic drugs and many other which are known, or not yet confirmed, to have deleterious effects on the foetus are still used by women of childbearing age and pregnant women to treat different illnesses [73]. Thus, there are insufficient safety studies in pregnancy on most drugs used for the treatment of tropical diseases [74].

Demographic Surveillance System (DSS) is an ideal platform to establish pharmacovigilance system in pregnancy. People in DSS area are routinely being followed to update their information in the database. It is therefore easy to identify early enough vital events such as pregnancy, birth and death. A link

between the DSS members and health care at the nearby facility can be established with facilitated follow-up of pregnant women. The present study aimed at assessing the levels of medication exposure during pregnancy and its relation to pregnancy outcome. It made use of the Health Demographic Surveillance System (HDSS) which was already in place in a rural district, Eastern Tanzania.

Materials and methods

Study site and HDSS platform

The study was conducted using the platform of the Rufiji Health Demographic Surveillance System (HDSS) which is located in Coastal region, Eastern Tanzania. The area has hot weather throughout the year and two rainy seasons. The asexual parasitaemia prevalence is 14%, and *Plasmodium falciparum* is the predominant species [75]. Rufiji HDSS monitors a population of about 97,000; they are all recorded in the database with their social and health characteristics. Data from all 13 health facilities within DSS catchment area are also routinely being collected. These health facilities have Reproductive and Child Health (RCH) clinic services. A prevalence of 74% of pregnant women deliver in the health facilities. Fertility rate is 4.8 and the maternal mortality ratio is 70 per 100,000 live births [76]. Details of the study area and population have been described elsewhere [77].

Study design and population

This was an observational prospective study conducted between April 2012 and March 2013. Pregnant women with a gestational age below 20 weeks and residing in the HDSS area were enrolled in the study and followed until delivery. Participants were recruited from both RCH clinic and in the community through monthly house visits. The set-up of HDSS facilitated early identification and recruitment of eligible pregnant women. All participants were followed up on a monthly basis until delivery. A structured questionnaire was used to interview for socio-demographic information, obstetrics and medical history. Physical examination, blood screening test for HIV, syphilis and haemoglobin were performed in the health facility. Patient's information from RCH card or medical registry was also used for addition information and for clarifying issues.

Participants were interviewed for any drug which was taken prior to the enrolment but during the current pregnancy. On the day of enrolment, all women were given a small exercise book as patient's medical record log. The latter was used whenever the woman went to health facility for treatment or to drug vender to fetch medication. Hence, all clinical information including drug used was filled in this personal medical record book. During each monthly follow-up visit, participants were asked for any new drug used, and in all cases evidence for the new used medication was verified from prescription sheet,

RCH card, hospital record or personal medical record log. Pregnancy risk of a drug exposure during pregnancy period was categorized in accordance to US Food and Drug Administration (FDA) [78].

Pregnancy information was recorded including birth outcomes (miscarriage, stillbirth or live birth), mother's complications at delivery, number of babies born, birth weight, gestational age at delivery (calculated from the last date of normal menstrual period), and any congenital abnormalities. For the case of home delivery, woman was advised to take the baby to the hospital within seven days post-delivery for proper examination.

Sample size

The sample size was pre-determined by the size of HDSS and the logistically feasible time frame of one year. The number of women in their early pregnancy which could be enrolled was estimated before (as 1000) to be sufficient for pilot implementation of pharmacovigilance system in pregnancy but no formal sample size calculation was performed. To comply with the reviewer's suggestion we consulted our senior statistician to guide us on the power calculation. However, he advised us not to perform a post-hoc power calculation. Calculations which make use of parameter estimates provided by the data invariably inflate the actual power, in fact post hoc power is a one-to-one function of the P-value obtained [79, 80].

Primary endpoints

The primary endpoint of the study was pregnancy outcome. Pregnancy outcome included miscarriage, stillbirth or live birth, birth weight and prematurity status at birth. Miscarriage was defined as loss of an embryo or foetus before the 28^{th} week of pregnancy. Stillbirth was defined as a baby born with no signs of life at or after 28 weeks of gestation. Low birth weight was defined as a birth weight below 2500 g, and premature was defined as birth before 37 weeks of gestational age.

Statistical analysis

STATA® 12.0 (Stata Corporation, College Station, Texas, USA) was used for data analysis. Numerical variables were summarized into median and range. Categorical variables were summarized using cross tabulation to estimate different proportion. Effects of demographic and pregnancy characteristics on primary endpoint of the study were assessed by bivariable analysis. Logistic regression models were used to estimate the crude odds ratio (OR) for the association between binary pregnancy outcomes (birth outcome, birth weight and birth maturity status) and medicines exposure. The multivariable adjusted logistic regression model included maternal age and parity as potential confounding variables.

Both were found to be associated with the study endpoints (P < 0.2). Two sided Wald test P-values are presented.

Ethics

Ethical approval for the study was granted by Ifakara Health Institute (IHI) ethical review board and National Institute for Medical Research (NIMR) ethical committee. Written informed consent was obtained from all participants.

Results

A total of 1089 pregnant women were enrolled into the study and 994 (91.3%) completed the follow-up. The latter constitutes the analysis population. 660 (66.4%) were recruited from the health facility during their routine RCH visits and 334 (33.6%) from the community through house visit. Overall, 323 (32.5%) women were recruited in first trimester of pregnancy with a mean gestational age of 10.4 [standard deviation (SD) 2.3] and 671 (67.5%) in first half of second trimester of pregnancy with mean gestational age of 16.9 (SD 1.7). Important demographic and clinical characteristics are shown in *table 5.1*.

Episodes of reported illnesses during pregnancy

Out of all 297 (29.9 %) of the enrolled pregnant women reported to have at least one episode of illness during pregnancy. Six diseases were reported: malaria 14.9% (148), urinary tract infection (UTI) 9.2% (91), sexually transmitted infections (STIs) 3.2% (32), upper respiratory tract infection (URTI) 1.5% (15), diarrhoea 1% (10) and chickenpox 0.1% (1).

Drugs exposure during pregnancy

15 (1.5%) of all study participants reported not to have used any drug during the pregnancy period. 974 (98%) used any of the three drug groups that are recommended by the Ministry of Health [81] for antenatal intervention, all of which used during second and third trimester of pregnancy. 931 (93.7) used vitamins and mineral supplements. 929 (93.5%) used anthelmintic (mebendazole). 946 (95.2%) used at least one dose of sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment of malaria (IPTp) [735 (73.9%) two doses and 211 (21.2%) one dose] [see *tables 5.2a&b*].

For anti-infective drugs used because of illnesses, 170 (17.1%) women used antibiotics, 148 (14.9%) antimalarial drugs, 59 (5.9%) antifungals and 29 (2.9%) antiretrovirals. Some women used more than one type of either of the mentioned anti-infective drugs during their pregnancy period. *Table 5.2a and 5.2b* summarize drugs exposures during pregnancy among study women.

Based on United State Food and Drug Administration (US FDA) risk categorization of drugs in pregnancy, the most common drugs used under category 'A' were ferrous sulfate and folic acid, category 'B' paracetamol, amoxicillin, erythromycin, metronidazole, benzathine benzylpenicillin and ceftriaxone, category 'C' antimalarial for treating illness (AL, quinine, SP), antiretroviral (ARV) for HIV infection (zidovudine, lamivudine and nevirapine), doxycycline, cotrimoxazole, aspirin, diclophenac, hyoscine butylbromide and promethazine, category 'D' traditional medicines and phenobarbitone, and no category 'X'.

Pregnancy outcome

Out of 994, 897 (90.2%) women delivered in health facilities, 94 (9.5%) at home, and 3 (0.3%) along the road side on their way to the health facility. There were three maternal deaths which all occur within 24 hours post-delivery, two of them due to post-partum haemorrhage and one secondary to eclampsia. Pregnancy outcomes included 28 (2.8%) abortions, 41 (4.1%) stillbirth and 925 (93.1%) live births. Regarding birth outcomes, 99 (10.0%) were premature and 55 (5.0%) babies had low birth weight. 12 (1.2%) of the newborns were identified as having congenital anomalies at the time of birth: 8 were polydactyl and the remaining 4 had clubfoot, spinal bifida, genital defect or cardiac defect. Two women with a newborn having polydactyl each were exposed to ARV and antitussive (coughing syrup), respectively and both drugs are under US FDA risk category 'C'. One woman with a newborn having *spinal bifida* was exposed to phenobarbitone in third trimester, the drug which is in US FDA risk category 'D'. The remaining women with congenital anomalies babies were not exposed to neither US FDA category 'C' nor category 'D' drugs.

Relation of medication exposure to pregnancy outcome

Maternal age and parity were assessed to determine their effect on pregnancy outcome (as potential confounders of drug effect). Maternal age had no significant effect on birth weight (0R 1.0; p value 0.356) but was associated with 3% increased risk of premature birth (OR 1.03; p value 0.038) and 4% increased risk of miscarriage/stillbirth (OR 1.04; p value 0.020). Parity had no significant effect on miscarriage/stillbirth (OR 1.1; p value 0.690) but was associated with a 60% increase risk of preterm birth (OR 1.6; p value 0.065) and 60% decreased risk of low birth weight (OR 0.6; p value 0.116).

Antimalarial exposure during pregnancy was not significantly associated with an increased risk of miscarriage/stillbirth (adjusted OR 1.3; 95%CI 0.7 -2.4; p=0.494), low birth weight (adjusted OR 0.7; 95CI% 0.3 – 1.8; p=0.460) or premature birth (adjusted OR 1.2; 95%CI 0.6 – 2.7; p=0.629). Antibiotics exposure was neither associated with an increased risk of miscarriage/stillbirth (adjusted OR 0.8; 95%CI

0.4 – 1.6; p=0.526), low birth weight (adjusted OR 0.6; 95%CI 0.2 – 1.6; p=0.295) or premature birth (adjusted OR 1.4; 95%CI 0.7 – 2.8; p=0.348) [*Table 5.3*].

Exposure to drugs under US FDA pregnancy risk category 'A', which mainly included ferrous sulfate and folic acid were associated with a reduced risk of miscarriage/stillbirth (adjusted OR 0.1; 95%CI 0.08 – 0.3; p< 0.001). There was no significant association of adverse pregnancy outcome in relation to exposure to drugs under category 'B', 'C' and 'D' [*Table 5.4*].

Discussion

The present study shows that there is a considerable amount and several types of drugs exposure during pregnancy in this region, as it may apply to other parts of Tanzania and sub Saharan countries. To our knowledge, it is the first prospective study conducted in a resource-limited setting that attempted to demonstrate the feasibility of establishing a reliable pregnancy exposure registry which followed a large group of pregnant women from their early pregnancy stage [59, 82]. All drugs exposure and related diseases during pregnancy period were carefully identified and recorded.

More than 98% of study women reported to have used at least one medication during pregnancy. This is more than twice to what was observed in Mozambique in a study conducted seven years ago [36]. Most of the drugs used were the ones covered under antenatal intervention program. The coverage of anthelmintic, haematemic and SP for IPTp in our study was almost twice that estimated at national level [83]. This high use of drugs may be the result of intense health promotion activities in the area under HDSS, in close collaboration with local and government authorities. A 94% coverage for iron and folic acid supplementations in this rural area is a remarkable achievement.

Apart from haematemic, anthelminthic and IPTp-SP exposure, analgesics were the most reported prescribed drugs. This observation is in agreement with two previous studies in sub-Saharan Africa [36, 84].

Malaria was the most often recorded illness during pregnancy (15%). This illustrates the high intensity of transmission in the study area (14) and the vulnerability of pregnant women to malaria [6]. It highlights the importance of having safe and effective drugs to clear parasites during pregnancy. AL was prescribed nearly 3 times more often than quinine. Some of these treatments correspond to inadvertent exposure, similarly to what has been observed in Sudan and Zambia [39, 40]. Others represent treatment that were probably administered during second and third trimester, as recommended [37]. A better

availability of AL when compared to quinine in health facilities and drug shops [66] may have also contributed to the frequent use of this drug.

When taken as a category and irrespective of the timing during pregnancy, antimalarial and antibiotic exposures were not associated with adverse pregnancy outcome. This result should be interpreted with caution since different types of medications, or the same medication but given at different time during pregnancy, may have different effects. A more detailed assessment of antimalarial exposure, taking into account the type and time of exposure during pregnancy will be reported elsewhere, including a larger sample size of pregnant women from another HDSS area. The present paper was more to pilot the feasibility of a pharmacovigilance system embedded in a HDSS in a developing country.

Iron and folic acid supplementation, the main drugs under US FDA pregnancy risk category 'A' were protective against miscarriage/stillbirth. However, adherence to these supplements and number of doses prescribed were not assessed. There was not much evidence yet to support the added benefits of these supplements in preventing miscarriage or stillbirth. Evidence mainly supports the use of these drugs to prevent anaemia and iron deficiency at term, to reduce the risk of low birth weight and early neonatal death, all factors that have shown to have a beneficial impact on child's survival [85-87]. The observed beneficial effects of recommended iron and folic acid supplementation in pregnancy validate the concept of pharmacovigilance system through HDSS.

About 3% of study women used traditional medicines which in most cases are under pregnancy risk category 'D'. The use of traditional medicines may have been higher than what is reported in the present study since participants were interviewed by health care providers who are trained to discourage patients to use herbs. Underreporting is a well-known phenomenon in other developing countries whereby study participants had difficult to disclose this to health care professionals [36, 88].

The observed prevalence of 1.2% congenital anomalies in the study is lower compared to the 3.0% global prevalence estimated by the WHO [89]. There is no national register to compare our rate with that in other parts of the country. However, there are possibilities of more anomalies to be identified later in life as the child grows. Hence, it would be important to follow all delivered babies prospectively at defined intervals, at least until the age of one year. Such a monitoring can be easily implemented in HDSS settings. In addition, it is also important to consider improving newborn's screening standards, training of health staff and detailed birth registry records to implement a reliable pregnancy pharmacovigilance system [82].

The present study demonstrates a way forward to establish a feasible, reliable and manageable active pharmacovigilance system in a resource-limited setting by taking advantage of existing monitoring platforms such as HDSS. Feasibility of the present proposed pharmacovigilance system is of merit over the probabilistic record linkage for monitoring antimalarial safety evaluated in Senegal [59] which is subjected to bias because of poor medical record system in most health facilities in developing countries and hence, some exposure cases and pregnancy outcome information may easily be missed. The drug exposure pregnancy registry proposed by Mehta U *et al*., [82] appears to be promising but its operational costs may be very high in most resource-limited countries where presence of skilled medical personnel is still a big problem.

Pharmacovigilance systems should reduce the uncertainty about safety of newly marketed medication against tropical diseases. Such a system that collects systematically and reliably data to determine whether a given medication is teratogenic or not through monitoring of a large exposure group could provide strong evidence on safety [73, 82]. It could help to overcome the current shortfall which is commonly seen in medical practice when treating a pregnant woman with medication that has suboptimal efficacy because of potential safety problems. Also, it could assist in the assessment of medicines that are not recommended during pregnancy, but are sometimes not avoidable to save the mother or the unborn child.

Conclusion

Almost all women are exposed to medication during pregnancy, either because drugs are recommended during this period, or because women are sick and need treatment. Since exposure to contraindicated drugs during pregnancy is sometimes inevitable in either trimester, safety monitoring mechanism should be in place in order to generate reliable information for the promotion of safe and effective treatment during pregnancy. HDSS sites can have a useful role in providing reliable pharmacovigilance data and the experience from its success will be helpful to expand the system to none HDSS areas.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

All authors of this manuscript were involved in design and interpretation of the study findings. DM and BG designed the study assisted by SA. DM was responsible for data collection and coordinating all procedures involved in the study. KI and SM assisted in field work activities. DM and FM were

responsible for data management and analysis. All authors reviewed and approved the final version of the manuscript.

Acknowledgements

We sincerely thank the pregnant women for their cooperation and all staff involved in the study. Special thanks are given to Advocatus Kakorozya, Athumani Mzuyu and Kusudi Bakari of Rufiji HDSS, and Jan Hattendorf of Swiss TPH for his inputs in statistical review. This study was co-funded by the Ifakara Health Institute (IHI) and European and Developing Countries Trial Partnership (EDCTP) through Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) project.

Author details

[1]Ifakara Health Institute, Rufiji HDSS, Tanzania. [2]Swiss Tropical and Public Health Institute, University of Basel, Switzerland. [3]Department of Ambulatory Care and Community Medicine & Division of Infectious Diseases, University Hospital, Lausanne, Switzerland

Tables

Table 5.1: Demographic and clinical characteristics of study women at the time of enrollment (n = 994)

Characteristics	**First trimester**	**Second trimester**	**Total**
	n = 323	**n = 671**	**n = 994**
Mean age, (years)*	26.4 (7.3; 14-49)	26.8 (7.0; 14-46)	26.6 (7.0; 14-49)
Mean BMI*	23.1 (3.8; 14.2-39.6)	23.4 (3.4; 14.0-42.5)	23.3 (3.6; 14.0-42.5)
Mean gestational age, (weeks)*	10.0 (2.2; 3-12)	16.6 (1.9; 13-20)	14.8 (3.6; 3-20)
Gravidity[#]			
Primigravidae	82 (25.4)	198 (29.5)	280 (28.2)
Secundigravidae	62 (19.2)	113 (16.8)	175 (17.6)
3 – 4 pregnancies	99 (30.7)	192 (28.6)	291 (29.3)
≥ 5 pregnancies	80 (24.8)	168 (25.1)	248 (24.9)
Recruited sites[#]			
Health facility	193 (59.8)	467 (69.6)	660 (66.4)
Home	130 (40.2)	204 (30.4)	334 (33.6)
Drinking alcohol[#]	3 (0.9)	3 (0.4)	6 (0.6)
Smoking cigarette[#]	2 (0.6)	0 (0)	2 (0.2)
Mean haemoglobin level, (g/dl)*	7.8 (4.7; 6.0-12.7)	7.5 (4.6; 5.2-14.3)	7.7 (4.6; 5.2-14.3)
HIV status[#]			
Negative	284 (88.0)	603 (89.9)	887 (89.2)
Positive	12 (3.7)	35 (5.2)	47 (4.7)
No results	27 (8.3)	33 (4.9)	60 (6.0)
Syphilis test[#]			
Negative	288 (89.2)	628 (93.6)	916 (92.2)
Positive	9 (2.8)	12 (1.8)	21 (2.1)
No results	26 (8.0)	31 (4.6)	57 (5.7)

*represents data presented in mean, (standard deviation [SD]; range)

[#]represents data presented in number (%)

Abbreviation: BMI = Body Mass Index

Table 5.2a: Classes of drugs reported to be used by the pregnant women

Class of drugs	**Number of women exposed (%)**
Vitamins and minerals	931 (93.7)
Anthelmintics[α]	929 (93.5)
Analgesics	237 (23.8)
Antibiotics	170 (17.1)
Antimalarials[α] *	148 (14.9)
Antifungals	59 (5.9)
Antiretroviral	29 (2.9)
Traditional medicine	27 (2.7)
Antihistamines	15 (1.5)
Antitussive	8 (0.8)
Antihypertensives	6 (0.6)
Antiasthmatics	5 (0.5)
Pregnancy risk Categories[#]	
A	931 (93.6)
B	253 (25.5)
C	233 (23.4)
D	46 (4.6)
X	0 (0.0)

[α] See table 5.2b for further details

*Excluding SP for IPTp

[#] Based on US FDA pregnancy risk categorization

Table 5.2b: RCH provided drugs and antimalarials exposure in pregnant women (n = 994)

Drug group	n (%)
SP for IPTp	
Single dose	211 (21.2)
Two doses	735 (73.9)
Not at all	48 (4.8)
Anthelminthic (Mebendazole)	
Yes	929 (93.5)
No	65 (6.5)
Iron and Folic acid supplementation	
Yes	93 (93.7)
No	63 (6.3)
Patients treated for malaria at least once*	
Yes	148 (14.9)
No	846 (85.1)
Types of antimalarials used in treating malaria	
AL only	94 (9.5)
Quinine only	28 (2.8)
SP only	11 (1.1)
AL and Quinine	11 (1.1)
AL and SP	4 (0.4)

*Some women were treated for malaria more than one time during pregnancy period

Abbreviation: SP = Sulfadoxine-pyrimethamine; IPPTp = Intermittent Preventive Treatment for malaria in pregnancy; AL = Artemether-lumefantrine

Table 5.3: Antimalarial and antibiotics exposure in relation to pregnancy outcome (n = 994)

Variables	Outcomes		Crude OR (95% CI)	P [μ]	Adjusted OR [α] (95% CI)	P [μ]
Birth outcome	**MC/SB n (%)**	**Live birth n (%)**				
Antimalarial exposure*						
Yes	12 (17.4)	136 (14.7)	1.2 (0.6 – 2.3)	0.546	1.3 (0.7 – 2.4)	0.494
No	57 (82.6)	789 (85.3)				
Antibiotics exposure						
Yes	10 (14.5)	160 (17.3)	0.8 (0.4 – 1.6)	0.551	0.8 (0.4 – 1.6)	0.526
No	59 (85.5)	765 (82.7)				
Birth weight (grams)	**< 2500 n (%)**	**≥ 2500 n (%)**				
Antimalarial exposure*						
Yes	5 (11.4)	131 (14.9)	0.7 (0.3 – 1.9)	0.523	0.7 (0.3 – 1.8)	0.460
No	39 (88.6)	750 (85.1)				
Antibiotics exposure						
Yes	5 (11.4)	155 (17.6)	0.6 (0.2 – 1.5)	0.291	0.6 (0.2 – 1.6)	0.295
No	39 (88.6)	726 (82.4)				
Maturity status at birth [a]	**Preterm n (%)**	**Term n (%)**				
Antimalarial exposure*						
Yes	8 (16.3)	128 (14.6)	1.1 (0.5 – 2.5)	0.742	1.2 (0.6 – 2.7)	0.629
No	41 (83.7)	748 (85.4)				
Antibiotics exposure						
Yes	11 (22.5)	149 (17.0)	1.4 (0.7 – 2.8)	0.329	1.4 (0.7 – 2.8)	0.348
No	38 (77.5)	727 (83.0)				

MC/SB = Miscarriage or stillbirth; OR = odds ratio; CI = confidence interval

* Excluding SP for IPTp

[μ] Estimated from the logistic regression model with Wald type P-value

[α] Adjusted for parity and maternal age

[a] Maturity status = Birth <37 weeks of gestation being preterm and ≥37 weeks of gestation being term

Table 5.4: US FDA pregnancy risk categories of drugs exposure in relation to pregnancy outcome

Variables	Outcomes		Crude OR (95% CI)	P [μ]	Adjusted OR [α] (95% CI)	P [μ]
Birth outcome	**MC/SB n (%)**	**Live birth n (%)**				
Drugs category 'A'						
Yes	51 (73.9)	880 (95.1)	0.1 (0.1 – 0.3)	< 0.001	0.1 (0.08– 0.3)	< 0.001
No	18 (26.1)	45 (4.9)				
Drugs category 'B'						
Yes	12 (17.4)	241 (26.1)	0.6 (0.3 – 1.1)	0.115	0.6 (0.3 – 1.1)	0.111
No	57 (82.6)	684 (73.9)				
Drugs category 'C'						
Yes	20 (29.0)	213 (23.0)	1.4 (0.8 – 2.3)	0.261	1.4 (0.8 – 2.4)	0.257
No	49 (71.0)	712 (77.0)				
Drugs category 'D'						
Yes	3 (4.3)	41 (4.4)	1.0 (0.3 – 2.9)	0.974	0.9 (0.3 – 3.2)	0.939
No	66 (95.7)	884 (95.6)				
Birth weight (grams)	**< 2500 n (%)**	**≥ 2500 n (%)**				
Drugs category 'A'						
Yes	40 (90.9)	840 (95.4)	0.5 (0.2 – 1.4)	0.191	0.5 (0.2 – 1.5)	0.207
No	4 (9.1)	41 (4.6)				
Drugs category 'B'						
Yes	7 (15.9)	234 (26.6)	0.5 (0.2 – 1.2)	0.122	0.5 (0.2 – 1.2)	0.125
No	37 (84.1)	647 (73.4)				
Drugs category 'C'						
Yes	8 (18.2)	205 (23.3)	0.7 (0.3 – 1.6)	0.436	0.7 (0.3 – 1.6)	0.387
No	36 (81.2)	676 (76.3)				
Drugs category 'D'						
Yes	1 (2.3)	40 (4.5)	0.5 (0.1 – 3.6)	0.485	0.6 (0.1 – 3.4)	0.449
No	43 (97.7)	841 (95.5)				
Maturity status at birth	**Preterm n (%)**	**Term n (%)**				
Drugs category 'A'						
Yes	45 (91.8)	835 (95.3)	0.6 (0.2 – 1.6)	0.277	0.5 (0.2 – 1.5)	0.231
No	4 (8.2)	41 (4.7)				
Drugs category 'B'						
Yes	17 (34.7)	224 (25.6)	1.5 (0.8 – 2.8)	0.160	1.5 (0.8 – 2.8)	0.175
No	32 (65.3)	652 (74.4)				
Drugs category 'C'						
Yes	14 (28.6)	199 (22.7)	1.4 (0.7 – 2.6)	0.345	1.4 (0.8 – 2.7)	0.269
No	35 (71.4)	677 (77.3)				
Drugs category 'D'						
Yes	4 (8.2)	37 (4.2)	2.0 (0.7 – 5.9)	0.201	2.0 (0.7 – 6.0)	0.200
No	45 (91.8)	839 (95.8)				

MC/SB = Miscarriage or stillbirth; OR = odds ratio; CI = confidence interval

[μ] Estimated from the logistic regression model with Wald type P-value

[α] Adjusted for parity and maternal age

Chapter 6: Safety of artemether-lumefantrine exposure in early pregnancy: an observational cohort

Dominic Mosha[1,2]*, Festo Mazuguni[1], Sigilbert Mrema[1], Esperanca Sevene[3], Salim Abdulla[1], Blaise Genton[2,4]

[1]Ifakara Health Institute, Rufiji HDSS, Tanzania

[2]Swiss Tropical and Public Health Institute, University of Basel, Switzerland

[3]Universidade Eduardo Mondlane, Faculdade de Medicina, Manhica Health Centre, Maputo, Mozambique

[4]Department of Ambulatory Care and Community Medicine & Division of Infectious Diseases, University Hospital, Lausanne, Switzerland

Accepted in *Malaria Journal*

Abstract

Introduction: There is limited data available regarding safety profile of artemisinins in early pregnancy. They are therefore not recommended by WHO as a first line treatment for malaria in first trimester due to associated embryo-foetal toxicity in animal studies. The aim of the study was to assess birth outcome among pregnant women inadvertently exposed to artemether-lumefantrine (AL) during first trimester in comparison to those of women exposed to other antimalarial drugs or no drug at all during the same period of pregnancy.

