UNIVERSITY OF NAIROBI

A COMPARISON OF DIFFERENT PARASITAEMIA LEVELS ON MALARIA TRANSMISSION POST-ASEXUAL DRUG TREATMENT

MUSYOKA DENNIS MUTINDA

I56/81235/2015

Thesis submitted in partial fulfillment for the award of Masters of Science in Biotechnology, University of Nairobi

October 2018

DECLARATION

I declare that this is my own work, and to the best of my knowledge has never been submitted as proposed work of study or examined for the award of degree in any university.

Musyoka Dennis Mutinda

Registration Number: I56/81235/2015

Signature: ……

Date: ……...

APPROVAL

We approve this thesis to be presented to the board of postgraduate studies as per the university regulations.

Dr. Isabella Oyier, PhD

Centre for Biotechnology and Bioinformatics (University of Nairobi)

Signature: ……

Date: ……..

Dr. Melissa Kapulu, PhD

KEMRI-Wellcome Trust Research Programme (Kilifi)

Signature: ……..

Date: …….....

ACKNOWLEDGEMENTS

I wish to thank Dr. Melissa Kapulu for believing in me to carry out this project under her supervision. My sincere appreciation for her guidance, mentorship, support and patience throughout the entire process. I also express my gratitudes to Dr. Isabella Oyier for supervision and support during the entire study period. For the much insightful Monday meetings with Prof Philip Bejon and Michelle Muthui I give all thanks.

I thank the IDeAL team led by Dr. Sam Kinyanjui for the studentship award and the constant support and attention to see me through this project. I also thank members of Centre for Biotechnology and Bioinformatics (University of Nairobi) for laying out a great support system in collaboration with KWTRP.

Much appreciation to Kevin Wamae, Emily Nyatichi, Polycarp Mogeni, MALPAC study team and colleagues in the IDeAL MSc students 1st Cohort for the invaluable assistance granted in lab work, analysis and write up.

Finally and with all the love I thank my family: my parents Thomas Musyoka and Jacinta Ndunge, my siblings Anthony Mutunga, Veronicah Mwikali and lovely Faith Mutanu. You have been my anchor through graduate school always encouraging me to push on and be better.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AL	Artemether-Lumefantrine
AP	Atovaquone-Proguanil
AS	Artesunate
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid
EIR	Entomological Infection Rate
G6PDH	Glucose-6-Phosphate Dehydrogenase
IRS	Insecticide Residual Spraying
LLITNs	Long Lasting Insecticide Treated Nets
MALPAC	Malaria Parasite Clearance
PCR	Polymerase Chain Reaction
PfMGET	*Plasmodium falciparum* male gametocyte-enriched transcript
PQ	Primaquine
RBCs	Red Blood Cells
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SP	Sulphadoxine-Pyrimethamine
WHO	World Health Organization
ΔΔCT	DeltaDelta C
KHDSS	Kilifi Health and Demographic Surveillance System

ABSTRACT

Malaria remains a public health concern despite remarkable progress towards its control. The current anti-malarial drug treatment, artemisinin-based combination therapy (ACT) is effective in clearing asexual stages and immature sexual stages, gametocytes, of *Plasmodium falciparum* but has limited effects on mature gametocytes which are responsible for human to mosquito transmission. Asymptomatic infections, generally of low parasitaemia, are common in endemic areas and yet their contribution to malaria transmission is not fully known. Moreover, the association between different parasitaemia levels and gametocyte carriage is not yet fully understood. Here, we determine gametocyte carriage of individuals with low parasitaemia and/or asymptomatic infections, kinetics of gametocyte appearance and clearance post-asexual anti-malarial drug treatment. Asexual parasitaemia in asymptomatic individuals was determined by quantitative polymerase chain reaction (PCR) targeting 18s ribosomal DNA in a Malaria Parasite Clearance study conducted along the Kenyan coast, an area of low to moderate transmission, between November 2013 and February 2014. A total of 90 healthy adults aged between 18-50 years were recruited and randomized to receive the following anti-malarial drugs: 1) atovaquone-proguanil and artesunate (for 3 days); 2) artesunate (for 7 days); and 3) sulphadoxine-pyrimethamine, artesunate and primaquine (1 dose/ 3 days/ 1 dose); and monitored for a period of 84 days. Samples from this study, covering 20 time points, were then used to determine gametocyte carriage as detected by reverse transcriptase polymerase chain reaction (RT-PCR) targeting the Pfs25 messenger RNA. Analysis of gametocyte kinetics was confined to subjects with more than two time points positive for asexual parasites (N=44 individuals) during the study period. Gametocyte prevalence was 25% (11/44) at enrollment. Individuals with gametocytaemia at enrollment presented with no carriage by day 9 post-treatment irrespective of the drug administered. Gametocyte carriage acquisition during follow up (up to 84 days) was 81.3% (26/44) for participants negative at enrollment irrespective of treatment (32/44). There was a significant difference between gametocyte proportions shortly after treatment (7 days post-treatment, 45.5% (20/44)) and at follow up (84 days post-treatment, 77.3% (34/44), p= 0.0022). After stratifying for different parasite levels in individuals presenting with parasites between 100-50,000 parasites/µl (moderate/microscopic parasitaemia), these parasite densities were positively associated with the prevalence of gametocyte carriage (p=0.0004) whilst a negative association was observed in individuals