Methods: Pregnant women with gestational age ≤ 20 weeks were recruited and followed prospectively until delivery. Women were recruited from Reproductive and Child Health (RCH) clinic or from monthly house visits (demography surveillance) and followed prospectively until delivery. A structured questionnaire was used to interview participants.

Results: Total of 2167 pregnant women were recruited and 1783 (82.3%) completed the study until delivery. 319 (17.9%) used antimalarials in first trimester, of whom 172 (53.9%) used AL, 78 (24.4%) quinine, 66 (20.7%) sulfadoxine-pyrimethamine (SP) and 11 (3.4%) amodiaquine. Quinine exposure in first trimester was associated with an increased risk of miscarriage/stillbirth (OR 2.5; 1.3 – 5.1; p=0.009) and premature birth (OR 2.6; 1.3 – 5.3; p=0.007) as opposed to AL with (OR 1.4; 0.8 – 2.5; p=295) for miscarriage/stillbirth and (OR 0.9; 0.5 – 1.8; p=0.865) for preterm birth. Congenital anomalies were identified in 4 exposed groups namely AL only (1/164 [0.6%]), quinine only (1/70 [1.4%]), SP (2/66 [3.0%]), and non-antimalarial exposed group (19/1464 [1.3%]).

Discussion and conclusion: Exposure to AL in first trimester was more common than to any other antimalarial drugs. Quinine exposure was associated with adverse pregnancy outcome, which was not the case following other antimalarial intake. Since AL and quinine were used according to their availability rather than to disease severity, it is likely that the effect observed was related to the drug, and not to the disease itself. Detailed information on developmental milestone up to 12 months is ongoing to rule out any adverse effect on infancy as a result of AL exposure in first trimester. Even with this caveat, a change of policy from quinine to AL for the treatment of uncomplicated malaria during the whole pregnancy period could be already envisaged.

Keywords: Pregnancy, safety, artemether-lumefantrine, exposure

Background

Over 60% of all pregnancies globally are at risk of malaria and more than 32 million are in sub-Sahara Africa [1]. Malaria infection is associated with high maternal and perinatal mortality in tropical and subtropical regions [90]. Severe maternal anaemia, intrauterine growth retardation, intrauterine death, stillbirth, premature delivery and low birth-weight are some of the reported substantial direct risks of malaria in pregnancy [2, 90]. Although malaria in pregnancy is a serious public health problem, there is limited information available regarding safety profile of most of licensed antimalarial in pregnancy because pregnant women are routinely not involved in clinical trials related to drug development for fear of harming the women and or developing foetus [91].

Artemisinin-based combination therapies (ACT) are the most effective drugs against *Plasmodium falciparum* and have been recommended by the World Health Organization (WHO) as a drug of choice for the treatment of *P falciparum* malaria [37]. ACTs are only recommended in pregnancy during second and third trimester, but not in first trimester, unless they are the only treatment available, or if the patient's life is threatened. Safety concerns of artemesinins in first trimester are the associated risks of visceral and skeletal anomalies following animal studies in early stage of pregnancy [92, 93]. Two previous small-scale studies assessing Zambian and Sudanese pregnant women exposed to artemisinin during first trimester could not find any association between drug exposure and maternal or birth adverse outcomes [39, 40]. However, evidence is still scarce to ensure safety of ACT during first trimester.

Artemether-lumefantrine (AL) (20 mg and 120 mg respectively) (Coartem©, Novartis Pharm AG) is one of the most popular and efficacious fixed dose of ACT which is currently available [94]. AL was introduced in Tanzania as a first line therapy for malaria in 2006 to replace sulphadoxine-pyrimethamine (SP) [95]. Inadvertent exposure to artemisinin during first trimester of pregnancy is possible due to its high availability at a subsidized cost in both private and public health facilities in the country [65, 66]. Furthermore, self-malaria treatment without consulting trained professional is common in sub-Sahara Africa; indeed, 70% of malaria episodes in rural Africa and 50% in urban areas are self-treated cases [96]. It is therefore important to take advantage of the latter to extend the margin safety information of artemisinin compounds in pregnancy by evaluating maternal and birth outcomes of inadvertently AL exposure to women in their first trimester.

There is increasing evidence supporting efficacy, safety and tolerability of ACT which outweigh advantages of quinine in treating malaria [41, 97]. Despite its reactogenicity profile and several reports

of resistant strains of *P falciparum* [98, 99], quinine remains the only recommended drug for treating both uncomplicated and complicated *P falciparum* malaria during first trimester of pregnancy [37, 95]. The present study aims at assessing the maternal and birth outcomes in pregnant women who were inadvertently exposed to AL during first trimester in comparison to those of women exposed to other antimalarial drugs or no drug at all during the same period of pregnancy using two Health Demographic Surveillance System (HDSS) platforms in Tanzania.

Methods

Study area

The study was conducted in Rufiji and Kigoma HDSS in Tanzania. Rufiji HDSS is in a rural setting while Kigoma HDSS is in an urban one, both areas have moderate to high malaria transmission intensity [100]. The study involved a total of 22 health facilities in the two HDSS sites. There was no clinical interventional research activity in the area during the study period.

Study design

The study enrolled pregnant women with gestational age of 20 weeks and below between March 2012 and April 2013. Only women residing in HDSS were eligible for the study. They were recruited from Reproductive and Child Health (RCH) clinic during their routine visits and from the community through monthly round-based house visits. The set-up of HDSS allows identification of pregnancy status in women of childbearing age through routine HDSS quarterly census. On the day of enrollment, participants were interviewed for obstetrics and previous medical history including history of chronic illness or disease, use of alcohol and smoking. Important laboratory test such as maternal haemoglobin level, screening for HIV and syphilis were performed. Use of any antimalarial during first trimester of the presenting pregnancy was the key question during interview. The reported drug use information by a participant was verified by assessing patient's medical record from the attended health facility, prescription sheet and maternal RCH card. Participants who had inadvertently used AL for malaria treatment in first trimester were compared with pregnant women who were treated with either quinine (Qn), sulphadoxine-pyrimethamine (SP), amodiaquine or women who had not used antimalarial drug(s) at all during the same period of pregnancy. Thus, women were not randomized but allocated to the study arm according to their antimalarial exposure history in first trimester.

Women were followed on monthly basis until delivery to monitor pregnancy and birth outcomes. The assessed pregnancy outcome included maternal mortality, spontaneous abortion (pregnancy lose ≤ 28 weeks of gestation), ectopic gestation, stillbirth and live birth. Birth outcome include birth weight,

maturity status at birth (estimated from the last normal menstrual period) and presence of congenital anomalies. All newborns were assessed for congenital abnormalities post-delivery by a study clinician or health facility midwife.

Primary endpoint

Primary endpoints of the study were pregnancy and baby outcomes. Pregnancy outcome included miscarriage, stillbirth or live birth whereas baby outcome included birth weight and maturity status at birth. Low birth weight was defined as a birth weight below 2500 grams and premature was defined as birth before 37 weeks of gestational age.

Statistical analysis

STATA® 12.0 (Stata Corporation, College Station, Texas, USA) was used for data analysis. Numerical variables were summarized into mean and standard deviation. Categorical variables were summarized using cross tabulation to estimate different proportion. The effect of demographic and pregnancy characteristics on primary endpoint of the study was assessed by bivariable analysis. Logistic regression model were used to estimate the crude odds ratio (OR) for the associated between binary pregnancy outcomes (birth outcome, birth weight and birth maturity status) and medicine exposure. The multivariable adjusted logistic regression model included maternal age and parity as potential confounding variables. Both were found to be associated with the study endpoints ($P < 0.2$). Two sided Wald test P-values are presented.

Ethics

Ethical approval was granted by the Ifakara Health Institute (IHI) ethical review board and the National Institute for Medical Research (NIMR) ethical committee. Written informed consent was obtained from all participants.

Results

A total of 2167 pregnant women were enrolled in the study and 1783 (82.3%) were followed until delivery [*Figure 6.1*]. 19.2% (342) were recruited from the community through house visit and 80.8% (1441) from the facility during their routine RCH clinic visits. 602 (33.8%) women were recruited in first trimester of pregnancy with mean gestational age of 10.5 [standard deviation (SD) 2.6] and 1181 (66.2%) during the first half of second trimester of pregnancy with mean gestation age of 16.9 (1.5). 559 (31.4%) were primigavidae, 336 (18.8%) secundigravidae and 888 (49.8%) were multigravidae with gravidity of 3 and above. Important demographic and clinical characteristics are summarized in *Table 6.1*.

Drug exposure

319 (17.9%) women used antimalarial in first trimester of pregnancy because of a morbid episode. 164 (51.4%) used AL only, 70 (21.9%) quinine only, 8 (2.5%) both AL and quinine, 66 (20.7%) SP and 11 (3.4%) amodiaquine. At least 88% of study women used three group of drugs that are in antenatal intervention as recommended by the Ministry of Health [95] namely, anthelminthic, sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment of malaria (IPTp) and iron and folic acid supplementation. Anthelminthic and IPTp-SP are prescribed in second and third trimester of pregnancy. 1579 (88.6%) used anthelminthic (mebendazole), 1626 (91.2%) used iron and folic acid supplementation and 1636 (91.8%) used at least one dose of IPTp-SP.

Pregnancy outcome and antimalarial exposure

Among 1783 deliveries, there were 5 maternal deaths that occurred within 24 hours, three were due to post-partum haemorrhage and the remaining two each was secondary to eclampsia and disseminated intravascular coagulopathy (DIC), respectively. Pregnancy outcomes included 44 (2.5%) abortions, 62 (3.5%) stillbirth and 1677 (94.1%) live births. Baby outcomes included 81 (4.8%) low birth weight babies and 113 (6.7%) premature births. 23 (1.3%) of the newborns were identified to have congenital anomalies at birth including, polydactyl 17 (73.9%), club foot 2 (8.7%), genital defect 2 (8.7%), *spinal bifida* 1 (4.3%) and cardiac defect 1 (4.3%). Congenital anomalies were identified in 4 exposed groups namely AL only (1 [0.6%] of 164), quinine only (1 [1.4%] of 70), SP (2 [3.0%] of 66), and non-antimalarial exposed group (19 of [1.3%] of 1464). *Table 6.2* summarizes pregnancy outcomes parameters in relation to antimalarial exposure status in first trimester.

Quinine exposure during first trimester was associated with an increased risk of miscarriage/stillbirth (adjusted OR 2.5; 95%CI 1.3 – 5.1; p=0.009) and premature birth (adjusted OR 2.6; 95%CI 1.3 – 5.3; p=0.007) as opposed to AL, SP and amodiaquine exposure which were not associated with increased risk of either miscarriage/stillbirth, low birth weight or premature birth [see details in *Table 6.3*].

Maternal age and parity were assessed to determine their effect on pregnancy outcome as potential confounders of the drug effect. Increase of maternal age in years was associated with 5% decreased risk of low birth weight (OR 0.95; p=0.009), 5% increased risk of miscarriage/stillbirth (OR 1.05; p=0.001), and 3% increased risk of preterm birth (OR 1.03; p=0.016). Multigravidae had 50% decreased risk of low birth weight (OR 0.5; p=0.006), 60% increased risk of miscarriage/stillbirth (OR 1.6; p=0.048), and 30% increased risk of preterm birth (OR 1.3; p=0.099) compared to primigravidae.

Discussion

The study findings provide further evidence on the safety profile of AL use in early pregnancy to treat malaria. It differs from previous first trimester artemisinine derivatives safety studies [39, 40] by having a larger sample size and a broader comparative exposure group. Also, the low mean gestational age at enrolment improves accuracy of drug exposure history, and thus reduces recall bias. It also increases the chances of identifying adverse pregnancy outcomes which commonly occurs during early stage of pregnancy, such as abortion [101].

Although AL is not recommended as first-line treatment for malaria during first trimester of pregnancy, it was used by 54% of women in this indication. Exposure to AL in first trimester was twofold higher than quinine, the drug of choice for malaria treatment during first trimester in Tanzania [95]. This observation suggests that AL is a popular drug. It reflects its high accessibility in most of the health facilities and by drug vendors in the country [65, 66]. In practice, quinine was frequently out of stock and its replacement could easily take several weeks, particularly in public health facilities. Since shortage of drugs is common in resource-limited settings [102, 103], inadvertent or voluntary exposure to contraindicated drugs is inevitable. Limited access to quinine may also explain the observed high SP and amodiaquine exposure, drugs which are currently not recommended for treating malarial illness [37].

Quinine exposure was associated with a twofold increased risk of miscarriage/stillbirth and preterm birth. The harmful effect of quinine during pregnancy has been known for a long time. Its abortive properties in relation to the induction of uterine contractions have long been reported by Maxwell JP [44]. The strength and prolongation of these contractions were reported to be dose dependent. A randomized control trial in Uganda showed oral quinine to have a twofold increased incidence of adverse effects compared to AL among pregnant women treated for uncomplicated malaria in second and third trimesters. There were nearly twofold increases in intrauterine foetal deaths in the quinine group than in the AL one, although the numbers were low. On the other hand, there was no difference in proportions of spontaneous abortions in the two study groups [41].

In the present study, these adverse pregnancy outcomes were not observed following AL, SP or amodiaquine exposure, which suggests that the deleterious effect of quinine was more related to the drug itself, rather than to the malaria episode. This is supported by the observation made by fieldworkers that quinine was not given to a particular group of women because of more severe disease, but just because AL was more readily accessible on the shelf of the health facility. Also, all women took quinine tablets, and not intravenous doses, which speaks for a similar degree of severity of the disease

in women who took quinine and AL. However, since it was not a proper randomized double-blind controlled trial, it is not possible to formally exclude a selection bias that would lead to different effects of the malaria disease itself. Whatever is the cause, the magnitude of the adverse effects associated with quinine exposure is alarming, when considering that this drug is viewed at present as the safest antimalarial drug in first trimester. There is a remote possibility of a deleterious effect of AL on the foetus, and hence on infant development, that could not be assessed at this stage in the study. We hope to be able to definitely exclude an adverse consequence of AL exposure during pregnancy on the infant when analysing the results of the 12-month follow-up of the offsprings. Precise information on neurological scores, including motor and sensory patterns, should assist policy decisions after careful analysis of the time of antimalarial exposure. The preliminary results of the first infant cohort are encouraging.

The observed prevalence of 1.3% congenital anomaly in the present study is lower than the global prevalence (3.0%) estimated by WHO [89]. No national figures of congenital anomalies are available for comparison in Tanzania. Also, there is a possibility of more anomalies be identified later in life as the child grows. Congenital anomalies was twice in the non-antimalarial exposed group compared to AL exposed group (1.3% vs 0.6%). Polydactyly was the most reported congenital anomaly (74%) but it is believed to be genetically determined rather than triggered by external exposure [104]. In animal studies, umbilical hernia has been reported to be associated with artemisinin exposure during pregnancy [92]. Our study had limitation to assess occurrence of umbilical hernia since the newborns were screened only once at the time of delivery. At this time, hernias may hardly present, and cannot therefore be identified. Also, umbilical hernia is commonly observed in most parts of Africa and is not viewed as an abnormality, it is often not brought to medical attention unless it manifest itself with complications such as intestinal obstruction [105, 106].

Conclusion

Exposure to AL in first trimester was more common than to any other antimalarial drugs. Quinine exposure was associated with adverse pregnancy outcome, which was not the case for other antimalarials. Since AL and quinine were used according to their availability rather than to disease severity, it is likely that the effect observed was related to the drug, and not to the disease itself. More information of developmental milestone up to 12 months is needed to rule out any adverse effect on infancy as a result of AL exposure in first trimester. Even with this caveat, a change of policy from

quinine to AL for the treatment of uncomplicated malaria during the whole pregnancy period could be already envisaged.

Competing interests

BG has received in the past a research grant from Novartis Pharma to work on the effect of artemether-lumefantrine introduction on child mortality and malaria transmission in Tanzania. Novartis Pharma had no involvement in the present project.

Funding

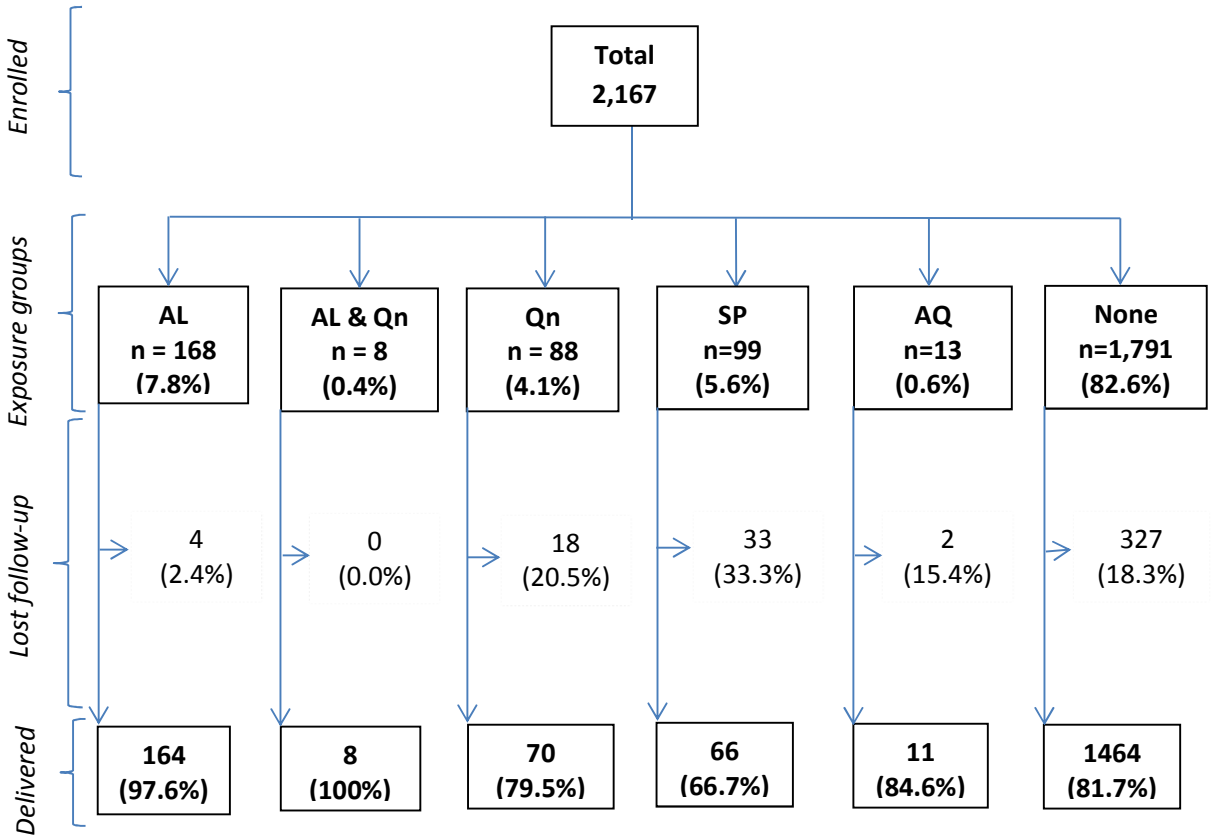
The study was co-funded by the Ifakara Health Institute (IHI) and the European and Developing Countries Trial Partnership (EDCTP) through the Malaria in Pregnancy Prevention Alternative Drugs (MiPPAD) project.

Acknowledgement

We thank the pregnant women for their cooperation and all staff involved in the study. Special thanks are given to Advocatus Kakorozya, Amri Shamte, Warren Temba, Essau Gharika, Matrida Lihenye, Athumani Mzuyu and Kusudi Bakari of Rufiji and Kigoma HDSS.

Figures and Tables

Figure 6.1: Flow chart of participants in the study



AL=Artemether-lumefantrine; Qn=Quinine; SP=Sulfadoxine-pyrimethamine; AQ=Amodiaquine; None=No antimalarial

Table 6.1: Characteristics of pregnant women enrolled in the study

Characteristics	**1st Trimester** **n = 602**	**First half of 2nd Trimester** **n = 1181**	**All Trimesters** **n = 1783**
Mean age, (years)*	25.7 (6.8; 14 – 49)	25.9 (6.7; 13 – 46)	25.8 (6.8; 13 – 49)
Mean BMI*	23.2 (3.9; 14.2-39.6)	23.3 (3.6; 14.0 – 42.5)	23.4 (3.7; 14.0 – 42.5)
Mean gestational age, (weeks)*	10.5 (2.6; 3 – 12)	16.9 (1.5; 13 – 20)	14.8 (3.7; 3 – 20)
Gravidity#			
Primigravidae	182 (30.0)	377 (31.9)	559 (31.4)
Secundigravidae	109 (18.0)	227 (19.2)	336 (18.8)
3 – 4 pregnancies	180 (29.6)	315 (26.7)	495 (27.8)
≥ 5 pregnancies	131 (21.4)	262 (22.2)	393 (22.0)
Recruited sites#			
Health facility	468 (22.3)	973 (82.4)	1441 (80.8)
Home	134 (77.7)	208 (17.6)	342 (19.2)
Drinking alcohol#	14 (2.7)	27 (2.3)	41 (2.3)
Smoking cigarette#	5 (0.8)	2 (0.2)	7 (0.4)
Haemoglobin level (g/dl)*	10.8 (1.5; 5.0 – 14.6)	10.7 (1.5; 5.4 – 14.9)	10.7 (1.5; 5.0 – 14.9)
HIV status#			
Negative	533 (88.5)	1086 (92.0)	1619 (90.8)
Positive	18 (3.0)	46 (3.9)	64 (3.6)
No results	51 (8.5)	49 (4.1)	100 (5.6)
Syphilis test#			
Negative	521 (86.5)	1082 (91.6)	1603 (89.9)
Positive	12 (2.0)	15 (1.3)	27 (1.5)
No results	69 (11.5)	84 (7.1)	153 (8.6)

*represents data presented in mean, (standard deviation [SD]; range)

#represent data presented in number (%)

Abbreviation: BMI = Body Mass Index

Table 6.2: Pregnancy and baby outcomes in relation to antimalarial exposure status during first trimester

Pregnancy outcome	**AL only**	**AL & Quinine**	**Quinine only**	**SP**	**Amodiaquine**	**None**
	164 (%)	**8 (%)**	**70 (%)**	**66 (%)**	**11 (%)**	**1464 (%)**
Abortion	5 (3.0)	2 (25.0)	3 (4.3)	0 (0.0)	0 (0.0)	34 (2.3)
Stillbirth	6 (3.7)	0 (0.0)	5 (7.1)	2 (3.0)	0 (0.0)	49 (3.3)
Live birth	153 (93.3)	6 (75.0)	62 (88.6)	64 (97.0)	11 (100)	1381 (94.3)
Birth maturity*						
Preterm birth	8 (5.2)	2 (33.3)	8 (12.9)	7 (10.9)	0 (0.0)	88 (6.4)
Full term birth	145 (94.8)	4 (66.7)	54 (87.1)	57 (89.1)	11 (100)	1293 (93.6)
Birth weight*						
Low birth weight	8 (5.2)	1 (16.7)	1 (1.6)	2 (3.1)	0 (0.0)	69 (5.0)
Normal birth weight	145 (94.8)	5 (83.3)	61 (98.4)	62 (96.9)	11 (100)	1312 (95.0)
Congenital anomalies	1 (0.6)	0 (0.0)	1 (1.4)	2 (4.1)	0 (0.0)	19 (1.3)

*Excluding abortion and stillbirth outcomes

Abbreviation: AL = Artemether-lumefantrine; SP = Sulfadoxine-pyrimethamine

Table 6.3: Pregnancy outcomes in relation to antimalarial exposure status in first trimester

Variables	Outcomes		Crude OR (95% CI)	P [μ]	Adjusted OR [α] (95%CI)	P [μ]
Birth outcome	**MC/SB n (%)**	**Live birth n (%)**				
AL exposure						
Yes	13 (12.3)	159 (9.5)	1.3 (0.7 – 2.4)	0.348	1.4 (0.8 – 2.5)	0.295
No	93 (87.7)	1518 (90.5)				
Quinine exposure						
Yes	10 (9.4)	68 (4.1)	2.5 (1.2 – 4.9)	0.011	2.5 (1.3 – 5.1)	0.009
No	96 (90.6)	1609 (95.9)				
SP exposure						
Yes	2 (1.9)	64 (3.8)	0.5 (0.1 – 2.0)	0.318	0.5 (0.1 – 2.0)	0.312
No	104 (98.1)	1613 (96.2)				
Amodiaquine exposure						
Yes	0 (0.0)	11 (0.7)	- (0)	-	- (0)	-
No	106 (100)	1666 (99.3)				
No antimalarial exposure						
Yes	83 (78.3)	1380 (82.3)	0.8 (0.5 – 1.3)	0.301	0.8 (0.5 – 1.2)	0.260
No	23 (21.7)	297 (17.7)				
Birth weight (grams)	**< 2500 n (%)**	**≥ 2500 n (%)**				
AL exposure						
Yes	9 (11.1)	150 (9.4)	1.2 (0.6 – 2.5)	0.608	1.2 (0.6 – 2.5)	0.573
No	72 (88.9)	1446 (90.6)				
Quinine exposure						
Yes	2 (2.5)	66 (4.1)	0.6 (0.1 – 2.4)	0.463	0.6 (0.1 – 2.4)	0.461
No	79 (97.5)	1530 (95.9)				
SP exposure						
Yes	2 (2.5)	62 (3.9)	0.6 (0.2 – 2.6)	0.520	0.7 (0.2 – 3.0)	0.639
No	79 (97.5)	1534 (96.1)				
Amodiaquine exposure						
Yes	0 (0.0)	11 (0.7)	- (0)	-	- (0)	-
No	100 (100)	1585 (99.3)				
No antimalarial exposure						
Yes	69 (85.2)	1311 (82.1)	1.3 (0.7 – 2.3)	0.485	1.2 (0.6 – 2.3)	0.564
No	12 (14.8)	285 (17.9)				
Maturity status at birth	**Preterm n (%)**	**Term n (%)**				
AL exposure						
Yes	10 (8.9)	149 (9.5)	0.9 (0.5 – 1.8)	0.812	0.9 (0.5 – 1.8)	0.865
No	103 (91.1)	1415 (90.5)				
Quinine exposure						
Yes	10 (8.9)	58 (3.7)	2.5 (1.3 – 5.1)	0.010	2.6 (1.3 – 5.3)	0.007
No	103 (91.1)	1506 (96.3)				
SP exposure						
Yes	7 (6.2)	57 (3.6)	1.7 (0.8 – 3.9)	0.177	1.8 (0.8 – 4.1)	0.160

No	106 (93.8)	1507 (96.4)				
Amodiaquine exposure						
Yes	0 (0.0)	11 (0.7)	- (0)	-	- (0)	-
No	113 (100)	1553 (99.3)				
No antimalarial exposure						
Yes	88 (77.9)	1292 (82.6)	0.7 (0.5 – 1.2)	0.205	0.7 (0.5 – 1.1)	0.168
No	25 (22.1)	272 (17.4)				

OR= Relative Risk; CI = Confidence Interval

$^{\mu}$ Estimated from the logistic regression model with Wald type P-value

$^{\alpha}$ = Adjusted for age and parity

Abbreviation: MC = Miscarriage; SB = Stillbirth; AL = artemether-lumefantrine; SP = Sulfadoxine-pyrimethamine

PART IV: EFFECTIVENESS OF ANTIMALARIAL DRUGS IN PREGNANCY

Chapter 7: Population pharmacokinetics and clinical response of artemether-lumefantrine in pregnant and non-pregnant women with uncomplicated Plasmodium *falciparum* malaria in Tanzania

Dominic Mosha[1,2*], Monia Guidi[3,4], Felista Mwingira[2], Salim Abdulla[1], Thomas Mercier[4], Laurent Arthur Decosterd[4], Chantal Csajka[3,4], Blaise Genton[2,5]

[1]Ifakara Health Institute, Rufiji HDSS, Rufiji, Tanzania

[2]Swiss Tropical and Public Health Institute, University of Basel, Switzerland

[3]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Lausanne, Switzerland

[4]Division of Clinical Pharmacology and Toxicology, Department of Laboratories, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland

[5]Department of Ambulatory Care and Community Medicine & Division of Infectious Diseases, University Hospital, Lausanne, Switzerland

Accepted in *Antimicrobial Agent and Chemotherapy Journal*

Abstract

Background: Artemether-Lumefantrine (AL) is the first line treatment for uncomplicated malaria in second and third trimester of pregnancy. Its efficacy has recently been challenged in pregnancy due to altered pharmacokinetic (PK) properties in this vulnerable group. The aim of this study was to determine the PK profile of AL in pregnant and non-pregnant women and assess their therapeutic outcome.