presenting with much higher densities (>50,000 parasites/µl). On the other hand, low level asexual parasitaemia (≤100 parasites/µl) was more likely to be positive for sexual stages than those above 100 parasites/µl (p= 0.1557). Low level asexual parasitaemia, here defined as ≤100 parasites/µl, is more likely to result in gametocyte carriage post-treatment in our setting, an area of changing malaria epidemiology. Even after anti-malarial drug treatment, there is an increased likelihood to develop gametocytes for those not presenting with sexual parasitaemia at baseline.

CHAPTER 1 : INTRODUCTION/LITERATURE REVIEW

1.1 Background of the study

Malaria is a mosquito-borne disease caused by *Plasmodium* parasites which are spread by an infected female *Anopheles* mosquito. People with uncomplicated malaria (no clinical signs to indicate severity or vital organ dysfunction) experience fever and flu-like symptoms. If untreated they may develop severe complications or even death. The incidence rate of malaria globally declined steadily from 76 to 63 cases per 1000 population at risk from 2010 to 2016 representing an 18% decline ("WHO | World malaria report 2017,"). However, in 2016, an estimated 216 million cases of malaria occurred worldwide (90% in the WHO African Region), compared with 237 million cases in 2010 and 211 million cases in 2015 ("WHO | World malaria report 2017,"). In 2016, there were an estimated 445000 deaths from malaria globally (WHO African Region accounting for 91%), compared to 446000 estimated deaths in 2015 ("WHO | World malaria report 2017,"). These statistics continue to highlight the significance of malaria as a critical obstacle to global health targets despite remarkable gains made in the past decades. Various interventions have been developed to prevent infection and/or cure disease ranging from vector control measures such as insecticide treated nets and indoor residual spraying to human vaccines and blood-stage parasite drug therapies.

1.2 Malaria: Vectors and species

Sub-Saharan Africa remains hard-hit by malaria based on the high morbidity and mortality reported and the vectors of human malaria in this region have been described (Sinka et al., 2010, Sinka et al., 2012). Among 41 vector species highlighted, the dominant *Anopheles gambiae* complex contributes to the increased burden of malaria experienced in sub-Saharan Africa. Within the *A. gambiae* complex, the *A. gambiae sensu stricto* is one of the most efficient vectors of *falciparum* malaria. To date only 5 species have been documented to cause human malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale* and *Plasmodium malariae*. These single-celled protozoan parasites have similar life cycles (except *P. malariae* which has a 3-day cycle) and cause varying severity of human malaria.

P. falciparum and *P. vivax* have the greatest health burden with *P. falciparum* being responsible for the majority of mortality cases in sub-Saharan Africa. *P. vivax* is mainly found

in South East Asia and infects people who are duffy positive (an antigen found on the surface of red blood cells (RBCs)) which is barely present in Africa (Howes et al., 2011) although there is increasing evidence showing emergence of vivax malaria in duffy-negative individuals (Abdelraheem et al., 2016, Woldearegai et al., 2013). It is less life-threatening in comparison to *falciparum* malaria although its dormant liver stages can activate months or years after an infection. *P. ovale* and *P. malariae* mono-infections are also found in Africa but in low prevalence (Doctor et al., 2016). *P. knowlesi* is found in South-East Asia and although originally known to be a macaque pathogen, it has been reported to cause zoonotic malaria (Singh et al., 2004).