Methods: Thirty-three pregnant women and 22 non-pregnant women with malaria were treated with AL (80/480mg) twice daily for 3 days. All patients provided five venous plasma samples for drug quantification at random times over 7 days. Inter- and intra-individual variability was assessed and covariates effects quantified using a nonlinear mixed-effect modeling approach (NONMEM®).

Results: A one-compartment model with first-order absorption and elimination with linear metabolism from drug to metabolite fitted the data best for both arthemether (AM), lumefantrine (LF) and their metabolites. Pregnancy status and diarrhea showed a significant influence on LF PK. Lumefantrine relative bioavailability and metabolism rate into desmethyl-lumefantrine were respectively 34% lower and 78% higher in pregnant women than in non-pregnant patients. Overall PCR-uncorrected treatment failure was 18% in pregnant women and 5% in non-pregnant women (OR = 4.04; p value 0.22). A high median day 7 lumefantrine concentration was associated with adequate clinical and parasitological response.

Conclusion: The observed reduction in lumefantrine relative bioavailability in pregnant women may explain the higher treatment failure in this group, mostly due to lower post-treatment prophylaxis. Hence, a modified treatment regimen of malaria in pregnancy should be considered.

Keywords: Pharmacokinetics, Arthemether, Lumefantrine, Pregnancy

Background

Malaria in pregnancy is a major public health problem, which is associated with high maternal and perinatal mortality in tropical and subtropical regions [90]. Pregnant women are at increased risk of clinical malaria compared to non-pregnant women because of the associated immunological and hormonal changes in pregnancy [10]. Substantial direct risks to pregnant women include severe maternal anaemia, and those affecting the baby are intra-uterine growth retardation, intrauterine death, stillbirth, premature delivery, low birth-weight, and perinatal and neonatal morbidity and mortality [2]. Because of all this, malaria in pregnancy should be treated effectively.

Artemether-lumefantrine (AL) (20mg and 120mg, respectively) is one of the most popular and efficacious fixed dose artemisinin-based combination therapies (ACT) against *Plasmodium falciparum*. It is currently available at a subsidized cost in most malaria endemic countries. AL has proved to be non-inferior to quinine in East Africa for the treatment of *P falciparum* infection in second and third trimester of pregnancy [41]. ACTs are recommended by the World Health Organization (WHO) as the first line treatment for uncomplicated malaria in second and third trimester of pregnancy [37]. Unfortunately, general inter-individual variability on drug absorption, distribution to different compartments of the body and tissues, plasma binding proteins, rate of metabolism, enterohepatic recirculation, and excretion may be associated with changes in bioavailability of a drug and consequently may affect the therapeutic efficacy [107].

Pregnancy has been reported to affect the efficacy of some drugs, including antimalarials. This is due to physiological changes which lower drug absorption, speed up drug clearance and increase body fluid volume of distribution [48, 108, 109]. Elevation of estrogens, progesterone, cortisol and prolactin hormones during pregnancy have been linked to altered metabolic activity of several hepatic cytochrome P450 enzymes. For instance, catalytic activity of CYP3A4, CYP2C9 and CYP2A6 enzymes increases during pregnancy [110, 111], and these enzymes are responsible for lumefantrine and artemether metabolism [112, 113]. Hence, it is expected that significant alteration of the pharmacokinetics (PK) of most antimalarial drugs during pregnancy occurs, which may be associated with lower drug concentrations and lower antimalarial cure rate, especially in advanced pregnancy [47, 51, 114]. A higher treatment failure rate has indeed been observed in pregnant women when compared to non-pregnant ones living in the same area [51]. Several PK studies on artemether (AM), lumefantrine (LF) and their respective metabolites, dihydroartemisinin (DHA) and debutyl-lumefantrine (DLF) have demonstrated low plasma concentration of these drugs in pregnant women compared to non-pregnant

adults. However, most of these studies included healthy male adult volunteers as a comparative group rather than female malaria patients [49, 51, 115, 116]. Because of various determinants of PK and therapeutic outcome, it is essential to have a comparative population of non-pregnant women of the same study area with the same disease.

An important concern during the course of AL treatment is to achieve adequate residual LF level after complete elimination of AM and DHA so that it may clear all residual malaria parasite [48]. Therefore, day 7 LF concentration level has been proposed as a surrogate marker for AL efficacy [64, 117]. Recent pharmacokinetics study of AL in Cambodia and Tanzania reported that the targeted day 7 LF concentration was also not achieved in a significant number of non-pregnant adult patients. In Tanzania, 35% of samples had LF concentration below the cut-off value of 175 ng/ml at day 7 [118]. In pregnancy, whereby host antiparasitic immunity is somehow compromised [10], a higher day 7 venous concentration of LF may be required than what has previously been proposed in studies involving non-pregnant adult patients i.e. a cut-off values of 175 ng/ml or 280 ng/ml in order to achieve effective therapeutic outcome and 600 ng/ml for maximal efficacy [119, 120]. Some predictive models have suggested that a twice-daily regimen of AL for 5 days would be preferable in later pregnancy in order to achieve sufficient drug concentration in plasma [49]. Increasing the duration of AL administration is indeed expected to increase the residual LF levels in the subsequent post-treatment cycle so as to reduce chances of recrudescence [118]. This should be interpreted with caution because extending the duration of treatment regimen may possibly lead to lower adherence. Doubling the dose might be another option but actually it may not be appropriate because absorption of LF is dose-limited [121].

The aim of the present study was to characterize the PK profile of AL and their metabolites, to determine their variability and to identify factors that might explain variations in drugs and metabolites levels in pregnant (second and third trimester of pregnancy) and non-pregnant women of the same area, and to assess cure rate and parasitological clearance in these two groups. The model developed for lumefantrine was used to simulate day 7 concentrations under standard and alternative dosage regimens and quantify the percentages of pregnant and non-pregnant women having concentrations below different proposed cut-off thresholds.

Material and methods

Study design and procedures

This study was conducted in Rufiji district, within a Coastal region in Eastern Tanzania. The asexual parasitaemia prevalence is 14% and *Plasmodium falciparum* is the predominant species [75]. The study

was carried out at Kibiti health center from April to September 2012. Approval for the study was granted by two independent ethical review bodies; (i) Research Ethics Committee of Ifakara Health Institute (IHI) and (ii) National Institute for Medical Research (NIMR) Ethical Committee. All women signed an informed consent prior to enrolment.

Pregnant and non-pregnant women diagnosed with uncomplicated malaria were recruited from either out-patient department or Reproductive and Child Health (RCH) clinic. Inclusion criteria were women aged 18 year and above, resident of Rufiji study area, pregnant during their second and third trimester, and having signs or symptoms suggestive of uncomplicated malaria with fever (axillary temperature ≥ 37.5^0C) or history of fever for the past 24 hours, *P falciparum* detected by microscopy, and hemoglobin level ≥ 7 g/dl. Exclusion criteria were known allergy to AL or quinine, history of renal, liver or heart problem, hyperparasitaemia above 200,000/μL, reported intake of any antimalarial within the past 28 days, unable to take oral medication, and vomiting the medication within 1 hour of first dose intake. The same criteria applied to non-pregnant women (control group) that were recruited concurrently during the same study period after informed consent. A full medical history including concomitant illness and concomitant medication was recorded. Clinical examination on the day of enrollment was performed by an experienced physician. Patients were also seen by the clinician during follow up visits at day 1, 2, 3, 7, 14, 28 and 42 whereby axillary temperature was measured as well as evaluation of malaria related symptoms [37]. Gestational age was determined from the estimated first day of the last normal menstrual period and compared to clinical examination of a fundal height. In case of any discrepancy, gestational age was recalculated from the estimated age at first RCH visit.

Drug regimen

Enrolled participants received four tablets of AL (Coartem® Novartis Pharma AG, Basel; 20 mg AM and 120 mg LF) over the course of 3 days at 0, 8, 24, 36, 48 and 60 hours. Each dose was administered with 200 ml of milk containing 4.5 g of fat because of the associated increase in LF bioavailability when taken with a fat rich meal [122]. All patients were asked to come back to the health center for each drug administration and observed for one hour after dose intake. None of the patient was admitted during the course of AL treatment but one pregnant woman who developed severe malaria at day 1 was admitted and the treatment was changed to intravenous quinine. A limited number of patients were administered drug at home by the study's clinician or field assistant.

Blood samples

To determine AM, DHA, LF and DLH concentration, 2 ml of venous blood sample was drawn from the patient at random times between 8 and 11 am on day 0, 1, 2, 3 and 7. Day 0 blood sample was collected before starting the medication as a baseline so as to determine the presence of any antimalarial in patient's plasma prior to treatment due to intake of non-declared drugs [123, 124]. The blood samples in an EDTA vacutainer® tube were centrifuged at 2,000 x g for 5 minutes and the plasma stored in cryotubes. Samples were kept at -25^{0}C for at most 6 weeks before transferred to Ifakara Health Institute (IHI) Bagamoyo clinical laboratory for temporary storage at -80^{0}C. All samples were packed in dry ice and shipped to clinical pharmacology laboratory of the University Hospital in Lausanne, Switzerland, to perform the drug bioassay.

To estimate the parasite density and clearance rate, capillary blood from a finger prick was taken at day 0, 3, 7, 14, 28 and 42. Samples were collected on blood slide Giemsa stained thick and thin blood smear were examined by two different experienced microscopists using light microscopy. Parasite in thick film fields were counted per 200 leukocytes and the parasite count was multiplied by a factor of 40 to give parasites per µl of blood. Approximately, 50µl of finger pricked blood was spotted onto Whatman® filter paper cards (3MM). DNA was extracted from Whatman® filter paper cards by Chelex method [125]. In order to differentiate between recrudescence and new infection, samples were genotyped by the most polymorphic marker the merozoite surface protein 2 (*MSP 2*) and the amplicons were visualized in a 2% agarose gel as described elsewhere [126].

Drug assay

Plasma concentrations of AM, DHA, LF and DLF were determined using a validated liquid chromatography-tandem mass spectrometry method (LC-MS/MS) [127]. The presence of 10 other antimalarial drugs and metabolites i.e. artesunate, amodiaquine, *N*-desethyl-amodiaquine, piperaquine, pyronaridine, mefloquine, chloroquine, pyrimethamine and sulfadoxine were also assessed at the same time. This is a standard procedure on how LC-MS/MS operates, so as to ensure that the malaria outcome that was observed was due to AL intake. The preciseness of the method is 3.1% – 12.6% for inter-day variation coefficient and its sensitivity is 0.15 – 3.0 ng/dl for lower limit quantification (LOQ) of basic or neutral antimalarial and 0.75 – 5 ng/dl for artemisinin derivatives. The bioassays were carried out at the Laboratory of clinical pharmacology of the Lausanne University Hospital, which takes part in the quality control system of the worldwide antimalarial resistance network (WWARN).

Efficacy assessment

AL efficacy was determined by cure rate and parasitological clearance. The definition of treatment response was according to WHO recommendations on the methods for surveillance of antimalarial drug efficacy [63]. Treatment response was thus classified into early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR). Participants who developed either clinical or parasitological failure as defined above received quinine 10 mg/kg of body weight three times a day for 7 days, quinine is a second line drug of choice.

Pharmacokinetic analysis

Drugs and their metabolites were modeled using the NONMEM computer program version 7.2 (NM-TRAN version II) [128]with the PsN-Toolkit version 3.5.3 [129]. The program uses mixed (fixed and random) effects regression to estimate population means and variances of the pharmacokinetic parameters and to identify factors that influence them.

Structural model. One and two-compartment models with first-order absorption and elimination and linear metabolisation to DLF and DHA were compared to describe, respectively, LF and AM pharmacokinetics with an additional compartment used to characterize metabolite data. The final estimated parameters were drug and metabolite systemic clearances (CL and CL_{met}), volume of distribution of the central compartment (V_C) and metabolism rate constant from the drug to the metabolite compartment (K_{23}). Owing to identification problems, the volume of distributions of DLF and DHA could not be estimated and were assumed to be equal to those of LF and AM, respectively. Because of the limited number of measurements in the absorption phase, the absorption rate constants (K_a) could not be adequately estimated and were thus fixed to 0.7 and 0.54 h^{-1} to achieve AM and LF peak plasma concentrations, respectively, 2 h and 6-8 h after drug intake [130]. Finally, the known pre-systemic conversion of AM into DHA was modeled estimating the fraction of the AM dose directly converted into the metabolite in the gut using $1-F_1$, with $F_1 = 1$ representing AM relative bioavailability. Since the drugs were given orally, these parameters represent apparent values. In case the analysis of baseline plasma samples showed non-zero concentration of the drugs (suggesting that AL was previously taken), a factor (F_0) was introduced in the model in order to estimate the residual doses from previous treatments.

Statistical model. Inter-patient variability of all the PK parameters was described by exponential errors following a log-normal distribution, as illustrated by the equation $\theta_j=\theta\cdot\exp(\eta_j)$, where θ_j is the pharmacokinetic parameter associated with the j^{th} individual, θ is the average population value, and η_j is

the j^{th} individual component of the inter-patient random effect, an independent, normally distributed variable with mean 0 and variance ω^2. In order to constrain individual F_1 to vary between 0 and 1, a logit function (logit F_1) was used. Correlations between PK parameters were also investigated. Finally, proportional, additive and combined proportional-additive error models were compared to describe the intrapatient (residual) variability for both drug and metabolite. The correlation between drug and metabolite concentration measurements was tested using the NONMEM® L2 item.

Covariate model. Available covariates were: pregnancy status, body weight, body mass index (BMI), age, gestational age and diarrhea. The covariate analysis was performed using a stepwise insertion/deletion approach. Visual inspection of the correlation between post hoc individual estimates of the PK parameters and the available patients' characteristics was first conducted. Potential covariates influencing the kinetic parameters were then incorporated sequentially and tested for significance in NONMEM®. This goal was achieved by modeling the typical value of the pharmacokinetic parameters θ to depend linearly on the covariate X (continuous covariates centered on the population median; dichotomous variables coded as 0 and 1) using $\theta=\theta_a \cdot (1+\theta_b \cdot X)$, where θ_a is the mean estimate and θ_b is the relative deviation of the mean due to the X covariate. In addition, body weight (BW) effect was modeled using the allometric function $\theta=\theta_a \cdot \left(\frac{BW}{MBW}\right)^{\vartheta_c}$, where MBW is the median population BW and θ_c was fixed to literature values, i.e. 0.75 for CL and 1 for V.

Selection of the model and parameter estimation. Drugs and metabolites were fitted by use of the first-order conditional (FOCE) method with interaction using the subroutine ADVAN5. Concentrations below the quantification limit (BQL) of the assay were generally treated using the M3 method described by Beal as implemented in the paper of Ahn et al [131, 132]. When using the L2 function, BQL data were replaced by LOQ/2 and handled with the M6 approach [132]. The log likelihood ratio test, based on differences in the OFV value (ΔOFV) provided by NONMEM®, was employed to discriminate between hierarchical models. Since a ΔOFV between any two models approximates a χ^2 distribution, a change of the objective function was considered statistically significant if it exceeded 3.84 ($p < 0.05$) or 6.63 ($p < 0.01$) for 1 additional parameter in model-building and backward-deletion steps respectively. Additional criteria for model selection were diagnostic goodness-of-fit plots, precision of pharmacokinetic parameters estimates, and the reduction of the parameters inter-patient variability.

Validation of the model. The stability of the final model was assessed by means of the bootstrap method implemented in PsN, generating two-thousand datasets by re-sampling from the original

dataset. Mean parameters values with their 95% confidential interval ($CI_{95\%}$) were derived and compared with the final pharmacokinetic model estimates. Model validation was performed by visual predictive checks (VPC), simulating data for 1000 individuals based on the final model and generating 2.5th, 50th and 97.5th percentiles. The observed concentrations were plotted against the 95% prediction interval ($PI_{95\%}$) of the simulated dataset at each time point and visually compared. Figures were generated with GraphPad Prism® (Version 6.00 for Windows, GraphPad Software, San Diego California USA, http://www.graphpad.com/).

Model-based stimulation for LF. The concentration-time profiles of LF in 1000 individuals receiving two different regimens of 6 doses over 3 days (at 0, 8, 24, 36, 48 and 60 h) and 5 days (at 0, 8, 24, 48, 72 and 96 h.) were derived by simulations based on the final model including inter-patient variability. Day 7 predicted median concentrations with their $PI_{95\%}$ for pregnant and non-pregnant women were derived. In addition, these simulations allowed quantifying the percentages of pregnant and non-pregnant women having a day 7 concentrations below different proposed cut-off thresholds of 175 ng/ml, 280 ng/ml and 600 ng/ml associated with treatment efficacy [64, 119].

Statistical analysis

Predicted lumefantrine day 7 concentrations for pregnant and non-pregnant women were compared using a t-test. The relationship between treatment failure and potential predictors associated with it, namely day 7 LF concentration, pregnancy status, gestational age, baseline parasite count, residual antimalarial and BMI was assessed by logistic regression estimating odds ratio (OR) and $CI_{95\%}$. A p-value below 0.05 was considered statistically significant. All the statistical analyses were performed using STATA® 12.0 (Stata Corporation, College Station, Texas, USA).

Results

Demographic and clinical parameters. Thirty-five pregnant women and 22 non-pregnant women with acute *Plasmodium falciparum* malaria were enrolled in the study from 23rd April to 5th September 2012. Two of the enrolled pregnant women were withdrawn from the study at day 2 and 7 because they refused to continue participating in the study. Two (9.1%) non-pregnant women were lost for follow-up at day 42. None of the pregnant women were lost for follow up. Baseline characteristics of pregnant and non-pregnant women are presented in *Table 7.1*. Two pregnant women presented with diarrhea at the day of enrollment and throughout the course of treatment. None of the study participants vomited the drug. All participants had normal physical condition on examination with no history of any chronic disease or smoking. Twenty-six women (14 pregnant and 12 non-pregnant) reported to have taken

paracetamol before enrollment. The median gestational age among pregnant women was 27 (14 – 37) weeks with relatively equal numbers of women in the second and third trimester of pregnancy.

Residual antimalarial. Blood samples from all 57 recruited participants in the study were screened to determine the presence of any antimalarial drugs prior to initiation of malaria treatment. Fifty-five (96.5%) had at least one antimalarial in their plasma: 89.5% (29 pregnant and 22 non-pregnant) of participants had plasma LF above the LOQ but the drug concentration was generally low with the average of 37.3 ng/ml. Other antimalarial drugss which were detected were DHL (14.0%) 8, AM (7%) 4, DHA (0.0%) 0, sulfadoxine (24.6%) 14, pyrimethamine (19.3%) 11 and quinine (1.8%) 1. Summarized statistics are shown in *Table 7.2*. Out of 14 participants detected with sulfadoxine, 13 were pregnant with a median baseline parasitaemia of 72086 (range 3920 – 198080) counts/µL [*Figure 7.1*]. Sulfadoxine concentration persisted at relative constant concentration throughout the first 7 days of monitoring plasma drug levels.

Population pharmacokinetic analysis

A total of 265 LF, 263 DLF, 146 AM, and 98 DHA plasma concentrations were included in the analysis. Twenty-five percent (n=37) AM, 7% (n=7) DHA and 2% (n=4) DLF concentrations were below the respective LOQs. The median (range) of samples available per study subject was 5 (4 – 5) for LF, 4 (3 – 5) for DLF, 3 (1 – 5) for AM and 2 (1 – 4) for DHA.

Artemether

AM and DHA pharmacokinetics were best described using a one-compartment model with first-order absorption from the gastrointestinal tract and linear metabolism to DHA, including pre-systemic conversion into the metabolite. Elimination of both compounds was modeled using a first-order process. The few basal AM concentrations did not allow estimating a residual dose from previous treatments. Inclusion of an inter-patient variability on V_c, CL_M, K_{23} or F_1 in addition to AM CL did not improve description of the data ($\Delta OFV \geq -1.9$, $p \geq 0.17$). A mixed error model best described residual intra-patient variability for AM and a proportional one for DHA. No correlations between the drug and the metabolite concentrations could be identified. Our results show that 21% of the AM dose is converted pre-systemically into DHA. None of the available covariates significantly affected AM or DHA pharmacokinetics ($\Delta OFV \leq 3.0$, $p \geq 0.08$). Although non-significant, an increase of 37% in drug CL in pregnant women compared to non-pregnant ones was however observed ($\Delta OFV = -1$, $p = 0.32$). The final model parameters' estimates and bootstrap evaluations are given in *Table 7.3*. The model was

considered reliable since the obtained parameter estimates laid within the bootstrap $CI_{95\%}$. VPC graphs of AM and DHA are shown in *Figure 7.2A*.

Lumefantrine

A one-compartment model with first-order absorption and elimination adequately described LF data. A two-compartment model did not improve the model fit (ΔOFV = -0.1, p = 0.75). Average dose from previous treatment (F_0) was estimated to be 3.2 mg with a large inter-individual variability (ΔOFV = -40, p = $2.5 \cdot 10^{-10}$). In addition to CL, an inter-patient variability on V_c (ΔOFV = -23, p = $1.6 \cdot 10^{-6}$) and a correlation between CL and V_c improved significantly the fit (ΔOFV = -117, p = $2.9 \cdot 10^{-27}$). The assignment of an inter-patient variability on LF bioavailability F_1 (fixed to 1) accounting for the correlation between CL and V_c and their variability resulted in additional improvement of the model fit (ΔOFV = -9.5, p = $8.7 \cdot 10^{-3}$). Metabolite concentrations were included in the model using a supplementary compartment with linear metabolism from the LF central compartment. The addition of an inter-individual variability on K_{23} improved significantly the description of the data (ΔOFV = -41, p = $1.5 \cdot 10^{-10}$), while no enhancement was observed when assigning variability on CL_M (ΔOFV = -0.02, p = 0.89). Residual intra-patient variability was best described using a proportional and mixed error model for LF and DLF, respectively. The model was further improved by including a correlation between drug and metabolite concentrations (ΔOFV = -85, p = $3.0 \cdot 10^{-20}$).