1.3 Life cycle of Plasmodium falciparum

During a blood meal, an infected female *Anopheles* mosquito injects sporozoites into the blood stream. Sporozoites invade hepatocytes where they multiply to produce schizonts which then release merozoites into the blood stream and invade red blood cells. Asexual parasite progression follows through a set of stages (ring, trophozoite and schizont) which are responsible for the symptoms associated with malaria. A small proportion of merozoites form gametocytes, which are the only parasite stage that is responsible for transmission from humans to mosquito vectors. Commitment of the *P. falciparum* parasites to sexual stages is described as a stress response to increasing unfavorable conditions such as antimalarial drugs and/or host immune system (Baker, 2010) which might be an evolutionary mechanism to ensure survival of the parasite. However, gametocytes have also been detected in asymptomatic individuals who were yet to receive treatment (Bousema et al., 2004) suggesting that several intrinsic factors affect the decision to initiate the sexual pathway. Early-stage (I-III) gametocytes sequester in bone marrow and within internal organs and only mature stage (IV/V) gametocytes circulate in the peripheral blood where they can be taken up by mosquitoes (Figure 1).

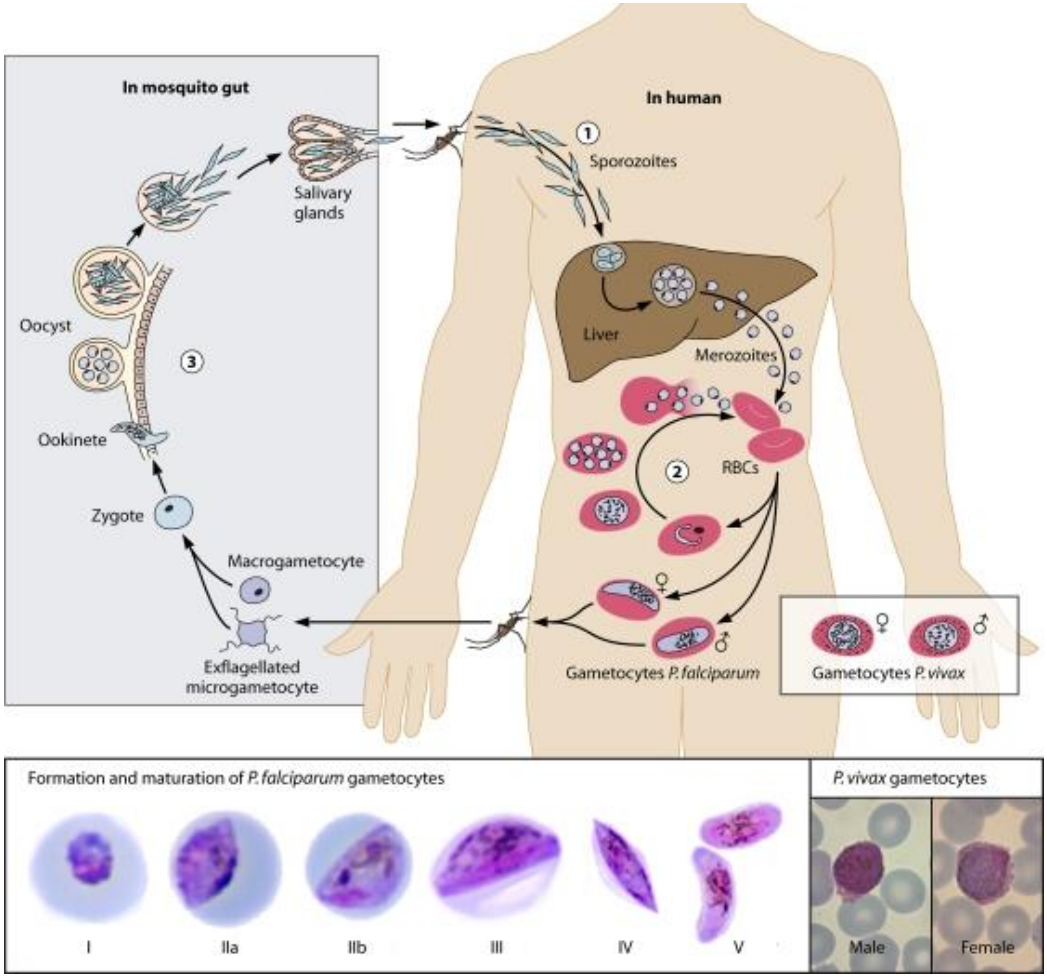

Figure 1.1: The life cycle of P. falciparum in both the human and mosquito host highlighting the main developmental stages. On the bottom is a photograph showing the distinct morphological stages of gametocytes from stage I to stage V. Adapted from Bousema and Drakeley (2011).