In univariable analyses, pregnancy and diarrhea were identified as significant covariates for both F_1 (ΔOFV = -5.1, p = 0.024 and ΔOFV = -15, p = $1.1 \cdot 10^{-4}$) and K_{23} (ΔOFV = -13, p = $3.1 \cdot 10^{-4}$ and ΔOFV = -4, p = 0.045). None of the remaining covariates influenced LF and DLF pharmacokinetics (ΔOFV ≥ -1.4, p ≥ 0.24). Multivariable combination of the significant covariates showed an additive influence of pregnancy and diarrhea on F_1 and pregnancy on K_{23} (ΔOFV = -33, p = $3.2 \cdot 10^{-7}$ with respect to the model without covariates). Our results show that relative bioavailability is 34% lower and metabolism rate 78% higher in pregnant women compared to non-pregnant patients. A decrease of 83% in F_1 was observed in women with diarrhea as compared to those who had no diarrhea. *Table 7.3* illustrates the final model parameters' estimates together with their bootstrap evaluations. The model was considered reliable since the obtained parameter estimates laid within the bootstrap $CI_{95\%}$. *Figure 7.2B* shows the concentration time-plots of LF and DLF for pregnant and non-pregnant women included in the analysis with average population predictions and 95% intervals.

Concentration-time simulation of lumefantrine

The day 7 predicted median concentrations of LF after administration of a 6-dose regimen over 3 days were 908 ($PI_{95\%}$: 217 – 3256) ng/ml for pregnant women and 1382 ($PI_{95\%}$: 386 – 5135) ng/ml for non-pregnant women (p = 0.10). While considering the large inter-patient variability in the kinetics of LF, 3% of the pregnant women would have day 7 concentrations below the cut-off value of 175 ng/ml, 9% below 280 ng/ml and 31% below 600 ng/ml. For non-pregnant women, 1% would exhibit day 7 concentrations below the cut-off value of 170 ng/ml, 2% below 280 ng/ml and 15 % below 600 ng/ml. Prolonging the time of drug administration over 5 days among pregnant women would provide median concentrations of 1374 ($PI_{95\%}$ 367 – 5536) ng/ml, with 0.1%, 2% and 16% of patients with concentrations below the cut-off value of 175 ng/ml, 280 ng/ml and 600 ng/ml, respectively [*Figure 7.3*].

Pharmacodynamics

There were a total of 7 therapeutic failures in the study, 6 (18.2%) pregnant women and 1 (4.5%) non-pregnant woman (OR = 4.04; p = 0.22). Among pregnant women, one developed ETF at day 1. She presented with signs and symptoms suggestive of severe malaria, was admitted and kept on full dose of intravenous quinine. One pregnant women had LCF, presented with fever (body temperature = 38.7^0C) at day 20, blood slide confirmed to have parasitaemia of 10,750 counts/μL. The remaining four pregnant women had LPF, one at day 28 and three at day 42. One non-pregnant woman had LPF at day 28. Hence, the overall PCR uncorrected efficacy of AL in the study was 87%, 82% (6/33) in pregnant women and 95% (1/22) in non-pregnant women. PCR investigation confirmed recrudescent infection in two women, one with ETF and the other with LCF, both pregnant; the remaining 5 (71%) had new infections.

The median and inter-quarterly range (IQR) for day 7 plasma concentration was 957 (409 – 1541) ng/ml in pregnant and 1179 (782 – 1807) ng/ml in non-pregnant women [*Figure 7.4A*]. Although non-significant, day 7 LF concentration was lower among women with therapeutic failure than those with ACPR. The median (and IQR) for LF concentration among women with ACPR was 1070 (751 – 1665) ng/ml whereas, for the women with LCF and LPF it was 730 (227 – 774) ng/ml (p = 0.075) [*Figure 4B*]. Twenty percent of study participants had day 7 LF concentrations below 600 ng/ml. Only two patients (33%) out of six among the ones who developed LCF and LPF had day 7 LF concentrations below 600 ng/ml and all were pregnant. Potential predictors of treatment failure in addition to day 7 LF concentration were pregnancy status, gestational age, baseline parasite count, residual antimalarial and BMI and none was statistically significant. No participant during the study period had miscarriage, stillbirth or any other severe adverse effect(s) related to AL.

Discussion

The study describes the pharmacokinetic properties of AM, LF and their active metabolites DHA and DLF in pregnant and non-pregnant women with malaria. The role of different covariates that could influence AL bioavailability, distribution and clearance in the two groups were carefully analyzed. The study differs from previous reports of population pharmacokinetics of AM and LF in pregnancy [49, 51, 116] by having a comparative group of non-pregnant women with malaria from the same population with relatively similar characteristics.

Prior treatment. Detectable residual antimalarial among recruited participants was unexpectedly high. This might be explained by uncontrolled prescription of AL, a first line malaria treatment, which is highly available and easily accessible from both private and public facilities [65, 66]. Prevalence of residual antimalarial among participants was higher than what was reported five years ago from *in vivo* studies in Ifakara (Tanzania) and Praeh Vihear (Cambodia) which was 74.3% and 50%, respectively [123, 124]. Such high prevalence of residual antimalarial levels in this population, particularly LF, is alarming because it can promote emergence and spread of drug resistance parasite. Also, the high residual prevalence of LF, irrespective of pregnancy trimester suggests a considerable AL exposure in the first trimester. There is an urgent need to monitor closely the implementation of standard malaria treatment guideline and discourage self-treatment by not acquiring antimalarial from drug venders without attended and screened for presence of malaria parasitaemia. Significant levels of detected sulfadoxine among pregnant women were probably the result of SP received from RCH clinic for Intermittent Preventive Treatment (IPTp).

Pharmacokinetics. The important study finding was the lower LF plasma concentration among pregnant patients compared to non-pregnant ones. This is similar to what has been observed in a Thailand study in which the concentration of LF was approximately half that of non-pregnant patients from historical data in the same population [51]. The reason for low LF concentration may be due to physiological changes related to pregnancy status which accounts for reduced absorption, expanded volume of distribution, elevated drug metabolism and clearance rate [107]. The observed increase in LF metabolism rate among pregnant women is explained by hormonal changes in pregnancy which increases catalytic activity of hepatic enzymes such as CYP3A4, an important enzyme for LF metabolism [111]. The design of the study did not allow displaying the effect of reduced absorption on LF bioavailability.

Altered bowel condition such as having diarrhea during malaria treatment has a significant effect on drug absorption and consequently lowers drug bioavailability. Increase of gastro-intestinal motility due to diarrhea reduces intestinal transit time of a drug, and this time is important to maximize drug absorption [133]. The latter explains why LF concentration, a high lipophilic compound, was 83% lower in women with diarrhea compared to the ones with no diarrhea. It is therefore important to assess for presence of diarrhea in patients and correct dosage regimens accordingly.

It is important to study concentration levels of a slowly eliminated partner antimalarial drug such as LF so as to determine minimum parasiticidal concentration (MPC) and minimum inhibitory concentration (MIC) of malaria parasite [117]. The observed day 7 median concentration of LF was lower in pregnant than in non-pregnant women. However, the concentration among pregnant women was twofold higher compared to what had been observed in Thai pregnant patients [49]. It is also higher than the concentrations previously reported in non-pregnant adults and paediatric patients in Ifakara-Tanzania, Thailand, Cambodia and the Lao People's Democratic Republic [118, 120, 134, 135]. Higher day 7 LF levels in the present study may be due to the administration of a standard recommended adult dose of AL with food [37] to all patients regardless of the patient's body weight.

.

The simulations under the standard 6 dose of AL over 3 days schedule show that a non-negligible number of pregnant women would have LF concentrations below various proposed therapeutic threshold targets at day 7. Splitting the same recommended total dose over a 5 day regimen would greatly improve the probability of exhibiting therapeutic drug concentrations. The latter has already been shown in other pharmacokinetics studies [118, 119, 136], but the benefit might be jeopardized by poor adherence to treatment in the prolonged regimen. Hence, a formal assessment of feasibility should be performed.

Pharmacodynamics. The observed cure rate and parasite clearance in pregnant women was lower compared to that of non-pregnant patients despite having the same median baseline parasitaemia. The observed lower LF concentration at day 7 among the patients with therapeutic failure could be one of the reasons explaining this difference. In order to improve therapeutic efficacy, it is therefore important to consider dose increase or modifying treatment regimen to allow higher day 7 LF concentrations. Day 7 LF concentration above 600 ng/dl was associated with 100% efficacy among pregnant patients in Thailand [137]. The latter was not observed in our study; indeed 3 out of the 5 (60%) pregnant women

with LCF or LTF had day 7 LF concentration above 600 ng/ml. This observation suggests that the proposed 600 ng/dl cut-off value better predicts parasite clearance of ongoing infection, rather than occurrence of new infection in the follow-up period. 600 ng/dl LF concentration at day 7 is not high enough to ensure post-treatment prophylaxis effect up to day 42. Indeed, reinfections were not all prevented with a day 7 LF concentration of 600 ng/ml. Partner drugs with longer half-life might offer better protection [117].

Baseline parasitaemia was not an important factor to determine therapeutic response among study participants. Indeed mean baseline parasite count in patients with ACPR was twofold higher compared to the ones with therapeutic failure. This is contrary to what has been reported in previous studies involving pregnant and non-pregnant patients in which patients with higher baseline parasitaemia were more likely to fail treatment [136, 137]. However, therapeutic failure rate among pregnant women in our study was much lower than that observed in Thailand in recent AL pharmacodynamics studies whereby therapeutic failure among pregnant patients was more than 30% [49, 137]. We have reason to believe that AL is more efficacious in Africa than in Southeast Asia where resistance to other antimalarial drugs such as quinine, mefloquine and artesunate has increased [47, 138].

Conclusion

The current AL treatment regimen in pregnancy is challenged by having low post-treatment prophylactic effect. Pregnancy is an important associated factor for low plasma concentration of LF probably due to reduced drug absorption, elevated drug metabolism and rapid clearance rate. It is therefore important to evaluate new treatment regimens of AL in this vulnerable group that would target higher day 7 LF concentration levels.

Competing interests

The authors declare that they have no competing interests

Acknowledgements

We sincerely thank the patients for their coorperation and all staff involved in the study. Special thanks are given to Sigilbert Mrema, Athumani Mzuyu, Bakari Kissa, Fadhili Mwakitete and Sajidu Ismail of Rufiji Health Demographic Surveillance System (HDSS), and Jackson Thomas and Happy Mkali of IHI Bagamoyo Clinical Laboratory. This work was supported by Ifakara Health Institute (IHI) and European and Developing Countries Trial Partnership (EDCTP) through Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) project.

Tables and figures

Table 7.1: Characteristics of study participants with *P falciparum* malaria on the day of enrollment

Characteristics	Pregnant women (n=33) Median (range)	Non-pregnant women (n=22) Median (range)
Age (years)	25 (18 -41)	21.5 (18 -35)
Body weight (Kg)	52 (40 – 80)	48.5 (41 – 79)
Height (cm)	158 (147 – 169)	157 (150 – 174)
BMI	21.8 (16.5 – 30.1)	20.3 (16.4 – 33.3)
Haemoglobin (g/dl)	10.2 (7.1 – 13.3)	13.4 (8 – 15.5)
Temperature (^{0}C)	37.1 (36.0 – 39)	37.2 (36.0 – 39.6)
Parasitaemia (counts/µL)	25,280 (560 – 198,080)	22,280 (560 – 195,680)
Gestation age (weeks)	27 (14 – 37)	NA
**Pregnancy – trimesters*		
Second trimester (%)	17 (52)	NA
Third trimesters (%)	16 (48)	NA

*Trimester presented in number (%). NA means not applicable

Abbreviation: BMI = Body Mass Index

Table 7.2: Plasma concentration of residual antimalarial drugs detected prior to treatment with AL in 57 recruited study patients [ng/ml]

Antimalarial	Patients (%)	Plasma concentration [ng/ml]			
		Mean	Median	Minimum	Maximum
Lumefantrine	51 (89.5)	37.3	24.9	5.4	205.5
Desbutyl-limefantrine	8 (14.0)	2.5	1.5	0.3	6
Artemether	4 (7)	26.4	2.9	0.4	157.3
Sulfadoxine	14 (24.6)	1,334.3	1,298.1	5.3	3,615.6
Pyrimethamine	11 (19.6)	6.9	5.3	1.7	18
Quinine	1 (1.8)	12.3	12.3	12.3	12.3

Table 7.3: Final population parameter estimates of artemether, lumefantrine and their metabolites and their bootstrap evaluations in 2000 replicates

Population pharmacokinetics analysis					**Bootstrap evaluation**			
Parameter	**Estimate**	**SE[a] (%)**	**IIV[b] (%)**	**SE[c] (%)**	**Estimate**	**$CI_{95\%}$[d]**	**IIV[b] (%)**	**$CI_{95\%}$[d]**
Artemether								
CL (L/h)	98	24	99	65	102	69-140	93	66-120
V_c (L)	373	16			354	225-492		
LogitF_1	1.4	27			1.5	0.7-2.6		
K_a (h^{-1})	Fixed to 0.70							
V_M (L)	Fixed to V_c							
K_{23} (h^{-1})	0.084				0.088	0.05-0.16		
CL_M(L/h)	71	46			69	38-136		
$\sigma_{prop,AM}$ (CV%)	72	26			69	49-87		
$\sigma_{add,AM}$ (µmol/L)	0.13	7			0.13	0.03-0.20		
$\sigma_{prop,DHA}$ (CV%)	53	14			51	44-59		
Lumefantrine								
CL (L/h)	2.8	12			2.8	2.2-3.6		
V_c (L)	134	14			134	101-174		
F_1	Fixed to 1	65	50				61	43-77
θ_{PregF1}	-0.33	37			-0.31	-(0.52-0.05)		
$\theta_{diarrF1}$	-0.84	15			-0.78	-(0.95-0.44)		
K_a (h^{-1})	Fixed to 0.54							
V_M (L)	Fixed to V_c							
F_0 (mg)	2.7	18	87	46	2.95	1.9-4.4	116	70-164
K_{23} (h^{-1})	$1.6\cdot10^{-4}$		46	54	$1.6\cdot10^{-4}$	$(1.2\text{-}2.0)\cdot10^{-4}$	44	31-57
$\theta_{PregK23}$	0.80	32			0.80	0.4-1.3		
CL_M(L/h)	2.6	15			2.6	1.9-3.5		
$\sigma_{prop,LF}$ (CV%)	51	32[c]			51	45-56		
$\sigma_{prop,DLF}$(CV%)	39	40[c]			38	32-44		
Correlation LF/DLF	68	18			67	63-69		
$\sigma_{add,DLF}$(µmol/L)	$4.4\cdot10^{-3}$	17[c]			$4.9\cdot10^{-3}$	$(3.8\text{-}6.1)\cdot10^{-3}$		

Abbreviations: CL: clearance, V_C: central volume of distribution, logitF_1: logit F_1 expressed as a logit function, k_a: first-order absorption rate constant, V_M: volume of distribution of the metabolite, F_0:

residual amount from the previous treatment, k_{23}: metabolism rate constant, CL_{met}: metabolite clearance, σ_{prop}: exponential residual error, σ_{add}: additive residual error, $\theta_{X\ PAR}$: effect of the X covariate on the parameter PAR expressed as (1- $\theta_{X\ PAR}$ X).

[a] Standard error (S.E.) of the estimate θ_i defined as S.E estimate/estimate, expressed as a percentage

[b] Inter-individual variability

[c] Standard error (S.E.) of the coefficient of variation or the additive component of the residual error defined as √S.E estimate/estimate, expressed as a percentage

[d] 95% confidence interval (C.I.)

Figure 7.1: Relationship between parasite density at enrollment and plasma residual levels of sulfadoxine prior treatment in 14 pregnant women

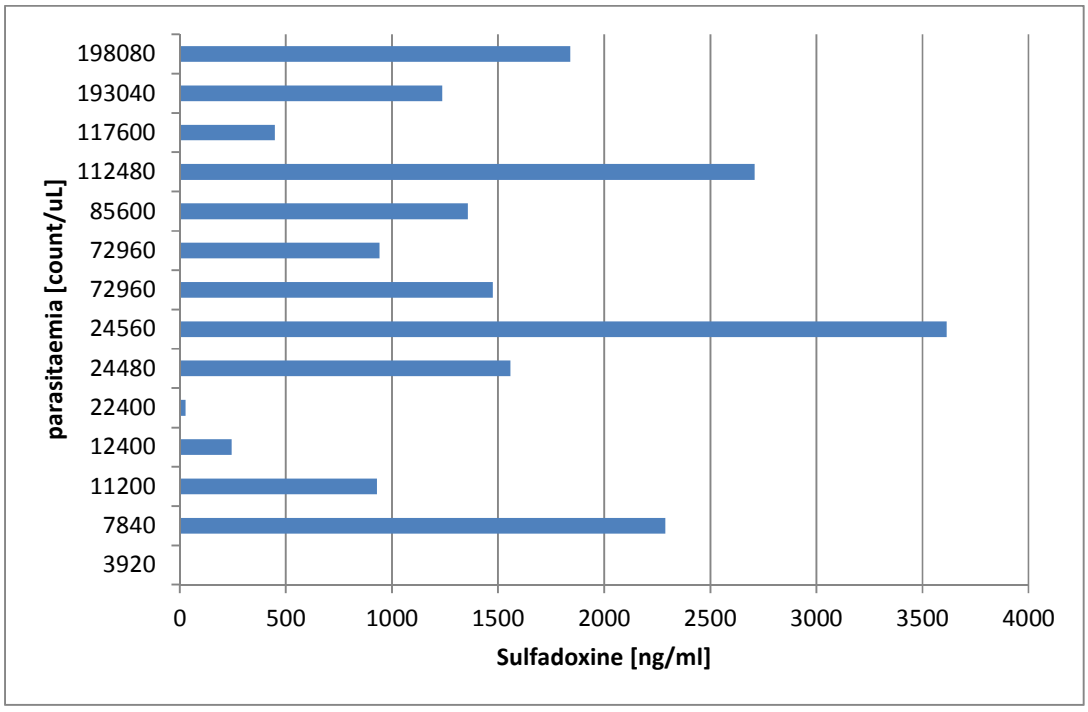


Figure 7.2A: Observed AM (left panel) and DHA plasma concentrations (right panel). Filled and empty circles represent pregnant and non-pregnant women, respectively. The solid line represents the average predicted concentrations and the dashed lines the 95th prediction intervals.

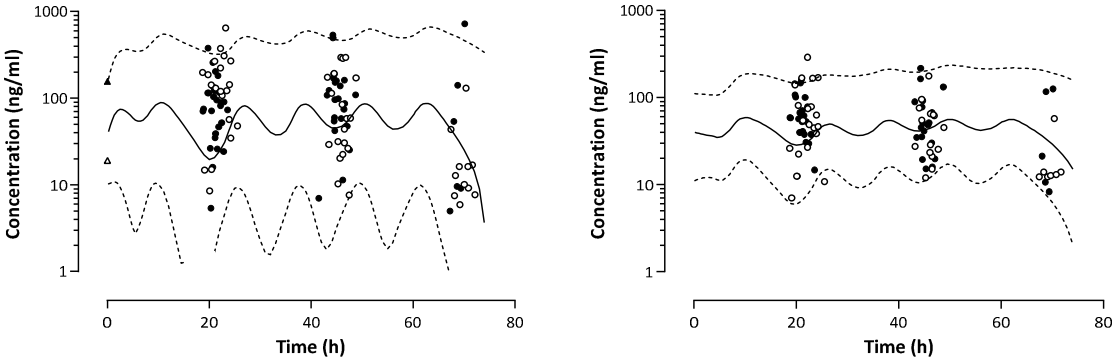


Figure 7.2B: Observed LF (upper panels) and DLF plasma concentrations (lower panels) in pregnant and non-pregnant women. Triangles residual plasma concentrations of LF and DLF found prior treatment initiation. The solid lines represent the mean population prediction and the dotted lines $PI_{95\%}$.

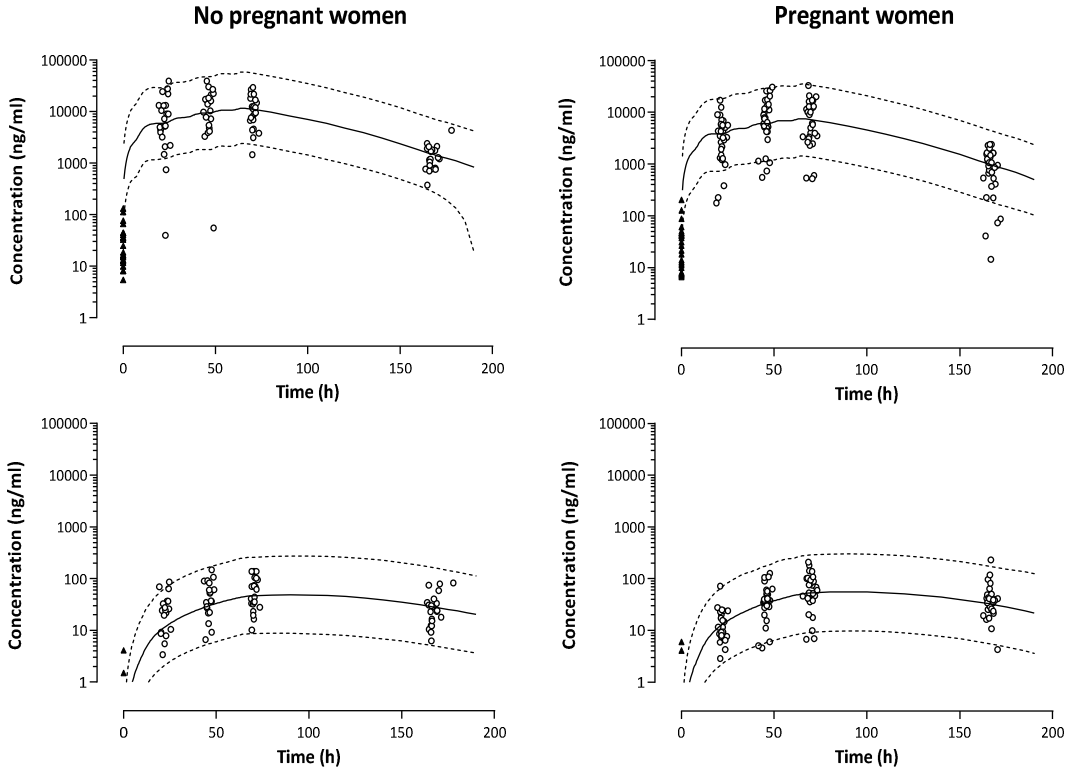


Figure 7.3: Predicted median concentration of lumefantrine (LF) after administration of 6·480 mg regimen over 3 (continuous line) and 5 days (dotted line) in pregnant women. Day 7 (168h) median predicted concentrations (circles) with their $PI_{95\%}$ are shown for the two dosage regimens.

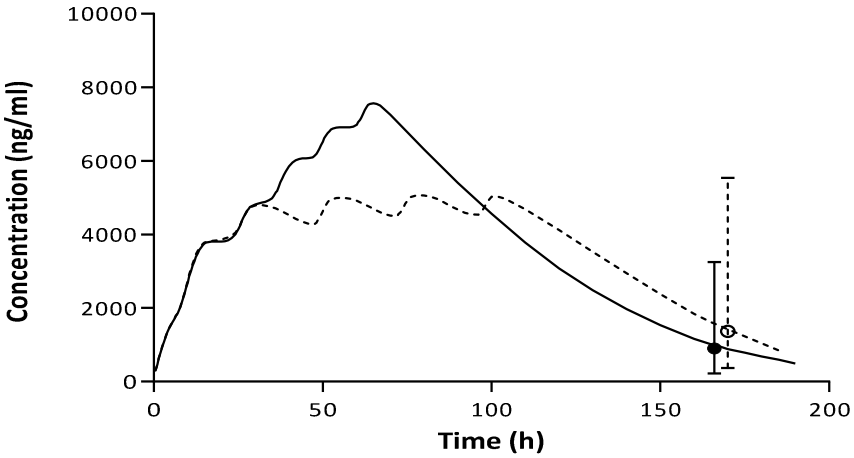


Figure 7.4A: Day 7 plasma concentration of lumefantrine in pregnant (n = 32) and non-pregnant (n = 22) study women

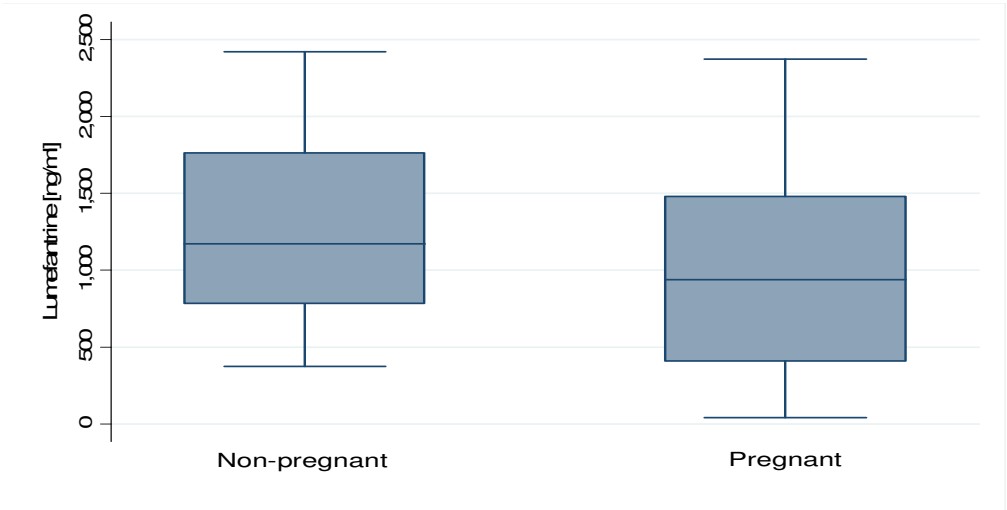
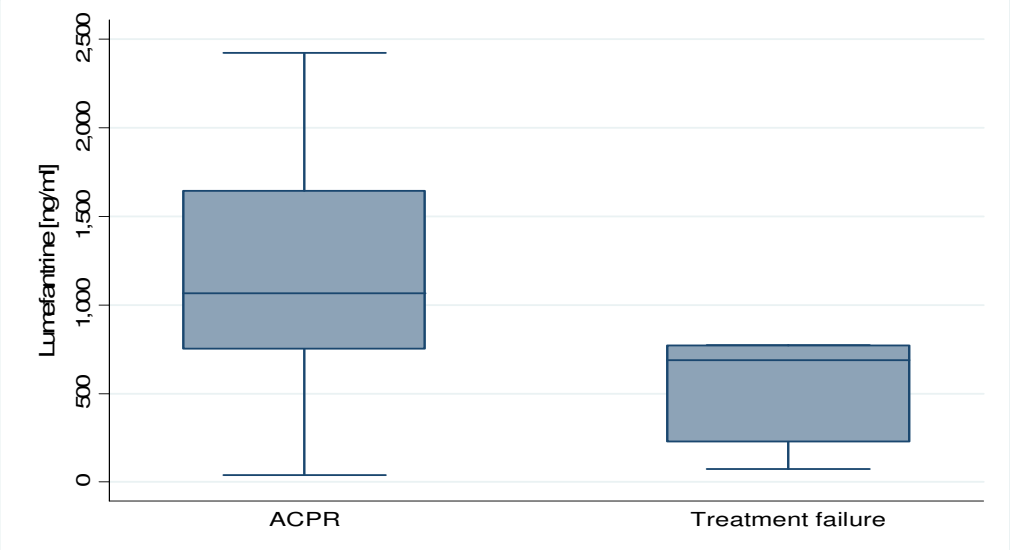


Figure 7.4B: Day 7 plasma concentration of lumefantrine in women with ACPR (n = 48) and those with treatment failure (n = 6) *



* Day 7 lumefantrine concentration could not be assessed in one woman since a rescue treatment with quinine was given at day 1 because of early treatment failure.

Chapter 8: Effectiveness of Intermittent Preventive Treatment with sulfadoxine-pyrimethamine during pregnancy on placental malaria, maternal anaemia and birth weight in areas with high and low malaria transmission intensity in Tanzania

Dominic Mosha*[1,2,3], Jaff Chilongola[3], Rabi Ndeserua[4], Felista Mwingira[2], Blaise Genton[2,5]

[1]Ifakara Health Institute, Rufiji HDSS, Rufiji, Tanzania

[2]Swiss Tropical and Public Health Institute, University of Basel, Switzerland

[3]Kilimanjaro Christian Medical University College, Moshi, Tanzania

[4]Muhimbili Orthopaedic Institute, Dar es Salaam, Tanzania

[5]Infectious Disease Service & Department of Ambulatory Care and Community Medicine, University of Lausanne, Lausanne, Switzerland

Under review in *Tropical Medicine & International Health Journal*

Abstract

Background

Intermittent Preventive Treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) has been shown to decrease placental malaria and pregnancy-associated malaria morbidity, and improve birth outcomes in highly endemic areas, but its benefit is unclear in areas with moderate to low transmission intensity and where parasite resistance to SP is high. The aim of the study was to assess the effectiveness of IPTp in areas with two different malaria transmission intensity.

Method

A prospective study recruiting pregnant women was conducted in two health facilities situated in areas with high and low malaria transmission intensity. A structured questionnaire was used to interview for socio-demographic characteristics and obstetrics history. Placental parasitaemia was screened using both light microscopy and real-time quantitative PCR.

Results

350 pregnant women were recruited and screened for placental parasitaemia, 175 from each area. Prevalence of placental parasitaemia was 16.6% (CI 11.4–22.9) in high transmission area and 2.3% (CI 0.6–5.7) in low transmission area. Primigravidae and residing in high transmission area were significant risk factors for placenta malaria (OR 2.4; CI 1.1–5.0; p=0.025) and (OR 9.4; CI 3.2–27.7; p< 0.001), respectively. IPTp was associated with decreased risk of placental malaria (OR 0.3; CI 0.1–1.0; p=0.044); the effect was more pronounced in high transmission area (OR 0.2; CI 0.06–0.7; p=0.015) than in low transmission area (OR 0.4; CI 0.04–4.5; p=0.478). IPTp use was not associated with decreased risk of maternal anaemia or low birth weight, regardless of transmission intensity. The number needed to treat (NNT) was 4 (CI 2 – 6) women in high transmission area and 33 (20 – 50) in low transmission area to prevent one case of placental malaria.

Conclusion

IPTp may have effect on lowering the risk of placental malaria in areas of high transmission, but this effect did not translate into a benefit on risk of maternal anaemia or low birth weight. The NNT needs to be considered, and weighted against that of other protective measures, eventually targeting areas which are above a certain threshold of malaria transmission in order to maximize the benefit.

Keywords: IPTp-SP, Placental malaria, Anaemia, Low birth weight

Background

Approximately 85 million pregnant women throughout the world are at risk of *Plasmodium falciparum* infection every year, two-third are in sub-Sahara Africa [1]. Pregnancy-associated malaria (PAM) is an important known cause of maternal and neonatal morbidity such as severe maternal anaemia, intra-uterine growth retardation, low birth weight (LBW), premature delivery, intrauterine death, stillbirth, and can lead to maternal or neonatal mortality [90]. Apart from malaria, anaemia in pregnancy may be secondary to iron, folate and vitamin B deficiency, sickle cell diseases, HIV or helminthic, which may also lead to LBW [139].

Human placenta is a preferred site for *P. falciparum* to accumulate and hence lead to deleterious consequence to both mother and the unborn baby [140].Infected erythrocytes express a unique variant surface antigen (VSA) which mediates adherence to chondroitin sulphate-A (CSA) receptors in the placental endothelium and sequester in the intervillous spaces. Sequestration leads to placental insufficiency which is due to inflammatory reaction to the infected erythrocytes. The reaction causes vasoconstriction and vascular damage which jeopardize haemodynamics of placenta [10, 19]. Primigravidae are particularly vulnerable to placental malaria than multigravidae because they lack neutralizing antibodies to CSA binding parasites that cause placental sequestration [141]. Placental parasitaemia, maternal anaemia and LBW are known to be more frequent in areas with stable malaria transmission because of the considerable higher level of acquired malaria immunity among women than in unstable transmission areas [142].

Prompt and effective case management of malaria illness and anaemia, use of insecticide treated nets (ITNs), and use of at least 2 doses for intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) after quickening have long been recommended by the WHO for malaria control especially in areas with stable malaria transmission [27]. IPTp regimen has recently been modified: it is now required that the first IPTp-SP dose to be administered as early as possible during second trimester and SP doses be given at least one month intervals up to delivery [32]. There is a growing concern that IPTp-SP effectiveness may be jeopardized by the high degree of resistance of *P falciparum* to SP. The latter is reported to be a major concern in East Africa and hence questions the viability of IPTp-SP use in this area [33, 143-145]. However, a meta-analysis by Kayentao *et al* [34] confirms the beneficial effects of 3 or more doses of IPTp in reducing the risk of LBW.

With the reported significant decline of malaria in most areas of East Africa including Tanzania [100, 146, 147], it becomes essential to evaluate the benefit of routine IPTp-SP, especially in areas with low malaria

transmission. This may assist decision-making on the relevance of advocating universal IPTp in areas where the rate of SP adverse reactions may outweigh the benefit of the treatment. For example, SP is not recommended to HIV infected women receiving cotrimoxazole prophylaxis or antiretroviral drugs due to fear of an increased occurrence of adverse drug reaction [148]. Little is known regarding effectiveness of IPTp-SP over other preventive measures for PAM morbidity in areas with low malaria transmission [32]. The aim of the study was to assess the effect of IPTp on placental malaria, PAM morbidity and birth outcomes in areas with high and low malaria transmission intensity.

Methods

Study area

The study was carried out in Moshi municipal, north-eastern Tanzania and Rufiji district in the eastern, coastal area of the country. Moshi is a low malaria transmission area with malaria prevalence of 1.0% while Rufiji district is a moderate to high malaria transmission area with prevalence of 20.8% [149]. The prevalence of *P falciparum* dihydropteroate synthase (Pfdhps) gene 581G mutation was 56% in infected malaria cases, according to the evaluation conducted six years ago in Tanga, a region adjacent to the two study areas [150]. This same area had a day 14 SP treatment failure rate as high as 68% among children [151]. Mawenzi Hospital in Moshi and Kibiti health Centre (HC) in Rufiji were involved to recruit study participants. In the year 2012, malaria accounted for 4% of the total out-patient cases in Mawenzi Hospital and 51% in Kibiti HC [152]. Both health facilities are public own with free antenatal care and delivery services. The standard of care and capacity to handle obstetrics emergences in the two facilities is similar.

Study design

A prospective study was conducted from July to October 2012, enrolling pregnant women who came for delivery in Mawenzi Hospital and Kibiti HC obstetric wards. The selection criteria for recruiting the participants included residency in the study area for at least one year, age of 16 years and above, gestational age of 28 weeks and above, and presence of up-to-date medical information. Cases of multiple pregnancies, severe conditions such as eclampsia, haemorrhage and sepsis were excluded. A structured questionnaire was used to interview women. Information from the mother's medical registry and Reproductive and Child Health (RCH) clinic card were used to verify and complement the generated information. Recorded information included social demographic characteristics, parity, ITNs use, history of malaria illness during pregnancy, use of IPTp-SP, haematemics and anthelmintic drugs. Gestational age was estimated based on the date of last normal menstrual period and compared with the estimated

fundal height recorded during first RCH booking. Birth weight below 2,500 grams was defined as LBW, preterm birth below 37 weeks of gestational age, and maternal anaemia was haemoglobin level below 11 g/dl. Placental malaria infection was defined as parasite positive results based on either blood slide smear reading or Polymerase Chain Reaction (PCR) results. Purpose of performing the two malaria screening tests was to maximize chances of detecting infected placentas.

Sample collection and examination

Placental blood was collected within one hour after delivery. Incision was made from at least three different sites of placenta on the maternal side where accumulated intervillous blood was collected using a blunt syringe. About two drops of collected blood (about 100 µL) were spotted onto a 903whatman® filter paper (3MM), air dried and preserved in plastic zipped locked bags for PCR genotyping. Filter papers were then transferred to the laboratory and DNA was extracted using Chelex® method [125]. The DNA was stored at -20^{0}C until further used. Malaria parasite positivity was determined by quantitative real-time PCR targeting the *P falciparum* S-type gene as described by Wampfler *et al* [153].

Thick and thin blood smear were also prepared and stained with Giemsa. Blood slide were examined independently by two experienced laboratory technicians using light microscope. Discrepancy findings were reviewed by a third independent technician and hence a consensus on positivity was reached. Parasite in thick film fields were counted per 200 leukocytes and the parasite count was multiplied by a factor of 40 to give parasites per µl of blood.

Statistical analysis

Data analysis was performed using STATA® 12.0 (Stata Corporation, College Station, Texas, USA). Numerical variables were summarized into median and range. Categorical variables were summarized using cross tabulation to estimate different proportion. The primary outcome was the proportion with placental malaria. The bivariable models included IPTp, maternal age, gravidity, transmission intensity level, history of malaria illness in pregnancy, HIV status, and ITNs as explanatory variables. Variables associated with the outcome having a p value < 0.2 in the bivariable model were retained in the final adjusted logistic regression model to estimate odds ratio (OR) and 95% confidence intervals (CI). The same method was done for secondary outcomes, i.e maternal anaemia and LBW.

Ethics

Ethical approval for the study was granted by the Kilimanjaro Christian Medical University College (KCMUCo) research ethics committee, recognized by the National Institute for Medical Research (NIMR). Written informed consent was obtained from all participants after each was informed individually regarding study's procedures. For a participant below 18 years, written consent was provided by a guardian or husband who was above 18 years.

Results

A total of 350 pregnant women were recruited and screened for placenta parasitaemia, 175 from the high malaria transmission area (Rufiji) and 175 from low malaria transmission area (Moshi). There was no early maternal death or referral to a tertiary health facility during the study period. The mean maternal age and gestational age at the time of recruitment was 25.2 years (standard deviation [SD] 6.9) and 37.2 (SD 2.2) weeks, respectively. 319 (91%) of recruited women reported to have used one or more dose of IPTp. The median (interquartile range [IQ] gestational age when the first IPTp dose was administered was 24 weeks (19 – 32) while, the median (IQ) gestational age at the second administration of IPTp dose was 30 weeks (24 – 36). Main demographic, screening parameters and pregnancy outcome information are presented in *table 8.1.*

Prevalence of placental malaria and associated factors

Prevalence of placental malaria was 16.6% (29/175) in the high transmission area and 2.3% (4/175) in the low malaria transmission. In high malaria transmission area, the prevalence of placenta malaria was 8% (14/175) by light microscopy and 15.4% (27/175) by PCR, whereas in low transmission area, it was 1.1% (2/175) and 1.7% (3/175) respectively.

Details of variables associated with placental malaria by bivariable and logistic regression model are shown in *Table 8.2.* Women living in high transmission areas were nine times more likely to have placental malaria than those living in low transmission areas (adjusted OR 9.4; CI 3.2 – 27.7; p< 0.001). Primigravidae were twice more likely to have placental malaria than multigravidae (adjusted OR 2.4; CI 1.1 – 5.0; p=0.025). There was no evidence of the association between placental malaria and HIV status or history of malaria during pregnancy.

Effectiveness of IPTp on placental malaria

In high transmission area, out of the 163/175 (93.1%) women who reported to have used at least one dose of IPTP, 24 (14.7%) were found to have placental malaria, while 139 (85.3%) had no placental

malaria. Among the 12/175 (6.9%) who reported not to have used IPTp during their pregnancy, 5 (41.7%) were found to have placental malaria. In low transmission area, of the 156/ 175 (89%) women who reported to have used at least one dose of IPTp, 3 (2.0%) were found to have placental malaria. Among the 19 (10.9%) who reported not to have used IPTp, one had placental malaria. In multivariate analysis, one dose or more of IPTp had 80% protective efficacy against placental malaria in high transmission area (adjusted OR 0.2; CI 0.06 – 0.7; p=0.015), while it was 60%,in low transmission area (adjusted OR 0.4; CI 0.04 – 4.5; p=0.478) [*Table 8.3a and 8.3b*]. There was no significant relationship between number of IPTp doses taken and prevalence of placental malaria in high transmission area or in low malaria transmission (Figure 8.1).

Number needed to treat (NNT) with IPTp to prevent placental malaria

In high transmission area, 24 out of 163 women who used IPTp had placental malaria versus 5 out of 12 women who did not use IPTp. This gives an absolute reduced risk of 27% for women to have placental malaria after using at least one dose of IPTp. Thus, in high malaria transmission area, 4 (CI 2 – 6) pregnant women needed to be treated with IPTp to prevent one case of placental malaria (NNT = 1/0.27 = 3.7 = ~ 4 women). By stratifying for gravidity, 14 out of 54 primigravidae who used IPTp in the same transmission area had placental malaria versus 2 out of 4 primigravidae who did not use IPTp. Among multigravidae, 10 out of 109 who used IPTp had placental malaria versus 3 out of 8 women who did not use IPTp. The absolute reduced risk in primigravidae in this area was 24% (0.5 – 0.26 * 100) while it was 29% (0.38 – 0.09 * 100) in multigravidae. Therefore, 4 (CI 2 – 4) primigravidae needed to be treated in high transmission area with at least one dose of IPTp to prevent one case of placental malaria (NNT = 1/0.24 = 4.2), versus 3 (CI 3 – 11) for multigravidae (NNT = 1/0.29 = 3.4).

In low transmission area, 3 out of 156 women who used at least one dose of IPTp had placental malaria versus 1 out of 19 women who did not use IPTp. This gives an absolute reduced risk of 3% (0.05 – 0.02 * 100). Therefore, in low transmission area, 33 (CI 20 – 50) pregnant women needed to be treated with at least a single dose of IPTp to prevent one case of placental malaria (NNT = 1/0.03 = 33.3 = about 33 women). One out of 69 primigravidae in the same transmission area had placental malaria whereas there was none among those who did not use IPTp at all. For multigravidae in this transmission area, 2 out of 87 who used IPTp had placental malaria versus one out of 15 of who did not use IPTp. The absolute reduced risk in primigravidae in this area was less than 0 and for multigravidae was 4% (0.067-0.023 * 100). Therefore, 25 (CI 14 – 50) multigravidae needed to be treated in low transmission area with at least a single dose of IPTp to prevent one placental malaria (NNT = 1/0.04 = 25).

IPTp and maternal anaemia

A total of 223/350 (63.7%) study women had haemoglobin concentration measured before delivery. The prevalence of maternal anaemia in high transmission area was 60.4% (81/134; CI 51.6 – 68.8) whereas it was 43.8% (39/89; CI 33.3 – 54.7) in low transmission area. Hence, living in area of high malaria transmission was associated with a significant increased risk of maternal anaemia when compared to low endemic area (adjusted OR 1.8; CI 1.0 – 3.2; p =0.036) [*Table 8.4*]. The prevalence of maternal anaemia in high transmission area among women who used at least one dose of IPTp was 61% (76/125), not much different than the 56% (5/9) among those who did not use IPTp (adjusted OR 1.2; CI 0.3 – 4.8; p = 0.755) [*Table 8.3a*]. In low transmission area, prevalence of anaemia was 46.2% (36/78) among women who used at least one dose of IPTpversus27.3% (3/11) among those who did not (adjusted OR 2.6; CI 0.6 – 10.7; p = 0.191) [*Table 8.3b*].Among other explanatory variables, placental malaria, gravidity, history of malaria infection during pregnancy, HIV status, anthelminthic and use of iron and folate supplementation at least a month during pregnancy period all had no statistical significant effect on maternal anaemia [*Table 8.4*].

IPTp and low birth weight

The prevalence of LBW in the high transmission area was 6.3% (11/175; CI 3.2 – 11.0) versus 4% (7/175; CI 1.6 – 8.1) in low transmission area (adjusted OR 1.7; CI 0.6 – 4.5; p = 0.293) [*Table 8.5*]. The prevalence of LBW in high transmission area among women who used at least one dose of IPTp was 5.5% (9/163) versus 16.7% (2/12) for those who did not use IPTp (adjusted OR 0.3; CI 0.1 – 1.5; p = 0.146) [*Table 8.3a*]. The prevalence of LBW in low transmission area among women who used at least one dose of IPTp was 3.8% (6/156) versus 5.3% (1/19) for those who did not (adjusted OR 0.7; CI 0.1 – 6.4; p = 0.757) [*Table 8.3b*]. Among other explanatory variables, placental malaria, gravidity, history of malaria infection during pregnancy, HIV status, anthelminthic and use of iron and folate supplementation at least a month during pregnancy period all had no statistical significant effect on the risk of LBW [*Table 8.5*].

Discussion

To our knowledge, the present study is the first to evaluate the effectiveness of IPTp in relation to placental malaria in areas with different malaria transmission intensities and high parasite resistance to SP. The evaluation takes into account other malaria preventive measures such as ITNs, and other preventive measures against maternal anaemia such as routine anthelminthic, iron and folate

supplementation. The study responded to a call from WHO that emphasized the importance of enhanced regular monitoring of IPTp effectiveness [32].

The prevalence of placental malaria was eight times higher in the high transmission area compared to the low transmission area (17% vs 2%). The overall prevalence of 9% in the present study corresponds to 8% prevalence which was observed nine years ago in Ifakara, another part of the country [154]. It shows that malaria in pregnancy is still an important health issue in Tanzania, especially in high transmission settings, that need to be addressed by effective preventive measures. Self-reporting of ITNs use by 95% of pregnant women is encouraging considering the reported ITNs efficacy of 23% against placental parasitaemia, 33% against miscarriage/stillbirth and 23% against LBW according to a systematic reviews of randomized trials [30]. In the present study, all women with placental malaria reported to have used ITNs, which precludes any effectiveness calculation. Our assessment of IPTp effectiveness applies thus only in condition of full ITN coverage.

Use of IPTp was associated with 80% protection against placental malaria in the high transmission area, and 60% in the low transmission one. The study findings agree with previous studies and reviewed evidence of IPTp to reduce the risk of placental malaria [34, 155-157]. This is achieved because each IPTp dose clears or suppresses any concurrent malaria parasites in the placenta and provides about 6 weeks post treatment prophylaxis [27, 158]. There was no IPTp dose increase relationship with reduced risk of placental malaria. The latter is opposite to what was reported by Kayentao K *et al* [34] in a systematic review that risk of placental infection decreases with increased number of IPTp doses which lead to the new WHO IPTp regimen of monthly IPTp administration from early second trimester[32]. However, our study was not powered to determine the effect of increasing dose on placental malaria. On the other hand, it may well be that the time interval between the last IPTp administrated dose and screening for placental malaria is a stronger determinant for detecting parasitaemia rather than the cumulative number of doses a woman received during her pregnancy. Indeed, the prophylactic effect of a drug declines when its plasma concentration decreases with time. The present study had a limitation to document the period interval between the last IPTp dose and delivery date, the moment when placenta sample was collected for screening of parasitaemia. This is essential to consider, particularly in high transmission areas whereby daily chances for a woman to have infectious mosquito bites are higher.

Due to the observed significant risk of having placental malaria when residing in high transmission area, the value for money of IPTp was much higher in areas of high transmission intensity due to its observed effect of reducing risk for placental malaria. Indeed, it was generally required to treat eight times less women in this area compared to low transmission area with at least one dose of IPTp to prevent one case of placental malaria. Because of the known higher risk of placental malaria in primigravidae, we attempted to estimate the NNT in this group versus the multigravida ones in both areas, but the small sample size and uneven representation of the exposure groups, both being potential biases of the study did not allow having meaningful results. In view of the importance to determine NNT in other malaria interventions in pregnancy such as ITN use, 18 and 50 pregnant women are the NNT with ITN to prevent at least one placental malaria and a LBW respectively, observed in a randomized trial of ITN in high malaria transmission area in Kenya [29]. The NNT should be more regularly used as criterion to prioritize interventions, especially so in moderate to low transmission areas. This estimation is essential in an era of declining malaria. Other preventive measures of malaria in pregnancy, such as intermittent screening and treatment (IST) with an effective antimalarial, have shown to be non-inferior to IPTp-SP against placental malaria, severe maternal anaemia and LBW in a randomized control trial in Ghana [159]. Effectiveness and/or value for money of IST in East Africa region may be higher than IPTp-SP because of the considerable higher SP resistance in this area as opposed to West Africa. Combining both IST and ITN use could help to overcome unnecessary monthly exposure to SP, especially in low transmission areas. However, having an effective, cheaper and user-friendly malaria screening test in pregnancy for universal coverage in antenatal clinics is an important challenge. The additional benefit of indoor residual spraying (IRS) in different level of malaria endemicity should probably also be part of this exercise

It is important to note that the coverage of IPTP second dose in most of sub Saharan countries is below 60% [31]. In the present study, IPTp second dose coverage was 51% with no difference between either of the two transmission areas. The key question is if the observed coverage of IPTp second dose is so low, what will be the coverage achieved for third and more doses. All this call for effective evaluation of the proposed IPTp regimen in comparison with novel proposed preventive measures of malaria in pregnancy.

The primary aim of malaria preventive measures in pregnancy is to prevent deleterious effects of malaria in woman and the baby. The usual concept is that IPTp reduces maternal parasitaemia, and hence maternal anaemia, placental malaria and LBW. IPTp effectiveness was challenged by Harrington

et al., [33] who conducted a study in Muheza, Tanzania, an area known to have high SP resistance, where IPTp did not decrease the risk of placental malaria, nor had an effect on improving maternal anaemia or LBW. A further challenging finding was reported by Gutman *et al*., [160] in Malawi which showed that IPTp did not reduce the frequency of placental malaria but was associated with improved birth outcomes. We ourselves found that IPTp did reduce placental malaria, but had no effect on maternal anaemia. For LBW, there was still a beneficial effect but the latter was not significant, probably due to the low rate in both study areas. Women deliver at home may have had an adverse outcome but our study had limitation of recruiting at delivery. However, inconsistences of IPTp effectiveness on improving maternal anaemia and LBW have also been reported in a systematic review by McClure EM *et al.,* [161]. It is likely that malaria is only one of the important contributors of maternal anaemia and LBW in developing countries. It will be thus essential in the coming years to monitor the changes in maternal malaria morbidity and the dynamic of LBW rates in areas of declining malaria transmission [146, 147] to better understand the respective role of malaria, malnutrition, infections (apart from malaria) and social-economic factors on maternal and baby outcomes.

Conclusion

The study shows that IPTp was associated with a lower rate of placental malaria, but this effect did not translate into protection against maternal anaemia and low birth weight. The NNT may suggest IPTp as an appropriate malaria control intervention, at least in areas with high level of malaria transmission. IPTp benefit is questionable in areas of low transmission. The NNT should be regularly evaluated in different level of malaria transmission and parasite resistance, different geographic settings, and on both mother and infant outcomes to best maximize benefit at reasonable costs.

Competing interest

The authors declare that they have no competing interest

Acknowledgements

We sincerely thank the pregnant women for their cooperation and all staff of Mawezi hospital and Kibiti HC who were involved in the study. Special thanks go to Athumani Mzuyu, Fadhili Mwakitete and Bakari Kissa of Rufiji HDSS, Jackson Thomas and Happy Mkali of IHI Bagamoyo Clinical Laboratory, and Alutu Masokoto of KCMC Teaching Hospital in Moshi.