P. falciparum gametocytes require about 10 days to grow from stage I to stage V. Changes in temperature and pH activate gametocytes within the mosquito to form gametes. In the mosquito midgut, gametes fuse producing a zygote which develops into an ookinete, which can penetrate the midgut wall and form oocysts. Oocysts enlarge over time and burst to release sporozoites that migrate to the mosquito salivary gland, from where they can infect humans during the next blood meal.

1.4 Malaria interventions

1.4.1 Insecticide treated nets

Conventional insecticide-treated nets and long lasting insecticide-treated mosquito nets (LLITNs) have been the most widely used intervention for malaria control in Africa (Bhatt et al., 2015) to prevent malaria transmission through mosquito bites. LLITNs use a new bioactive technology that releases insecticides to the surface of the netting fibers. They do not require re-treatment and have minimal potential for environmental impact. To fully realize the full benefits of LLITNs, wide coverage and distribution to the most vulnerable populations (children under 5 years and pregnant women) is needed and the routine use of nets. However, the use of these nets has been low despite wide distribution (Pulford et al., 2011) which has become a limitation to realizing full potential of LLITNs owing partly to behavior change communication.

1.4.2 Indoor Residual Spraying (IRS)

IRS has been adopted as a possible way to vector control. IRS repels and kills mosquitoes resting inside houses. Spraying of insecticides is done on surfaces within a homestead once to thrice-a-year depending on transmission intensity or the type of insecticide. IRS has been linked to reduced morbidity and mortality although this has been shown to wane with time (Tukei et al., 2017). Its effectiveness heavily relies on total community coverage to ensure resting surfaces within homesteads are sprayed. Although insecticide-impregnated bed nets and indoor residual spraying of insecticides have been used for vector control, their efficacy is thought to be limited without concerted efforts directed against the parasite (Cui et al., 2015).

1.4.3 Malaria vaccines

The idea of a malaria vaccine was first put forward by Clyde DF (Clyde et al., 1973) involving a volunteer who was repeatedly exposed to X-ray irradiated *Plasmodium falciparum* sporozoites and developed antisporozoite antibodies on follow up. A number of vaccine candidates have since then renewed hope for malaria eradication although these have also failed due to limited efficacy (Patarroyo et al., 1992, Alonso et al., 1994). Despite considerable progress, we still lack a highly effective vaccine. However, continued development into pre-erythrocytic vaccines, blood-stage vaccines and transmission blocking vaccine is still ongoing with the RTS,S vaccine pilot implementation due to start in 2018 (“PATH and GSK welcome

progress toward RTS,S malaria vaccine pilot implementation with selection of countries," 2017). The RTS,S vaccine is the only advanced vaccine candidate to have undergone safety and efficacy clinical trials (Olotu et al., 2016, RTS,S Clinical Trials Partnership, 2014, Asante et al., 2011). However, results from a long-term phase II trial of the malaria vaccine strongly indicated that its efficacy falls over time, leading to rebounds in later years (Olotu et al., 2016).

1.4.4 Antimalarial drugs.

Antimalarial drugs are important in eradication measures because they not only offer disease resolution and prevent progression to severe disease but also prevent transmission to uninfected individuals. Antimalarial drugs target different stages of the *P. falciparum* life cycle and their effectiveness especially in interrupting transmission can reduce incidence and prevalence of malaria with greater effects experienced in areas of low transmission where the infectious reservoir is symptomatic and receives antimalarial treatment (White, 2008).

Quinoline derivatives (chloroquine, amodiaquine, mefloquine, primaquine, piperaquine, lumefantrine) accumulate in the parasitophorus food vacuole, forming a complex with heme thus preventing crystallization in the food vacuole (Medicine and School, 2000). Inhibition of heme polymerase enzyme results in accumulation of free heme which is toxic to the parasite. Primaquine has been shown to block transmission to mosquitoes (Shekalaghe et al., 2007) and is thought to act in this way. Antifolates (sulfonamides, pyrimethamine, and proguanil) target enzymes involved in folate synthesis, a pathway central to the synthesis of parasite DNA (Hyde, 2005). Atovaquone is used in combination with proguanil mainly for malaria chemoprophylaxis (McKeage and Scott, 2003). While atovaquone blocks the parasite mitochondrial electron transport chain, proguanil inhibits parasite dihydrofolate reductase through its active metabolite, cycloguanil (Srivastava and Vaidya, 1999). Artemisinin compounds (artemether, arteether, dihydroartemisinin and artesunate) are thought to bind iron leading to the generation of free radicals that damage parasite proteins (Eckstein-Ludwig et al., 2003). In addition, they have the fastest parasite clearance times compared to any other antimalarial drugs (White, 1994).