Tables and figures

Table 8.1: Characteristics of study participants from the Moshi and Rufiji study sites

Characteristics	**Moshi (n = 175) n (%)**	**Rufiji (n = 175) n (%)**	**Total 350 (%)**
Age (years)			
< 20	35 (20.0)	50 (28.6)	85 (24.3)
20 – 35	126 (72.0)	104 (59.4)	230 (65.7)
> 35	14 (8.0)	21 (12.0)	35 (10.0)
Gravidity			
Primigravidae	73 (41.7)	58 (33.1)	131 (37.4)
Multigravidae	102 (58.3)	117 (66.9)	219 (62.6)
IPTp use			
Not at all	19 (10.9)	12 (6.9)	31 (8.9)
Single dose	66 (37.7)	72 (41.1)	138 (39.4)
Two or more doses	90 (51.4)	91 (52.0)	181 (51.7)
ITNs use			
Yes	161 (92.0)	170 (97.1)	331 (94.6)
No	14 (8.0)	5 (2.9)	19 (5.4)
HIV status			
Positive	8 (4.5)	4 (2.3)	12 (3.4)
Negative	160 (91.4)	171 (97.7)	331 (94.6)
No results	7 (4.0)	0 (0.0)	7 (2.0)
Haemoglobin level (g/dl)*	11.1 (1.7)	10.5 (1.4)	10.7 (1.6)
Parasite density (count/µl)*	18 (14.1)	269.9 (336.5)	238.4 (324.9)
Pregnancy outcome			
Birth outcome			
Live birth	172 (98.3)	172 (98.3)	344 (98.3)
Stillbirth	3 (1.7)	3 (1.7)	6 (1.7)
Birth weight			
≥ 2500 gram	168 (96.0)	164 (93.7)	332 (94.9)
< 2500 gram	7 (4.0)	11 (6.3)	18 (5.1)
Gestational age at birth			
Term	149 (85.1)	150 (85.7)	174 (49.7)
Preterm	26 (14.9)	25 (14.3)	176 (50.3)

*Haemoglobin level and placenta parasite density presented in mean (sd)

Table 8.2: Strength of association between placental malaria and other factors

Variable	Placental malaria		Crude OR (95% CI)	p	Adjusted OR$^{\alpha}$ (95% CI)	p$^{\mu}$
	Yes 33 (%)	No 317 (%)				
Age (years)						
< 25	14 (42)	146 (46)	0.9 (0.4 – 1.8)	0.860	2.5 (0.7 – 9.5)	0.164
≥ 25	19 (58)	171 (54)				
Gravidity						
Primigravidae	17 (52)	114 (36)	1.9 (0.9 – 3.9)	0.083	2.4 (1.1 – 5.0)	0.025
Multigravidae	16 (48)	203 (64)				
Transmission						
High	29 (88)	146 (46)	8.5 (2.9 – 24.7)	< 0.001	9.4 (3.2 – 27.7)	< 0.001
Low	4 (12)	171 (54)				
History of malaria						
Yes	6 (18)	62 (20)	0.9 (0.4 – 2.3)	0.849	1.1 (0.4 – 2.9)	0.846
No	27 (82)	255 (80)				
HIV status*						
Positive	1 (3)	11 (4)	0.9 (0.1 – 7.0)	0.904	1.2 (0.1 – 11.2)	0.883
Negative	31 (97)	300 (96)				
ITNs use						
Yes	33	317	-	-	-	-
No	0	0				

*Seven women had no HIV results
$^{\mu}$Estimated from the logistic regression model
$^{\alpha}$Adjusted for gravidity and area of malaria transmission

Table 8.3a: IPTp use in relation to placenta malaria, maternal anaemia and low birth weight in high malaria transmission areas

Variable	Frequency		Crude OR (95% CI)	p	Adjusted OR$^{\alpha}$ (95% CI)	p$^{\mu}$
	Yes	*No*				
Placental malaria	*29 (%)*	*146 (%)*				
IPTp use						
Yes	24 (82.8)	139 (95.2)	0.2 (0.1 – 0.8)	0.023	0.2 (0.1 – 0.7)	0.015
No	5 (17.2)	7 (4.8)				
*Maternal anaemia**	*Yes*	*No*				
	81 (%)	*53 (%)*				
IPTp use						
Yes	76 (93.8)	49 (92.5)	1.2 (0.3 – 4.8)	0.756	1.2 (0.3 – 4.9)	0.755
No	5 (6.2)	4 (7.5)				
Low birth weight	Yes	No				
	11 (%)	164 (%)				
IPTp use						
Yes	9 (81.8)	154 (93.9)	0.3 (0.1 – 1.5)	0.146	0.3 (0.1 – 1.5)	0.146
No	2 (18.2)	10 (6.1)				

*No haemoglobin level information in 41 women
$^{\mu}$Estimated from the logistic regression model
$^{\alpha}$Adjusted for gravidity

Table 8.3b: IPTp use in relation to placenta malaria, maternal anaemia and low birth weight in low malaria transmission areas

Variable	Frequency		Crude OR (95% CI)	p	Adjusted OR$^{\alpha}$ (95% CI)	p $^{\mu}$
	Yes	*No*				
Placental malaria	*4 (%)*	*171 (%)*				
IPTp use						
Yes	3 (75.5)	153 (89.5)	0.4 (0.03 – 3.6)	0.378	0.4 (0.04 – 4.5)	0.478
No	1 (25.5)	18 (10.5)				
*Maternal anaemia**	*Yes*	*No*				
	39 (%)	*50 (%)*				
IPTp use						
Yes	36 (92.3)	42 (84.0)	2.3 (0.7 – 9.3)	0.247	2.6 (0.6 – 10.7)	0.191
No	3 (7.7)	8 (16.0)				
Low birth weight	Yes	No				
	7 (%)	168 (%)				
IPTp use						
Yes	6 (85.7)	150 (89.3)	0.7 (0.1 – 6.3)	0.767	0.7 (0.1 – 6.4)	0.757
No	1 (14.3)	18 (10.7)				

* No haemoglobin level information in 41 women 86

$^{\mu}$Estimated from the logistic regression model

$^{\alpha}$Adjusted for gravidity

Table 8.4: Strength of association between maternal anaemia and other factors

Variable	Anaemia		Crude RR (95%CI)	p	Adjusted RR$^{\alpha}$(95% CI)	p$^{\mu}$
	Yes 120 (%)	No 103 (%)				
Placental malaria						
Infected	20 (16.7)	10 (9.7)	1.9 (0.8 – 4.2)	0.133	1.5 (0.6 – 3.4)	0.362
Not infected	100 (83.3)	93 (90.3)				
Gravidity						
Primigravidae	42 (35.0)	44 (42.7)	0.7 (0.4 – 1.2)	0.238	0.7 (0.4 – 1.3)	0.261
Multigravidae	78 (65.0)	59 (57.3)				
Transmission						
High	81 (67.5)	53 (51.5)	2.0 (1.1 – 3.4)	0.015	1.8 (1.0– 3.2)	0.036
Low	39 (32.5)	50 (48.5)				
History of malaria						
Yes	24 (20.0)	19 (18.4)	1.1 (0.6 – 2.1)	0.769	1.1 (0.6 – 2.2)	0.737
No	96 (80.0)	84 (81.6)				
HIV status*						
Positive	4 (3.4)	4 (3.9)	0.9 (0.2 – 3.5)	0.835	0.9 (0.2 – 3.9)	0.912
Negative	115 (96.6)	99 (96.1)				
Iron & folates$^{\neq}$						
Yes	105 (88.2)	91 (89.2)	0.8 (0.4 – 2.1)	0.819	0.8 (0.3 – 1.8)	0.552
No	14 (11.8)	11 (10.8)				
Anthelminthic						
Yes	108 (90.0)	89 (86.4)	0.3 (0.4 – 1.2)	0.406	1.1 (0.5 – 2.6)	0.804
No	12 (10.0)	14 (13.6)				

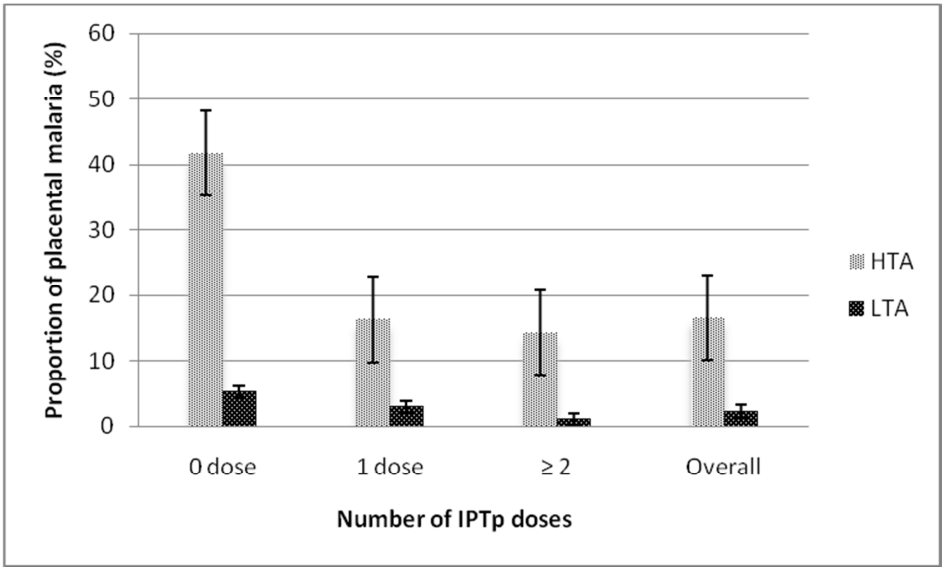
*One woman had no HIV result; $^{\neq}$missing iron and folate use information in two women;
$^{\mu}$Estimated from the logistic regression model
$^{\alpha}$Adjusted for placental malaria, and transmission intensity

Table 8.5: Strength of association between low birth weight and other risk factors

Risk factor	Low birth weight		Crude RR	p	Adjusted RR[α]	p[μ]
	Yes	No	(95% CI)		(95% CI)	
	18 (%)	332 (%)				
Placental malaria						
Infected	3 (16.7)	30 (9.0)	2.0 (0.6 – 7.4)	0.290	2.1 (0.6 – 7.5)	0.279
Not infected	15 (83.3)	302 (91.0)				
Gravidity						
Primigravidae	7 (38.9)	124 (37.3)	1.1 (0.4 – 2.8)	0.895	1.1 (0.4 – 3.1)	0.809
Multigravidae	11 (61.1)	208 (62.7)				
Transmission						
High	11 (61.1)	164 (49.4)	1.6 (0.6 – 4.3)	0.337	1.7 (0.6 – 4.5)	0.293
Low	7 (38.9)	168 (50.6)				
History of malaria						
Yes	6 (33.3)	62 (18.7)	2.2 (0.8 – 6.0)	0.134	2.2 (0.8 – 6.1)	0.130
No	12 (66.7)	270 (81.3)				

[μ]Estimated from the logistic regression model
[α]Adjusted for history of malaria

Figure 8.1: Prevalence of placental malaria in relation to IPTp doses taken in low and high malaria transmission areas



HTA = High transmission area; LTA = Low transmission area

PART V: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Chapter 9: Discussion

This chapter discusses the main study findings on antimalarial drugs used for the treatment of malaria during pregnancy. It includes important challenges and pending answers regarding safety and effectiveness of antimalarial drugs in pregnant women. The feasibility of having active pharmacovigilance system in pregnancy in Tanzania, as may apply to other resource-limited countries, has also been assessed. This thesis responds to pronounced uncertainties on safety and effectiveness of antimalarial in this vulnerable group. The chapter also presents general thesis recommendations on practical application and potential further studies based on the main study findings.

9.1 Safety of antimalarial drugs in pregnancy and potential platform for generating reliable drug safety information during pregnancy

There is insufficient safety information on both antimalarial and non-antimalarial drugs administer in pregnancy to establish the effect of medication on embryo or developing fetus. This is a significant public health problem which leaves the healthcare providers with limited means to weigh risks versus benefits when using a given medication. Limited medicine safety information during pregnancy is due to the genuine reason that pregnant women are routinely excluded from drug development trials for fear of harming the mother and/or the fetus. Part of pregnancy safety information is based on findings from animal studies which are not always easy translated to humans and hence, medicines come to market with limited safety information available for their use in pregnancy [35]. The latter explains why most medicines are not recommended for use during pregnancy period which may be due to justifiable safety uncertainties rather than causal relationship derived from rigorous trials. This lack of evidence based risk-benefit information may explain why pregnant women sometimes end up treated with less effective drugs or a non-friendly treatment regimen as opposed to non-pregnant individuals. However, contraindicated medicines in pregnancy cannot always be avoided in chronic diseases such as epilepsy, HIV, TB or in case of acute medical emergencies that are life-threatening for the mother and/or the fetus if not treated appropriately. Furthermore, most women of child bearing age use these medicines without knowing that they are pregnant which, in most cases, tends to be the consequence of unplanned pregnancy. Use of contraindicated drugs among pregnant women is also inevitable because over-the-counter prescription is common in Africa, including Tanzania [162]. The latter is of a great concern with the current observed increasing number of private drug vendors in most developing countries where monitoring of safety practice by the responsible authorities may be a problem too.

Drug safety in pregnancy can best be assessed through active PV system or pregnancy exposure registry system. Active PV involves active cohort event monitoring and reporting of adverse drug effects when

the drug is already on the market (post-approval). It assists to determine any minor or rare drug reactions which were not detected during phase III clinical trials. In pregnancy, PV system helps to determine whether the drug has an effect in inducing foetal risks or not [73]. Active cohort event monitoring such as use of pregnancy exposure registry do exist in most developed countries and its operation is strongly supported by the (i) US Food and Drug Administration (US FDA) in USA and (ii) European Medicine Agency for drugs or products in European countries, so as to provide reliable drug safety information following exposure in pregnant women or women of childbearing age. This facilitates additional safety information of products, especially the ones for which safety in pregnancy is questionable secondary to pre-clinical animal data that suggest teratogenicity, for example ACTs [163, 164].

PV databases on drug safety information in pregnancy or pregnancy exposure registries do not exist in almost all developing countries, including Tanzania, due to some reported complexities such as limited health skilled personnel, inadequate clinical record of the data and lack of quality birth registry system [36]. These challenges have existed for long time and it appears that there is no promising solution yet in place to overcome these challenges. Under TFDA in Tanzania, drug safety information is collected spontaneously, but the reporting rate is very low which is one of disadvantages of passive PV system [61]. Furthermore, in passive PV system, it is difficult to detect adverse drug reaction that takes longer to appear in a patient following exposure, as it may be the case in pregnancy whereby adverse event that affects the baby in a womb may be detected after delivery or later in child's life. The latter will not be missed in active PV system since it involves monitoring of a cohort for events in a defined period of time.

It is an ample time to consider having an effective mechanism in place for generating reliable drug safety information during pregnancy in a tropical developing country. Chapter 4 of the thesis shows that there is a potential opportunity to overcome this challenge in the country, and thus to achieve having a feasible, reliable and manageable PV system for cohort event monitoring among pregnant women in the country. The latter is possible by using HDSS platform which has existed for quite some time in monitoring various vital events such as pregnancies, births and deaths. Added advantages of using HDSS platform for a successful and reliable PV system in pregnancy include: well-established HDSS database of its residence which is routinely updated on quarterly basis in a year during HDSS census, have experienced field workers who are equipped with skills and transport means to facilitate follow up, strong established relationship between health facility personnel with HDSS staff team who had long

been cooperating in various cohort studies which involves gathering various clinical data from patients and finally, good relation between HDSS staff team and HDSS residence or patients which facilitates confidentiality and corporation that is important to ensure effectiveness in gathering health information. It is important to note that implementation of monitoring drug safety in pregnancy in a resource limited settings is also supported by the WHO protocol that encourage individual countries and sentinel sites such as HDSS to contribute for data pooling into a common WHO database on safety of medicine exposure in pregnancy [82]. To my knowledge, there is no developing country, particularly in sub-Saharan Africa which has tried so far to come forward and implement active PV system in pregnancy that would involve monitoring of all drugs exposure during pregnancy.

Achieving the establishment of an effective PV system for cohort event monitoring during pregnancy in Tanzania using HDSS platform will be a get way through of cascading this success story to other HDSS and non-HDSS areas in resource-limited countries. It is important to emphasize that other countries should buy in the use of sentinel sites such as HDSS platform through INDEPTH network to facilitate the set-up of active cohort event monitoring of anti-infective agents. Having a larger and reliable safety data pool will ensure a timely risk-benefit profile assessment of a given medicine and a strong scientific power to conclude the observed safety results. The latter will assist to overcome the current challenge whereby nearly all developing countries rely on medicine safety data from industrialised countries in which there are no pregnancy safety data for medicines that aim at treating tropical diseases because they are hardly being used in these countries [74].

There are 49 HDSS sites registered under INDEPTH network in 20 countries; 36 in Africa, 12 in Asia and 1 in Papua New Guinea (Oceania) [165]. Implementation of active PV system using HDSS sites should have a geographical representation of countries and continents in the respective sites. This will allow a wide diversity assessment of different medicines exposure and diseases in relation to pregnancy outcome because there is no a common treatment guideline to all these countries and furthermore, health seeking behavior among patients is not always the same. The selection criteria of study sites for PV system should be (i) having a large population of HDSS residence, (ii) high number of health facilities with good coverage of antenatal services in HDSS catchment area, and (iii) HDSS experience to carry clinical related cohort studies. It is important to have strong scientific and capacity building support during the initial implementation stage of PV system in HDSS sites. This is possible because most HDSS sites in the South have a long-standing scientific collaboration with research centers in the North, for example Ifakara HDSS in Tanzania with Swiss TPH in Switzerland, Manhica HDSS in Mozambique with

Barcelona center for international health research in Spain and Farafenni HDSS in Gambia with London School of Hygiene and Tropical Medicine (LSHTM). Having such a bounding in the operation will assist to enhance this system through technical support that would ensure an effective monitoring mechanism. The latter will facilitate having reliable and quality PV reports that would be produced on a timely basis. The success story of achieving the proposed active PV system in HDSS areas should later on be applied in areas with no HDSS. In none HDSS areas, criteria for consideration should be a country with good health information system, good coverage of antenatal services and commitment of the ministry of health to support the system through its available resources. In addition, the facilitation and supportive role of pharmaceutical companies should be clearly defined, as well as that of donor agencies.

The pilot PV system reported in chapter 4 has shown that almost all women (98%) had used at least one medication during pregnancy. Anti-infective including anthelminthic, antibiotics and antimalarial were highly used among pregnant women. Such a high magnitude of anti-infective exposure justifies an active PV monitoring system in the country. High anti-infective exposure among pregnant women in resource-limited countries is known. A study conducted in Mozambique seven years ago reported antibiotics and antimalarial exposure among 3105 studied pregnant women was 41% and 24%, respectively. Exposure to these drugs in pregnancy was associated with increased risk of stillbirth [36]. It is important to emphasize that having effective PV system in pregnancy should go parallel with effective improvement of newborn screening and birth registry. The latter will facilitate proper detection of any adverse birth outcome and good data linkage between pregnancy exposure information and birth outcomes records.

The first 12 weeks of pregnancy (first trimester) is an important pregnancy period in baby's development and is highly sensitive to drug safety issues because it is when organogenesis takes place and therefore, congenital malformation or miscarriage commonly occurs if harmful chemicals or biological agents are used in pregnant woman [71]. There is a limited armamentarium of antimalarial drugs recommended in early pregnancy period. So far, chloroquine, proguanil, clindamycin and quinine are the only recommended antimalarial, knowing that the first two are no more effective [37]. It is important to evaluate the safety of quinine when compared to more recent drugs such as ACT, due to ongoing queries of clinicians regarding potential adverse pregnancy outcome. Quinine has long been associated with abortive properties which are often linked with overdose. The first report questioning quinine safety was published for the first time almost a century ago [44]. Quinine overdose in treating patients is inevitable in most health facilities in developing countries because dosage is mainly provided

based on age group rather than body weight. This is a matter of concern, especially in poor rural communities whereby malnutrition is also a problem too.

It is time to explore the appropriateness of using ACTs as first line drugs during first trimester . Currently, there is no safety signal in humans to question the introduction of these drugs during first trimester [39, 40, 166]. Furthermore, additional information from interim analyses of two studies in Africa, at present are in their final stages, collecting ACTs safety information in first trimester do support the evidence that ACTs are safe in early pregnancy (*ter Kuile FO, personal communication*). One may argue that the available information is not yet sufficient enough to justify policy change, especially well designed studies that include infant development follow-up. However, it is also not clear how much safety information is needed to change recommendation. Based on the available promising information of artemisinins safety in first trimester, it is time to plan a randomized controlled trial that would involve ACTs versus quinine for uncomplicated malaria and parental artesunate versus parental quinine for severe malaria, both in first trimester. The latter may be important for consideration following AQUAMAT trial (compared artesunate verses quinine in treating children with severe malaria) that recommended artesunate to replace quinine as a drug of choice for severe *P. falciparum* malaria [97].

9.2 Antimalarial efficacy in relation to alteration of PK properties due to pregnancy condition

It is essential to consider that pregnant women should be treated with effective medicine that will ensure good therapeutic outcome within a minimum period of time. However, there are key factors that determining therapeutic outcome of any given disease condition and that includes; (i) host or patient factor that influence drug properties (pharmacokinetic) in terms of drug absorption, distribution, biotransformation and excretion, (ii) host immunity against infectious agent, and (iii) the efficacy of therapeutic agent or a drug in eliminating the infectious agent from the host and to prevent new infection into the host for a defined period of time. The two host factors are greatly altered during pregnancy as opposed to non-pregnant individuals. In addition to safety issues, all these factors are important parameters which drug manufacturers in most cases have to consider during drug development process and while modeling to determine appropriate dose regimen.

It is unfortunate that doses used in pregnancy are often extrapolated from adult because of the same reason that pregnant women are not involved in PK studies of a pre-licensed medicine. Hence, PK alterations that occur during pregnancy period are not reflected in the formulated dose that a pregnant woman will need to take to clear or prevent a given infection. This is a key weakness in malaria chemotherapy in pregnancy which may explain lower effectiveness of artemether-dihydroartemisinin,

lumefantrine, artesunate-dihydroartemisinin, dihydroartemisinin, piperaquine, atovequone and proguanil in pregnancy [49, 51, 114, 167]. The PK alteration may increase the likelihood of malaria treatment failure in pregnancy due to lowered plasma drug levels and considering that host immunity against malaria and many other infections is compromised during pregnancy [10]. The sub-optimal dose which will be circulating in a woman's body system will lead to incomplete elimination of a parasite and hence recrudescence, but also to a lower post-treatment prophylactic effect. It is therefore essential to reassess the current recommended dose regimen of all antimalarial drugs in pregnancy so as to reassure the expected therapeutic outcome in this vulnerable group.

Most antimalarial PK studies preferably monitor the longer partner drug with a higher half-life clearance in plasma such as LF in AL and not the shorter one because the longer partner drug is more informative to display its minimum parasiticidal concentration (MPC) and minimum inhibitory concentration (MIC) of the study drug. Chapter 6 of the thesis reported LF bioavailability among pregnant women in the study was lower by 34% as opposed to non-pregnant women. There was a higher metabolism rate of LF into DLF by 78% among pregnant women as opposed to non-pregnant women and this may be one of the underlying reasons for lower LF bioavailability in pregnancy. This shows that pregnancy is an important responsible factor in the alteration of PK properties of AL. The latter has also been reported in other PK studies conducted in Thailand and hence agrees that physiological changes in pregnancy remain the most responsible factor for lowering plasma drug level in pregnant women [51].

Higher MIC and prolonged post-treatment prophylaxis is one of the merits for an effective antimalarial. This may be a weakness of AL when compared to other ACTs, particularly dihdroartemisinin-piperaquine (DP). DP has been reported to be the ACT with the longest post-treatment prophylactic effect because of the prolonged half-life of piperaquine [168-170]. Beneficial effect of post-treatment prophylaxis is more visible in area with high malaria transmission as opposed to low transmission due to difference in chances of having new infection. Protecting a pregnant woman from new infection for a reasonable interval of time post treatment will help her body to recover effectively from weakness associated with the existed infection which is important too for the health gain of unborn baby in the womb. DP has shown to be efficacious, safe and tolerable for treating uncomplicated malaria in Ghanaians pregnant women [171].

To increase bioavailability of LF, it is advisable to take AL with a meal rich in fat because LF is a hydrophobic lipophilic compound which is absorbed slowly. In our study, the median day 7

concentration of LF in pregnant women was 908 ng/ml as opposed to non-pregnant women who had a much higher concentration of 1382 ng/dl. These values in either of the two study groups are higher than the median day 7 LF concentrations from another PK study conducted in Tanzania and Cambodia [118] where meal rich in fat was not concomitantly administered during AL intake. Encouraging AL administration with a meal rich in fat should be considered as an immediately solution to increase bioavailability of this drug in pregnancy while planning for the possibility of further study that would propose a modified AL treatment regimen in pregnant women.

9.3 Malaria preventive measures in pregnancy and IPTp-SP effectiveness

Pregnant women are known to be important vulnerable group for malaria infection. One of the reasons that may explain their vulnerability are the changes that occur in a placenta which makes human placenta a favorable harbor site for malaria parasite [10, 19]. With time, malaria infection in the placenta may lead to harmful consequences to a woman and hence may be responsible for pregnancy-associated malaria morbidities. High malaria transmission areas have been reported to carry a big burden as opposed to low transmission areas because of likelihood of having asymptomatic malaria infection which is explained by their developed immunity [6]. Thus, there have been effective preventive measures advocated for preventing malaria in pregnancy especially in malaria endemic areas. Cost-effectiveness of some of these preventive measures are challenged because of the reported decline of malaria in most areas which were previously known to have high transmission [146, 147] and hence call for the need to determine the role of other non-malarial factors in relation to adverse pregnancy outcome. Furthermore, there is a growing concern of parasite resistance to antimalarial drugs [172] and mosquito resistance to pyrethroids, insecticide commonly used in ITN and indoor residual spraying [173, 174]. All these changes call for a continuous evaluation of the cost-effectiveness of the existing and newly proposed malaria preventive measures in pregnancy, and identify the effective gears which will be applied in the defined range of malaria transmission settings [5].

Use of at least two doses of SP for IPTp from second trimester of pregnancy and ITNs are important malaria preventive measures which have been in place to prevent malaria and associated morbidities in pregnancy in malaria endemic areas for almost a decade [27]. SP resistance jeopardizes the reliability of the existing IPTp regimen for controlling malaria in pregnancy considering that there is no alternative for SP which have been recommended to date. Little is known regarding effectiveness of the current IPTp regimen for malaria control in pregnancy particularly in low malaria transmission settings. Interaction of SP with other drugs is also a questionable issue due to safety and efficacy reasons, a greater concern is

its interaction with anti-retroviral (ARV) or cotrimoxazole in HIV individual, consider the high prevalence of HIV in sub-Sahara Africa and most infected people are on ARV [175]. However, IPTp use is still routinely advocated and practice in most of sub Saharan countries regardless the presence of an existing chronic illness or intensity of malaria transmission to which a given pregnant woman is residing.

Prevention of pregnancy associated malaria morbidity such as maternal anaemia and LBW are the primary benefits of IPTp use [176]. There have been inconsistences to support the latter effects of IPTp [161], even in chapter 8 of the thesis which also did not support IPTp effectiveness on preventing maternal anaemia and LBW in either area of transmission intensity. WHO has currently issued a new IPTp guideline which requires monthly administration of SP early from second trimester following studies that supported this regimen in order to enhance net benefits of IPTp [34, 157]. However, it is still important to question the added benefits of this novel regimen in areas with low malaria transmission considering that unnecessary drug exposure in pregnancy is not an ideal practice. It is also questionable in terms of feasibility and acceptability of having high coverage of all proposed IPTp doses in the newly regimen since the coverage of IPTp second dose in the previous regimen was still below 60% in most malaria countries in sub-Sahara [31]. We therefore expect the coverage of third or fourth IPTp dose to be even lower, and the expected added benefit may thus be very low.

ITN has long been advocated for use as an effective means of malaria prevention in both pregnant and non-pregnant individuals. ITNs have reduced more than 15% of all pregnancy associated malaria morbidities [143]. Thus, it is important to promote ITNs use in pregnancy and, unlike IPTp, it does not expose directly a pregnant woman to a drug. Added benefit to ITN is infancy protection because most women sleep with their newborns. Acceptability of ITNs is also higher in the community as observed in chapter 8, 95% ITN coverage versus 51% IPTp second dose although, the figures may differ with the findings observed in other malaria areas within and outside Tanzania.

Indoor residual spray in an effective means of malaria vector control and should be considered for use in prevention of malaria in pregnancy albeit, its specific effect in this vulnerable group has not yet been evaluated [5]. However, the emerging and spread of pyrethroids resistance [174] is a key challenge to scientists because pyrethroids are well tolerated by pregnant women and there is no reported toxicity to the fetus when used according to the given safety procedure [177]. The role of mosquito repellant in preventing malaria in pregnancy has not well been evaluated despite of diethyltoluamide (DEET), a common active ingredient of mosquito repellent, to be known of being safe in pregnancy [178].

Mosquito repellents are not part of malaria control intervention in pregnancy. This preventive measure might be beneficial particularly for pregnant women living in high transmission areas during their late out-door activities or women travelling from low to high transmission areas. Effectiveness and safety of mosquito repellents in pregnancy should be evaluated.

Intermittent screening with a rapid diagnostic test (RDT) and treatment to those whose result is positive (IST) in every antenatal visit has been thought as the right alternative for IPTp. IST with an effective antimalarial has shown to be non-inferior to IPTp-SP in West Africa [159]. Implementation of IST will help to reduce unnecessary SP exposure to pregnant women, particularly the ones in low transmission areas. And if implemented in areas with high SP resistance, it will ensure more effective cure of parasitaemia when compared to SP. The type of screening test to detect parasitaemia in pregnancy is important and RDT might have the advantage over microscopy to detect circulating antigenemia, eventually from parasites sequestered in the placenta. This is more theoretical than practical. It has indeed been shown that RDTs are more sensitive than microscope to detect placenta malaria (81% vs 72%). Histidine rich protein 2 (HRP2)-based RDTs may be preferred than plasmodium lactate dehydrogenase (pLDH)-based RDTs because they are more sensitive and thermo stable [179].

It is important to evaluate the effectiveness of malaria preventive measures in pregnancy according to levels of transmission intensity. Proposed interventions should be considered based also on the perceptions, acceptability and cost implication in the targeted community. All these parameters are important to assure effective coverage of a particular intervention.

9.3 Conclusion

There is a big gap of knowledge about malaria treatment in pregnancy which is due to limited information available on the potential effective and safer treatment to be used in this vulnerable group. It is an ample time to have effective mechanism of addressing the knowledge gap of safety therapies used during pregnancy. The latter is possible by establishing an active cohort even monitoring mechanism of drug exposure in pregnancy so as to contribute busting a safety data pool which would be useful for evidence-based medicine. It is time to make effective use of the available AL safety information so as to increase its marginal safety for use in all trimesters as this drug may be safer than the current recommended quinine. Effectiveness of IPTp use should actively be monitored and evaluate its potential benefits especially in areas with high SP resistance.

9.4 Recommendation

Malaria treatment and prevention is still a major challenging area in pregnancy with a lot of pending questions which need concrete answers and immediate solutions. It needs both local and international partners to come together and address these challenges in order to improve maternal and neonatal health. The answers to these challenges should address both immediate and long term action plan on malaria chemotherapy in pregnancy. It is also recommended that pharmaceutical companies and other global financial bodies should invest more on building drug monitoring capacity that could assess and reporting PV issues of antimalarial drugs in malaria endemic areas. We therefore recommend on the following practical application and further studies as outlined below:

Practical application areas

a. Establish a safety monitoring mechanism of drug exposure during pregnancy period in malaria endemic countries. This will increase the safety data pool particularly for newly marketed medicines and other anti-infective drugs for treating tropical diseases in which there is more need to be addressed regarding their safety use in pregnancy.
b. Use of HDSS platform in limited-resource countries to establish pregnancy exposure registry system. This should go along with the improvement of birth registry system as well as maternal and neonatal care services.
c. Continuous train and advocate to health care provider on abiding to standard treatment guidelines for treatment and care among pregnant women. This should effectively involve both public and private health sectors, including drug venders. It should also target to discourage use of non-recommended medicines in pregnancy and the use of anti-infective after standard screening of a particular infection in the facility.
d. Reduce the intensity of pierce statement in the WHO malaria treatment guideline that cautions ACTs use and the risk of embryo-foetal toxicity because such risk has not yet been confirmed in any human studies to date. Furthermore, a plan should be developed for the use of alternative drugs such as ACTs that are safer than quinine and hence to be considered as first line malaria treatment for uncomplicated malaria during first trimester of pregnancy.
e. Restrict quinine use to severe cases of malaria in all trimesters of pregnancy. This is due to its safety problems and secondly it has high possibility to develop resistance, being a monotherapy.
f. Modify ACT regimen in pregnancy to improve its efficacy in this vulnerable group. It should target to increase MPC and MIC.

g. Continuously advocate on the administration of AL with meal rich in fat so as to maximize LF absorption, especially in pregnant women.
h. Regularly estimate the Number Needed To Treat with IPTp and eventually apply this intervention in selected groups in defined transmission level areas.

Further research areas

a. Determine safety of antimalarial and other anti-infective drugs in pregnancy in relation to their specific period of exposure during pregnancy using active PV system (Cohort event monitoring).
b. Assess the strength of achieving establishment of active PV system in HDSS area and how best can the experience be applied to none HDSS areas. Cost-effective analysis and sustainability should also be evaluated.
c. Evaluate the available evidence on ACTs safety in first trimester by the policy makers and advise a way forward on expanding its marginal safety in pregnancy.
d. Consider a way forward for planning and conduct a randomized control trial that would target to assess ACTs safety in first trimester.
e. Reevaluate quinine safety in pregnancy and advise on whether there is a need to keep on using this drug in pregnant women or it is time to substitute it with a safer medicine. Studies should be conducted to assess the safety and efficacy of artesunate as first-line treatment for severe malaria during pregnancy
f. Assess the effectiveness and compliance of the proposed 5 days AL regimen for treating uncomplicated malaria in pregnancy.
g. Assess the effectiveness of the newly proposed IPTp regimen by the WHO in East Africa region, an area with the highest SP resistance.
h. Assess the effectiveness and cost implication of active screening and treatment of malaria in pregnancy verses the newly proposed IPTp regimen in East Africa region.

Reference

1. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO: **Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study.** *PLoS Med* 2010, **7:**e1000221.
2. Steketee RW, Nahlen BL, Parise ME, Menendez C: **The burden of malaria in pregnancy in malaria-endemic areas.** *Am J Trop Med Hyg* 2001, **64:**28-35.
3. CDC: **The rapid assessment of the burden of malaria during pregnancy: a toolkit.** *Centre for Disease Control and Prevention* 2010.
4. Enato EF, Okhamafe AO: **Plasmodium falciparum malaria and antimalarial interventions in sub-Saharan Africa: challenges and opportunity.** *Afr Jour Biotech* 2005, **4:**1598-1605.
5. Menendez C, D'Alessandro U, ter Kuile FO: **Reducing the burden of malaria in pregnancy by preventive strategies.** *Lancet Infect Dis* 2007, **7:**126-135.
6. Takem EN, D'Alessandro U: **Malaria in pregnancy.** *Mediterr J Hematol Infect Dis* 2013, **5:**e2013010.
7. Bouyou-Akotet MK, Adegnika AA, Agnandji ST, Ngou-Milama E, Kombila M, Kremsner PG, Mavoungou E: **Cortisol and susceptibility to malaria during pregnancy.** *Microbes Infect* 2005, **7:**1217-1223.
8. Ansell J, Hamilton KA, Pinder M, Walraven GE, Lindsay SW: **Short-range attractiveness of pregnant women to Anopheles gambiae mosquitoes.** *Trans R Soc Trop Med Hyg* 2002, **96:**113-116.
9. Lindsay S, Ansell J, Selman C, Cox V, Hamilton K, Walraven G: **Effect of pregnancy on exposure to malaria mosquitoes.** *Lancet* 2000, **355:**1972.
10. Rogerson SJ, Mwapasa V, Meshnick SR: **Malaria in pregnancy: linking immunity and pathogenesis to prevention.** *Am J Trop Med Hyg* 2007, **77:**14-22.
11. Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Warikar N, Seal A, McGready R, Sugiarto P, Tjitra E, Anstey NM, Price RN: **Adverse pregnancy outcomes in an area where multidrug-resistant plasmodium vivax and Plasmodium falciparum infections are endemic.** *Clin Infect Dis* 2008, **46:**1374-1381.
12. Saute F, Menendez C, Mayor A, Aponte J, Gomez-Olive X, Dgedge M, Alonso P: **Malaria in pregnancy in rural Mozambique: the role of parity, submicroscopic and multiple Plasmodium falciparum infections.** *Trop Med Int Health* 2002, **7:**19-28.
13. Agbor-Enoh ST, Achur RN, Valiyaveettil M, Leke R, Taylor DW, Gowda DC: **Chondroitin sulfate proteoglycan expression and binding of Plasmodium falciparum-infected erythrocytes in the human placenta during pregnancy.** *Infect Immun* 2003, **71:**2455-2461.
14. UNAIDS/WHO: **UNAIDA report on the global AIDS epidemic 2010. Joint United Nations Programme on HIV/AIDS.** *UNAIDS* 2010, **10.11E**.
15. Ladner J, Leroy V, Simonon A, Karita E, Bogaerts J, De Clercq A, Van De Perre P, Dabis F, Pregnancy, Group HIVS: **HIV infection, malaria, and pregnancy: a prospective cohort study in Kigali, Rwanda.** *Am J Trop Med Hyg* 2002, **66:**56-60.
16. Verhoeff FH, Brabin BJ, Hart CA, Chimsuku L, Kazembe P, Broadhead RL: **Increased prevalence of malaria in HIV-infected pregnant women and its implications for malaria control.** *Trop Med Int Health* 1999, **4:**5-12.
17. Steketee RW, Wirima JJ, Bloland PB, Chilima B, Mermin JH, Chitsulo L, Breman JG: **Impairment of a pregnant woman's acquired ability to limit Plasmodium falciparum by infection with human immunodeficiency virus type-1.** *Am J Trop Med Hyg* 1996, **55:**42-49.
18. Marchesini P, Crawley J: **Reducing the burden of malaria in pregnancy.** *Roll Back Malaria* 2004.
19. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW: **Malaria in pregnancy: pathogenesis and immunity.** *Lancet Infect Dis* 2007, **7:**105-117.

20. Tuikue Ndam NG, Salanti A, Bertin G, Dahlback M, Fievet N, Turner L, Gaye A, Theander T, Deloron P: **High level of var2csa transcription by Plasmodium falciparum isolated from the placenta.** *J Infect Dis* 2005, **192:**331-335.
21. Staalsoe T, Shulman CE, Bulmer JN, Kawuondo K, Marsh K, Hviid L: **Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated Plasmodium falciparum malaria.** *Lancet* 2004, **363:**283-289.
22. Achidi EA, Apinjoh TO, Titanji VP: **Malaria parasitemia and systemic cytokine bias in pregnancy.** *Int J Gynaecol Obstet* 2007, **97:**15-20.
23. Thevenon AD, Zhou JA, Megnekou R, Ako S, Leke RG, Taylor DW: **Elevated levels of soluble TNF receptors 1 and 2 correlate with Plasmodium falciparum parasitemia in pregnant women: potential markers for malaria-associated inflammation.** *J Immunol* 2010, **185:**7115-7122.
24. Conroy AL, McDonald CR, Silver KL, Liles WC, Kain KC: **Complement activation: a critical mediator of adverse fetal outcomes in placental malaria?** *Trends Parasitol* 2011, **27:**294-299.
25. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, Font F, Alonso PL: **The impact of placental malaria on gestational age and birth weight.** *J Infect Dis* 2000, **181:**1740-1745.
26. Suguitan AL, Jr., Cadigan TJ, Nguyen TA, Zhou A, Leke RJ, Metenou S, Thuita L, Megnekou R, Fogako J, Leke RG, Taylor DW: **Malaria-associated cytokine changes in the placenta of women with pre-term deliveries in Yaounde, Cameroon.** *Am J Trop Med Hyg* 2003, **69:**574-581.
27. WHO: **A strategic framework for malaria prevention and control during pregnancy in the Africa Region.** *WHO, Brazzaville* 2004.
28. Takken W: **Do insecticide-treated bednets have an effect on malaria vectors?** *Trop Med Int Health* 2002, **7:**1022-1030.
29. ter Kuile FO, Terlouw DJ, Phillips-Howard PA, Hawley WA, Friedman JF, Kolczak MS, Kariuki SK, Shi YP, Kwena AM, Vulule JM, Nahlen BL: **Impact of permethrin-treated bed nets on malaria and all-cause morbidity in young children in an area of intense perennial malaria transmission in western Kenya: cross-sectional survey.** *Am J Trop Med Hyg* 2003, **68:**100-107.
30. Gamble C, Ekwaru PJ, Garner P, ter Kuile FO: **Insecticide-treated nets for the prevention of malaria in pregnancy: a systematic review of randomised controlled trials.** *PLoS Med* 2007, **4:**e107.
31. van Eijk AM, Hill J, Alegana VA, Kirui V, Gething PW, ter Kuile FO, Snow RW: **Coverage of malaria protection in pregnant women in sub-Saharan Africa: a synthesis and analysis of national survey data.** *Lancet Infect Dis* 2011, **11:**190-207.
32. WHO: **WHO Evidence Review group: Intermittent Preventive Treatment of malaria in pregnancy (IPTp) with Sulfadoxine-Pyrimethamine (SP).** *WHO Geneva* 2012.
33. Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE: **Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance.** *Clin Infect Dis* 2011, **53:**224-230.
34. Kayentao K, Garner P, van Eijk AM, Naidoo I, Roper C, Mulokozi A, MacArthur JR, Luntamo M, Ashorn P, Doumbo OK, ter Kuile FO: **Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis.** *JAMA* 2013, **309:**594-604.
35. Howard TB, Tassinari MS, Feibus KB, Mathis LL: **Monitoring for teratogenic signals: pregnancy registries and surveillance methods.** *Am J Med Genet C Semin Med Genet* 2011, **157C:**209-214.
36. Sevene E, Bardaji A, Mariano A, Machevo S, Ayala E, Sigauque B, Aponte JJ, Carne X, Alonso PL, Menendez C: **Drug exposure and pregnancy outcome in Mozambique.** *Paediatr Drugs* 2012, **14:**43-49.
37. WHO: **Guideline for the treatment of Malaria: second edition.** *WHO Geneva* 2010.

38. Obonyo CO, Juma EA: **Clindamycin plus quinine for treating uncomplicated falciparum malaria: a systematic review and meta-analysis.** *Malar J* 2012, **11:**2.
39. Adam I, Elhassan EM, Omer EM, Abdulla MA, Mahgoub HM, Adam GK: **Safety of artemisinins during early pregnancy, assessed in 62 Sudanese women.** *Ann Trop Med Parasitol* 2009, **103:**205-210.
40. Manyando C, Mkandawire R, Puma L, Sinkala M, Mpabalwani E, Njunju E, Gomes M, Ribeiro I, Walter V, Virtanen M, et al: **Safety of artemether-lumefantrine in pregnant women with malaria: results of a prospective cohort study in Zambia.** *Malar J* 2010, **9:**249.
41. Piola P, Nabasumba C, Turyakira E, Dhorda M, Lindegardh N, Nyehangane D, Snounou G, Ashley EA, McGready R, Nosten F, Guerin PJ: **Efficacy and safety of artemether-lumefantrine compared with quinine in pregnant women with uncomplicated *Plasmodium falciparum* malaria: an open-label, randomised, non-inferiority trial.** *Lancet Infect Dis* 2010, **10:**762-769.
42. Fungladda W, Honrado ER, Thimasarn K, Kitayaporn D, Karbwang J, Kamolratanakul P, Masngammueng R: **Compliance with artesunate and quinine + tetracycline treatment of uncomplicated falciparum malaria in Thailand.** *Bull World Health Organ* 1998, **76 Suppl 1:**59-66.
43. Davis TM, Binh TQ, Thu le TA, Long TT, Johnston W, Robertson K, Barrett PH: **Glucose and lactate turnover in adults with falciparum malaria: effect of complications and antimalarial therapy.** *Trans R Soc Trop Med Hyg* 2002, **96:**411-417.
44. Maxwell JP: **The use of quinine during pregnancy, labour, and the puerperium.** *Trans R Soc Trop Med Hyg* 1908, **1:**229-235.
45. Nosten F, McGready R, d'Alessandro U, Bonell A, Verhoeff F, Menendez C, Mutabingwa T, Brabin B: **Antimalarial drugs in pregnancy: a review.** *Curr Drug Saf* 2006, **1:**1-15.
46. de Vries PJ, Bich NN, Van Thien H, Hung LN, Anh TK, Kager PA, Heisterkamp SH: **Combinations of artemisinin and quinine for uncomplicated falciparum malaria: efficacy and pharmacodynamics.** *Antimicrob Agents Chemother* 2000, **44:**1302-1308.
47. McGready R, Ashley EA, Moo E, Cho T, Barends M, Hutagalung R, Looareesuwan S, White NJ, Nosten F: **A randomized comparison of artesunate-atovaquone-proguanil versus quinine in treatment for uncomplicated falciparum malaria during pregnancy.** *J Infect Dis* 2005, **192:**846-853.
48. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R: **Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance.** *Lancet Infect Dis* 2007, **7:**136-144.
49. Tarning J, McGready R, Lindegardh N, Ashley EA, Pimanpanarak M, Kamanikom B, Annerberg A, Day NP, Stepniewska K, Singhasivanon P, et al: **Population pharmacokinetics of lumefantrine in pregnant women treated with artemether-lumefantrine for uncomplicated Plasmodium falciparum malaria.** *Antimicrob Agents Chemother* 2009, **53:**3837-3846.
50. Morris CA, Onyamboko MA, Capparelli E, Koch MA, Atibu J, Lokomba V, Douoguih M, Hemingway-Foday J, Wesche D, Ryder RW, et al: **Population pharmacokinetics of artesunate and dihydroartemisinin in pregnant and non-pregnant women with malaria.** *Malar J* 2011, **10:**114.
51. McGready R, Stepniewska K, Lindegardh N, Ashley EA, La Y, Singhasivanon P, White NJ, Nosten F: **The pharmacokinetics of artemether and lumefantrine in pregnant women with uncomplicated falciparum malaria.** *Eur J Clin Pharmacol* 2006, **62:**1021-1031.
52. WHO: **A practical handbook on the pharmacovigilance of antimalarial medicines.** *WHO Geneva* 2007.
53. WHO: **The importance of Pharmacovigilance: safety monitoring of medical products.** *WHO Geneva* 2002.

54. Pal S, Dodoo A, Olsson S: **The world medicine situation 2011: medicine expenditures.** *WHO Geneva* 2011.
55. Pirmohamed M, Atuah KN, Dodoo AN, Winstanley P: **Pharmacovigilance in developing countries.** *BMJ* 2007, **335:**462.
56. Brasseur P, Vaillant MT, Olliaro PL: **Anti-malarial drug safety information obtained through routine monitoring in a rural district of South-Western Senegal.** *Malar J* 2012, **11:**402.
57. Mehta U, Durrheim D, Mabuza A, Blumberg L, Allen E, Barnes KI: **Malaria pharmacovigilance in Africa: lessons from a pilot project in Mpumalanga Province, South Africa.** *Drug Saf* 2007, **30:**899-910.
58. Thiam S, Ndiaye JL, Diallo I, Gatonga P, Fall FB, Diallo NE, Faye B, Diouf ML, Ndiop M, Diouf MB, et al: **Safety monitoring of artemisinin combination therapy through a national pharmacovigilance system in an endemic malaria setting.** *Malar J* 2013, **12:**54.
59. Dellicour S, Brasseur P, Thorn P, Gaye O, Olliaro P, Badiane M, Stergachis A, ter Kuile FO: **Probabilistic record linkage for monitoring the safety of artemisinin-based combination therapy in the first trimester of pregnancy in Senegal.** *Drug Saf* 2013, **36:**505-513.
60. MOH T: **Tanzania Food and Drug Authority: National guideline for monitoring medicine safety** *TFDA(2ED)* 2010.
61. Authority TFaD: **Report on monitoring of adverse drug reaction due to antimalarial use in Tanzania.** *TFDA* 2009.
62. Kabanywanyi AM, Mulure N, Migoha C, Malila A, Lengeler C, Schlienger R, Genton B: **Experience of safety monitoring in the context of a prospective observational study of artemether-lumefantrine in rural Tanzania: lessons learned for pharmacovigilance reporting.** *Malar J* 2010, **9:**205.
63. WHO: **Methods for surveillance of antimalarial drug efficacy.** *WHO Geneva* 2009.
64. WHO: **Methods and techniques for assessing exposure to antimalarial drugs in clinical field studies.** *WHO Geneva* 2011.
65. Mbwasi R, Mlaki W: **Increasing access to medicines in Tanzania.** *Lancet* 2008, **372:**205-206.
66. Rutta E, Kibassa B, McKinnon B, Liana J, Mbwasi R, Mlaki W, Embrey M, Gabra M, Shekalaghe E, Kimatta S, Sillo H: **Increasing Access to Subsidized Artemisinin-based Combination Therapy through Accredited Drug Dispensing Outlets in Tanzania.** *Health Res Policy Syst* 2011, **9:**22.
67. Shields KE, Wiholm BE, Hostelley LS, Striano LF, Arena SR, Sharrar RG: **Monitoring outcomes of pregnancy following drug exposure: a company-based pregnancy registry program.** *Drug Saf* 2004, **27:**353-367.
68. Charlton R, C dV: **Systematic overview of data source for drug in pregnancy research.** *University of Bath* 2012.
69. Lawrence JM, Andrade SE, Avalos LA, Beaton SJ, Chiu VY, Davis RL, Dublin S, Pawloski PA, Raebel MA, Smith DH, et al: **Prevalence, trends, and patterns of use of antidiabetic medications among pregnant women, 2001-2007.** *Obstet Gynecol* 2013, **121:**106-114.
70. Mitchell AA, Gilboa SM, Werler MM, Kelley KE, Louik C, Hernandez-Diaz S, National Birth Defects Prevention S: **Medication use during pregnancy, with particular focus on prescription drugs: 1976-2008.** *Am J Obstet Gynecol* 2011, **205:**51 e51-58.
71. Parisi MA, Spong CY, Zajicek A, Guttmacher AE: **We don't know what we don't study: the case for research on medication effects in pregnancy.** *Am J Med Genet C Semin Med Genet* 2011, **157C:**247-250.
72. Levine M, O'Connor AD: **Obstetric toxicology: teratogens.** *Emerg Med Clin North Am* 2012, **30:**977-990.
73. Kennedy DL, Uhl K, Kweder SL: **Pregnancy exposure registries.** *Drug Saf* 2004, **27:**215-228.

74. White NJ, McGready RM, Nosten FH: **New medicines for tropical diseases in pregnancy: catch-22.** *PLoS Med* 2008, **5:**e133.
75. Khatib RA, Skarbinski J, Njau JD, Goodman CA, Elling BF, Kahigwa E, Roberts JM, MacArthur JR, Gutman JR, Kabanywanyi AM, et al: **Routine delivery of artemisinin-based combination treatment at fixed health facilities reduces malaria prevalence in Tanzania: an observational study.** *Malar J* 2012, **11:**140.
76. Welfare TMoHaS: **Tanzania Ministry of Health. 2010 District Health Profile.** *Tanzania MOH* 2011.
77. Shabani J, Lutambi AM, Mwakalinga V, Masanja H: **Clustering of under-five mortality in Rufiji Health and Demographic Surveillance System in rural Tanzania.** *Glob Health Action* 2010, **3**.
78. FDA U: **FDA Pregnancy Categories.** *Drugcom* 2013.
79. Good PI, Hardin JW: **Common errors in statistics (and how to avoid them).** *John Wiley & Sons, Inc* 2006, **2nd edition:**115.
80. Hoenig JM, Heisey DM: **The abuse of power: the pervasive fallacy of power calculations for data analysis.** *The American Statistician* 2001, **55**.
81. Welfare TMoHaS: **Standard treatment guidelines and the national essential medicines list for mainland Tanzania.** *Tanzania MOH 3rd ED* 2007.
82. Mehta U, Clerk C, Allen E, Yore M, Sevene E, Singlovic J, Petzold M, Mangiaterra V, Elefant E, Sullivan FM, et al: **Protocol for a drugs exposure pregnancy registry for implementation in resource-limited settings.** *BMC Pregnancy Childbirth* 2012, **12:**89.
83. Incorporation TNBoSaMI: **Tanzania Demographic and Health Survey 2004 - 05.** *NBS* 2005.
84. Eze UI, Eferakeya AE, Oparah AC, Enato EF: **Assessment of prescription profile of pregnancy women visiting antenatal clinics.** *Pharmacy Practice* 2007, **5:**135-139.
85. Balarajan Y, Subramanian SV, Fawzi WW: **Maternal iron and folic acid supplementation is associated with lower risk of low birth weight in India.** *J Nutr* 2013, **143:**1309-1315.
86. Pena-Rosas JP, Viteri FE: **Effects and safety of preventive oral iron or iron+folic acid supplementation for women during pregnancy.** *Cochrane Database Syst Rev* 2009**:**CD004736.
87. Titaley CR, Dibley MJ, Roberts CL, Hall J, Agho K: **Iron and folic acid supplements and reduced early neonatal deaths in Indonesia.** *Bull World Health Organ* 2010, **88:**500-508.
88. van Andel T, Carvalheiro LG: **Why urban citizens in developing countries use traditional medicines: the case of suriname.** *Evid Based Complement Alternat Med* 2013, **2013:**687197.
89. WHO: **Congenital anomalies.** *WHO Geneva* 2012.
90. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD: **Epidemiology and burden of malaria in pregnancy.** *The Lancet Infectious Diseases* 2007, **7:**93-104.
91. (CDER) CfDER: **Reviewer guidance evaluating the risks of drug exposure in human pregnancies.** *FDA US* 2005.
92. White TE, Clark RL: **Sensitive periods for developmental toxicity of orally administered artesunate in the rat.** *Birth Defects Res B Dev Reprod Toxicol* 2008, **83:**407-417.
93. WHO: **Assessment of the safety of artemisinin compounds in pregnancy: report of two informal consultations convened in 2006.** *WHO Geneva* 2007.
94. Lefevre G, Marrast AC, Grueninger H: **Novartis Malaria Initiative: best practice example of pharmaceutical industry's engagement in the fight against malaria.** *Ann N Y Acad Sci* 2011, **1222:**19-29.
95. MOH T: **National guideline for malaria diagnosis and treatment 2005.** *National malaria Control Program* 2006.
96. McCombie SC: **Treatment seeking for malaria: a review of recent research.** *Soc Sci Med* 1996, **43:**933-945.

97. Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, Bojang K, Olaosebikan R, Anunobi N, Maitland K, et al: **Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial.** *Lancet* 2010, **376:**1647-1657.
98. Okombo J, Ohuma E, Picot S, Nzila A: **Update on genetic markers of quinine resistance in Plasmodium falciparum.** *Mol Biochem Parasitol* 2011, **177:**77-82.
99. Parker D, Lerdprom R, Srisatjarak W, Yan G, Sattabongkot J, Wood J, Sirichaisinthop J, Cui L: **Longitudinal in vitro surveillance of Plasmodium falciparum sensitivity to common anti-malarials in Thailand between 1994 and 2010.** *Malar J* 2012, **11:**290.
100. Statistics TNBo: **Preliminary results of the 2011-12 Tanzania HIV/Aida and malaria indicator survey (THMIS).** *NBS* 2012.
101. Everett C: **Incidence and outcome of bleeding before the 20th week of pregnancy: prospective study from general practice.** *BMJ* 1997, **315:**32-34.
102. Lufesi NN, Andrew M, Aursnes I: **Deficient supplies of drugs for life threatening diseases in an African community.** *BMC Health Serv Res* 2007, **7:**86.
103. Twaweza: **Stock out or in stock? Acess to medicines in Tanzania.** *Twaweza* 2013, **Brief No. 5**.
104. Cheng B, Dong Y, He L, Tang W, Yu H, Lu J, Xu L, Zheng B, Li K, Xiao C: **Crossed polydactyly type I caused by a point mutation in the GLI3 gene in a large Chinese pedigree.** *J Clin Lab Anal* 2006, **20:**133-138.
105. David O, David M: **The prevalence, spontaneous resolution, and parent's attitudes towards umbilical hernia coexisting in Nigerian children presenting with other surgical pathology.** *Ann Pediatr Surg* 2009, **5:**16-20.
106. Meier DE, OlaOlorun DA, Omodele RA, Nkor SK, Tarpley JL: **Incidence of umbilical hernia in African children: redefinition of "normal" and reevaluation of indications for repair.** *World J Surg* 2001, **25:**645-648.
107. Pang KS: **Modeling of intestinal drug absorption: roles of transporters and metabolic enzymes (for the Gillette Review Series).** *Drug Metab Dispos* 2003, **31:**1507-1519.
108. Anderson GD: **Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach.** *Clin Pharmacokinet* 2005, **44:**989-1008.
109. Pavek P, Ceckova M, Staud F: **Variation of drug kinetics in pregnancy.** *Curr Drug Metab* 2009, **10:**520-529.
110. Isoherranen N, Thummel KE: **Drug metabolism and transport during pregnancy: how does drug disposition change during pregnancy and what are the mechanisms that cause such changes?** *Drug Metab Dispos* 2013, **41:**256-262.
111. Jeong H: **Altered drug metabolism during pregnancy: hormonal regulation of drug-metabolizing enzymes.** *Expert Opin Drug Metab Toxicol* 2010, **6:**689-699.
112. Piedade R, Gil JP: **The pharmacogenetics of antimalaria artemisinin combination therapy.** *Expert Opin Drug Metab Toxicol* 2011, **7:**1185-1200.
113. Staehli Hodel EM, Csajka C, Ariey F, Guidi M, Kabanywanyi AM, Duong S, Decosterd LA, Olliaro P, Beck HP, Genton B: **Effect of single nucleotide polymorphisms in cytochrome P450 isoenzyme and N-acetyltransferase 2 genes on the metabolism of artemisinin-based combination therapies in malaria patients from Cambodia and Tanzania.** *Antimicrob Agents Chemother* 2013, **57:**950-958.
114. McGready R, Stepniewska K, Edstein MD, Cho T, Gilveray G, Looareesuwan S, White NJ, Nosten F: **The pharmacokinetics of atovaquone and proguanil in pregnant women with acute falciparum malaria.** *Eur J Clin Pharmacol* 2003, **59:**545-552.
115. Onyamboko MA, Meshnick SR, Fleckenstein L, Koch MA, Atibu J, Lokomba V, Douoguih M, Hemingway-Foday J, Wesche D, Ryder RW, et al: **Pharmacokinetics and pharmacodynamics of**

artesunate and dihydroartemisinin following oral treatment in pregnant women with asymptomatic Plasmodium falciparum infections in Kinshasa DRC. *Malar J* 2011, **10:**49.

116. Tarning J, Kloprogge F, Piola P, Dhorda M, Muwanga S, Turyakira E, Nuengchamnong N, Nosten F, Day NP, White NJ, et al: **Population pharmacokinetics of Artemether and dihydroartemisinin in pregnant women with uncomplicated Plasmodium falciparum malaria in Uganda.** *Malar J* 2012, **11:**293.
117. White NJ, Stepniewska K, Barnes K, Price RN, Simpson J: **Simplified antimalarial therapeutic monitoring: using the day-7 drug level?** *Trends Parasitol* 2008, **24:**159-163.
118. Staehli Hodel EM, Guidi M, Zanolari B, Mercier T, Duong S, Kabanywanyi AM, Ariey F, Buclin T, Beck HP, Decosterd LA, et al: **Population pharmacokinetics of mefloquine, piperaquine and artemether-lumefantrine in Cambodian and Tanzanian malaria patients.** *Malar J* 2013, **12:**235.
119. Ezzet F, van Vugt M, Nosten F, Looareesuwan S, White NJ: **Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria.** *Antimicrob Agents Chemother* 2000, **44:**697-704.
120. Price RN, Uhlemann AC, van Vugt M, Brockman A, Hutagalung R, Nair S, Nash D, Singhasivanon P, Anderson TJ, Krishna S, et al: **Molecular and pharmacological determinants of the therapeutic response to artemether-lumefantrine in multidrug-resistant Plasmodium falciparum malaria.** *Clin Infect Dis* 2006, **42:**1570-1577.
121. Ashley EA, Stepniewska K, Lindegardh N, McGready R, Annerberg A, Hutagalung R, Singtoroj T, Hla G, Brockman A, Proux S, et al: **Pharmacokinetic study of artemether-lumefantrine given once daily for the treatment of uncomplicated multidrug-resistant falciparum malaria.** *Trop Med Int Health* 2007, **12:**201-208.
122. Ashley EA, Stepniewska K, Lindegardh N, Annerberg A, Kham A, Brockman A, Singhasivanon P, White NJ, Nosten F: **How much fat is necessary to optimize lumefantrine oral bioavailability?** *Trop Med Int Health* 2007, **12:**195-200.
123. Hodel EM, Genton B, Zanolari B, Mercier T, Duong S, Beck HP, Olliaro P, Decosterd LA, Ariey F: **Residual antimalarial concentrations before treatment in patients with malaria from Cambodia: indication of drug pressure.** *J Infect Dis* 2010, **202:**1088-1094.
124. Hodel EM, Kabanywanyi AM, Malila A, Zanolari B, Mercier T, Beck HP, Buclin T, Olliaro P, Decosterd LA, Genton B: **Residual antimalarials in malaria patients from Tanzania--implications on drug efficacy assessment and spread of parasite resistance.** *PLoS One* 2009, **4:**e8184.
125. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE: **Pyrimethamine and proguanil resistance-conferring mutations in Plasmodium falciparum dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa.** *Am J Trop Med Hyg* 1995, **52:**565-568.
126. Felger I, Irion A, Steiger S, Beck HP: **Genotypes of merozoite surface protein 2 of Plasmodium falciparum in Tanzania.** *Trans R Soc Trop Med Hyg* 1999, **93 Suppl 1:**3-9.
127. Hodel EM, Zanolari B, Mercier T, Biollaz J, Keiser J, Olliaro P, Genton B, Decosterd LA: **A single LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma.** *J Chromatogr B Analyt Technol Biomed Life Sci* 2009, **877:**867-886.
128. Beal SL, Sheiner LB, Boeckmann A, Bauer RJ: **NONMEM users guides (1989-2009). Icon Development Solution.** 2009.
129. Lindbom L, Pihlgren P, Jonsson EN: **PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM.** *Comput Methods Programs Biomed* 2005, **79:**241-257.
130. Novartis: **What are the ingredients of Coartem?** *Norvatis Pharmaceuticals Corporation* 2009.

131. Ahn JE, Karlsson MO, Dunne A, Ludden TM: **Likelihood based approaches to handling data below the quantification limit using NONMEM VI.** *J Pharmacokinet Pharmacodyn* 2008, **35:**401-421.
132. Beal SL: **Ways to fit a PK model with some data below the quantification limit.** *J Pharmacokinet Pharmacodyn* 2001, **28:**481-504.
133. Jamei M, Turner D, Yang J, Neuhoff S, Polak S, Rostami-Hodjegan A, Tucker G: **Population-based mechanistic prediction of oral drug absorption.** *AAPS J* 2009, **11:**225-237.
134. Denis MB, Tsuyuoka R, Lim P, Lindegardh N, Yi P, Top SN, Socheat D, Fandeur T, Annerberg A, Christophel EM, Ringwald P: **Efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in northwest Cambodia.** *Trop Med Int Health* 2006, **11:**1800-1807.
135. Stohrer JM, Dittrich S, Thongpaseuth V, Vanisaveth V, Phetsouvanh R, Phompida S, Monti F, Christophel EM, Lindegardh N, Annerberg A, Jelinek T: **Therapeutic efficacy of artemether-lumefantrine and artesunate-mefloquine for treatment of uncomplicated Plasmodium falciparum malaria in Luang Namtha Province, Lao People's Democratic Republic.** *Trop Med Int Health* 2004, **9:**1175-1183.
136. White NJ, van Vugt M, Ezzet F: **Clinical pharmacokinetics and pharmacodynamics and pharmacodynamics of artemether-lumefantrine.** *Clin Pharmacokinet* 1999, **37:**105-125.
137. McGready R, Tan SO, Ashley EA, Pimanpanarak M, Viladpai-Nguen J, Phaiphun L, Wustefeld K, Barends M, Laochan N, Keereecharoen L, et al: **A randomised controlled trial of artemether-lumefantrine versus artesunate for uncomplicated plasmodium falciparum treatment in pregnancy.** *PLoS Med* 2008, **5:**e253.
138. Na-Bangchang K, Ruengweerayut R, Mahamad P, Ruengweerayut K, Chaijaroenkul W: **Declining in efficacy of a three-day combination regimen of mefloquine-artesunate in a multi-drug resistance area along the Thai-Myanmar border.** *Malar J* 2010, **9:**273.
139. Savage EJ, Msyamboza K, Gies S, D'Alessandro U, Brabin BJ: **Maternal anaemia as an indicator for monitoring malaria control in pregnancy in sub-Saharan Africa.** *BJOG* 2007, **114:**1222-1231.
140. Brabin BJ, Romagosa C, Abdelgalil S, Menendez C, Verhoeff FH, McGready R, Fletcher KA, Owens S, D'Alessandro U, Nosten F, et al: **The sick placenta-the role of malaria.** *Placenta* 2004, **25:**359-378.
141. Mayor A, Rovira-Vallbona E, Machevo S, Bassat Q, Aguilar R, Quinto L, Jimenez A, Sigauque B, Dobano C, Kumar S, et al: **Parity and placental infection affect antibody responses against Plasmodium falciparum during pregnancy.** *Infect Immun* 2011, **79:**1654-1659.
142. Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, Brabin B: **Malaria in pregnancy and the endemicity spectrum: what can we learn?** *Trends Parasitol* 2004, **20:**425-432.
143. Feng G, Simpson JA, Chaluluka E, Molyneux ME, Rogerson SJ: **Decreasing burden of malaria in pregnancy in Malawian women and its relationship to use of intermittent preventive therapy or bed nets.** *PLoS One* 2010, **5:**e12012.
144. Harrington W, McGready R, Muehlenbachs A, Fried M, Nosten F, Duffy P: **Intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine: the times they are a-changin'.** *Clin Infect Dis* 2012, **55:**1025-1026; author reply 1026-1027.
145. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, Macete E, Berenguera A, David C, Dobano C, et al: **A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic.** *PLoS One* 2008, **3:**e1934.
146. D'Acremont V, Kahama-Maro J, Swai N, Mtasiwa D, Genton B, Lengeler C: **Reduction of anti-malarial consumption after rapid diagnostic tests implementation in Dar es Salaam: a before-after and cluster randomized controlled study.** *Malar J* 2011, **10:**107.

147. O'Meara WP, Mangeni JN, Steketee R, Greenwood B: **Changes in the burden of malaria in sub-Saharan Africa.** *Lancet Infect Dis* 2010, **10:**545-555.
148. WHO: **WHO expert consultation on cotrimoxazole prophylaxis in HIV infection.** *WHO Geneva* 2006.
149. TACAIDS Z, NBS, OCGS, ICF International: **Tanzania HIV/AIDS and Malaria Indicator Survey: 2011 - 2012.** March 2013.
150. Alifrangis M, Lusingu JP, Mmbando B, Dalgaard MB, Vestergaard LS, Ishengoma D, Khalil IF, Theander TG, Lemnge MM, Bygbjerg IC: **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* 2009, **80:**523-527.
151. Gesase S, Gosling RD, Hashim R, Ord R, Naidoo I, Madebe R, Mosha JF, Joho A, Mandia V, Mrema H, et al: **High resistance of Plasmodium falciparum to sulphadoxine/pyrimethamine in northern Tanzania and the emergence of dhps resistance mutation at Codon 581.** *PLoS One* 2009, **4:**e4569.
152. Welfare TMoHaS: **MTUHA Annual Report.** *Tanzania MOH* 2013.
153. Wampfler R, Mwingira F, Javati S, Robinson L, Betuela I, Siba P, Beck HP, Mueller I, Felger I: **Strategies for Detection of Plasmodium species Gametocytes.** *PLoS One* 2013, **8:**e76316.
154. Kabanywanyi AM, Macarthur JR, Stolk WA, Habbema JD, Mshinda H, Bloland PB, Abdulla S, Kachur SP: **Malaria in pregnant women in an area with sustained high coverage of insecticide-treated bed nets.** *Malar J* 2008, **7:**133.
155. Hommerich L, von Oertzen C, Bedu-Addo G, Holmberg V, Acquah PA, Eggelte TA, Bienzle U, Mockenhaupt FP: **Decline of placental malaria in southern Ghana after the implementation of intermittent preventive treatment in pregnancy.** *Malar J* 2007, **6:**144.
156. Taylor SM, Antonia AL, Chaluluka E, Mwapasa V, Feng G, Molyneux ME, ter Kuile FO, Meshnick SR, Rogerson SJ: **Antenatal receipt of sulfadoxine-pyrimethamine does not exacerbate pregnancy-associated malaria despite the expansion of drug-resistant Plasmodium falciparum: clinical outcomes from the QuEERPAM study.** *Clin Infect Dis* 2012, **55:**42-50.
157. ter Kuile FO, van Eijk AM, Filler SJ: **Effect of sulfadoxine-pyrimethamine resistance on the efficacy of intermittent preventive therapy for malaria control during pregnancy: a systematic review.** *JAMA* 2007, **297:**2603-2616.
158. White NJ: **Intermittent presumptive treatment for malaria.** *PLoS Med* 2005, **2:**e3.
159. Tagbor H, Bruce J, Agbo M, Greenwood B, Chandramohan D: **Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: a randomised controlled non-inferiority trial.** *PLoS One* 2010, **5:**e14425.
160. Gutman J, Mwandama D, Wiegand RE, Ali D, Mathanga DP, Skarbinski J: **Effectiveness of intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy on maternal and birth outcomes in Machinga district, Malawi.** *J Infect Dis* 2013, **208:**907-916.
161. McClure EM, Goldenberg RL, Dent AE, Meshnick SR: **A systematic review of the impact of malaria prevention in pregnancy on low birth weight and maternal anemia.** *Int J Gynaecol Obstet* 2013, **121:**103-109.
162. Yusuff KB, Omarusehe LD: **Determinants of self medication practices among pregnant women in Ibadan, Nigeria.** *Int J Clin Pharm* 2011, **33:**868-875.
163. Center for Drug Evaluation Research CfBEaR: **Guidance for industry establishing pregnancy exposure registries (2002).** *US Food and Drug Administration* 2002.
164. Agency EM: **Guideline on the exposure to medical products during pregnancy: need for post-authorisation data.** *EMEA/CHMP/313666/2005* 2005.
165. Network I: **Brief profile of member centres.** *INDEPTH Network*.

166. Manyando C, Kayentao K, D'Alessandro U, Okafor HU, Juma E, Hamed K: **A systematic review of the safety and efficacy of artemether-lumefantrine against uncomplicated Plasmodium falciparum malaria during pregnancy.** *Malar J* 2012, **11:**141.
167. Tarning J, Rijken MJ, McGready R, Phyo AP, Hanpithakpong W, Day NP, White NJ, Nosten F, Lindegardh N: **Population pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated malaria.** *Antimicrob Agents Chemother* 2012, **56:**1997-2007.
168. Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, Menendez C, Nambozi M, Valea I, Nabasumba C, Sasi P, et al: **Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial.** *PLoS One* 2009, **4:**e7871.
169. Four Artemisinin-Based Combinations Study G: **A head-to-head comparison of four artemisinin-based combinations for treating uncomplicated malaria in African children: a randomized trial.** *PLoS Med* 2011, **8:**e1001119.
170. WorldWide Antimalarial Resistance Network DPSG: **The effect of dosing regimens on the antimalarial efficacy of dihydroartemisinin-piperaquine: a pooled analysis of individual patient data.** *PLoS Med* 2013, **10:**e1001564; discussion e1001564.
171. Osarfo J: **Efficacy, safety and tolerability of dihydroartemisinin-piperaquine for treatment of uncomplicated malaria in pregnancy in Ghana.** *MCDC* 2014.
172. Klein EY: **Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread.** *Int J Antimicrob Agents* 2013, **41:**311-317.
173. Curtis CF, Jana-Kara B, Maxwell CA: **Insecticide treated nets: impact on vector populations and relevance of initial intensity of transmission and pyrethroid resistance.** *J Vector Borne Dis* 2003, **40:**1-8.
174. Protopopoff N, Matowo J, Malima R, Kavishe R, Kaaya R, Wright A, West PA, Kleinschmidt I, Kisinza W, Mosha FW, Rowland M: **High level of resistance in the mosquito Anopheles gambiae to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania.** *Malar J* 2013, **12:**149.
175. Negin J, Cumming RG: **HIV infection in older adults in sub-Saharan Africa: extrapolating prevalence from existing data.** *Bull World Health Organ* 2010, **88:**847-853.
176. Programme WGM: **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** *WHO Geneva* 2013.
177. Communicable Disease Control PaEWpESWaPotHEPoCSP: **safety of pyrethroids for public health use.** *WHO Geneva* 2005.
178. Koren G, Matsui D, Bailey B: **DEET-based insect repellents: safety implications for children and pregnant and lactating women.** *CMAJ* 2003, **169:**209-212.
179. Abba K, Deeks JJ, Olliaro P, Naing CM, Jackson SM, Takwoingi Y, Donegan S, Garner P: **Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries.** *Cochrane Database Syst Rev* 2011**:**CD008122.

CURRICULUM VITAE

Personal information

Surname	Mosha
Other names	Dominic Franklin
Date of birth	29th April, 1978
Sex	Male
Nationality	Tanzanian by Birth
Contact address	Ifakara Health Institute, P. O. Box 78373, Dar es Salaam, Tanzania.
E-mail	dmosha@ihi.or.tz and dfmosha@hotmail.com

Academic qualification

2011 – 2014	**PhD in Epidemiology** (Basel University, Switzerland).
2006 – 2007	**Masters of International Health, MIH** (Copenhagen University, Denmark).
1999 – 2004	**Doctor of Medicine, MD** (KCMUCollege of Tumaini University, Tanzania)

Publications

- **Dominic Mosha**. Comparing health services and patients care between Northumbria HealthTrust in the United Kingdom and KCMC Hospital in Tanzania: Elective experience. *Sbmj* 2004: 386
- **Mosha D,** Mahande M, Ahaz J, Mosha M, Njau B, Kitali B, Obure J. Factors associated with management of cervical cancer patients at KCMC Hospital, Tanzania: a retrospective cross-sectional study. *Tanzania Journal of Health Research 2009, 11(2):70-74*
- **Dominic Mosha**, Anja Poulsen, Hugh Reyburn, Frank Mtei, Elimsaada Kituma, Ib Bygbjerg: Quality of paediatric blood transfusion at district level in Tanzania. *BMC Pediatric 2009, 9:51*
- Amon Exavery, Sigilbert Mrema, Amri Shamte, Kristin Bietsch, **Dominic Mosha**, Godfrey Mbaruku, Honorati Masanja. Levels and correlates of non-adherence to WHO recommended inter-birth intervals in Rufiji, Tanzania. *BMC Pregnancy Childbirth 2012, 12:152*
- Mohammed A, Ndaro A, Kalinga A, Manjurano A, MoshaJ, **Mosha D**, van Zwetselaar M, Koenderink J, Mosha F, Alifrangis M, Reyburn H, Roper C, Kivishe R. Trends in chloroquine resistance marker, Pfcrt-K76T mutation ten years after chloroquine withdrawal in Tanzania. *Malaria Journal* 2013, 12:415
- **Dominic Mosha**, Festo Mazuguni, Kahema Irema, Sigilbert Mrema, Salim Abdulla, Blaise Genton. Medication exposure during pregnancy: a pilot pharmacovigilance study using health demographic surveillance platform. (*Under review in BMC Pregnancy & Childbirth journal*).

- **Dominic Mosha,** Monia Guidi, Felistas Mwingira, Salim Abdulla, Thomas Mercier, Laurent Arthur Decosterd, Chantal Csajka, Blaise Genton. Population pharmacokinetics and clinical response of artemether-lumefantrine in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* malaria in Tanzania. (Accepted *in Antimicrobial Agent & Chemotherapy journal*).

- **Dominic Mosha,** Festo Mazuguni**,** Sigilbert Mrema, Esperanca Sevene, Salim Abdulla, Blaise Genton. Safety of artemether-lumefantrine exposure in early pregnancy: an observational cohort (Accepted *in Clinical Infectious Diseases Journal*)

- **Dominic Mosha,** Jaff Chilongola, Rabi Ndeserua, Felista Mwingira, Blaise Genton. Effectiveness of Intermittent Preventive Treatment with sulfadoxine-pyrimethamine during pregnancy on placental malaria, maternal anaemia and birth weight in areas with high and low malaria transmission intensity in Tanzania (Under review in *Tropical Medicine & International Health Journal*)

Work experience

2009 – To date	**Research Scientist,** Ifakara Health Institute (Rufiji HDSS).
2007 – 2009	**Assistant Lecturer,** KCMUCollege of Tumaini University, Department of Public Health.
2005 – 2007	**Medical Officer,** KCMC Referral Hospital, Department of Emergency Medicine.
2004-2005	**House officer,** KCMC Referral Hospital

Referees

1. Prof. Blaise Genton, *MD, MSc, PhD*
   Project leader,
   Swiss Tropical and Public Health Institute,
   Socinstrasse 57, 4002 Basel,
   Switzerland.
   Tel : +41 79 556 5868
   Fax : +41 61 284 8105
   Email: Blaise.Genton@unibas.ch
2. Dr. Godfrey Mbaruku, *MD, MMed, PhD*
   Chief Research Scientist and IHI Deputy Director,
   Ifakara Health Institute (IHI),
   P.O Box 78373,
   Dar es Salaam. Tanzania.
   Tel: +255 784 492129
   Fax: +255 222 771714
   Email: gmbaruku@ihi.or.tz