Resistance to antimalarial drugs has been on the rise resulting from target modification or alteration (White, 2004). Since the probability of resistance developing simultaneously to two

chemotherapeutic agents with different mechanisms of action is extremely low (White, 1998), combination therapy was introduced whereby highly effective short-acting artemisinins are combined with a longer-acting partner drug. Artemisinin-based combination therapies (ACTs) have greatly reduced morbidity and mortality rates and are the first-line drugs recommended for uncomplicated malaria (Organization, 2006). Despite the high efficacy reported on ACTs there is still an increase in new infections resulting from human-mosquito transmission. Thus as antimalarial drugs kill the asexual parasites, transmission stages (gametocytes) continue to be passed to the mosquito vector. Due to this, malaria elimination requires new interventions that not only target intra-erythrocytic stages but also block transmission by either reducing gametocyte formation or killing late-stage gametocytes. More important is the role of gametocytes in the transmission of resistant parasites which underscores the need to prevent transmission if malaria elimination is to be realized.

Factors such as parasite drug susceptibility, parasite density before initiation of treatment, individual differences in antimalarial pharmacokinetics and immunity influence parasite clearance times (White, 2011) and by extension gametocyte appearance and clearance. In addition, asexual parasite clearance times are difficult to estimate due to varying parasite densities and different drug regimens that are in use in different African regions. Consequently, the density and clearance of asexual parasites will affect gametocyte appearance and carriage. A recent study observed high gametocyte carriage in children treated with a non-ACT (mefloquine) and this was associated primarily with the slow clearance of asexual parasites which are the source of gametocytes (Sowunmi et al., 2009c). After drug or immune clearance of asexual parasites, the duration of gametocyte carriage is determined by the maximum duration of gametocyte sequestration (a process of deliberate isolation for instance in the extracellular spaces of the bone marrow which experience reduced circulatory flow and may provide protection from the host immune system) and the maximum circulation time following their release into the bloodstream (Bousema et al., 2010). As a consequence, gametocyte carriage in individual patients may persist for as long as 55 days (non-ACT) or 13 days (ACT) after a radical cure of infection (Bousema et al., 2010).

Artemether-Lumefantrine (AL) which is widely used in Africa, rapidly clears gametocytes in a median time of 72 hours although its effect is specific to the early sexual stages (Lefèvre et al.,

2001). Due to its pronounced effect on malaria transmission shortly after treatment, it has been proposed as the first-line choice for reducing community-wide transmission of *P. falciparum* in settings of low endemicity (Makanga, 2014). Primaquine (PQ) is the only drug effective against mature gametocytes (Shekalaghe et al., 2007) although it is not suitable for large scale use owing to its hemolytic effects in individuals with glucose-6-phosphate dehydrogenase (G6PDH) deficiency. However, WHO recommends a single dose of 0.25mg base/kg on the first day of treatment in combination with ACTs ("WHO | Updated WHO policy recommendation,"). A single dose of PQ added to sulphadoxine-pyrimethamine (SP) + artesunate (AS) was shown to efficiently clear gametocytes to concentrations below 0.1 gametocytes/ml in a hyper-endemic malaria zone in northeastern Tanzania (Shekalaghe et al., 2007). This suggests that, to reduce malaria transmission, the mode of action and target for antimalarial drugs as well as the half-life of these drugs should be considered. While the effect of artemisinins is restricted to immature gametocytes, PQ would therefore be a better option to target the mature forms of gametocytes in any comprehensive regimen. Moreover, in therapeutic efficacy studies on atovaquone-proguanil (AP), the emergence and clearance of gametocytes post-treatment have been less frequently evaluated in general. Since AP is widely used for chemoprophylaxis, it is desirable to evaluate its effects on gametocyte carriage and sex ratio owing to its diverse mode of action and long half-life.

1.5 Association between parasitaemia and gametocyte positivity

P. falciparum strategically invests in gametocytes in response to in-host conditions. Changes in the conversion rate optimize parasite survival and transmission during infection. The parasite thus 'decides' between in-host survival and between-host transmission. Using an evolutionary framework, Reece Se and colleagues show that high conversion early during infection increases chances of transmission though this risks investment in asexual stages thus resulting in a short duration of infection (Reece et al., 2009). On the other hand, focus on asexual parasite replication reduces chances of transmission at any time. While increased conversion is perceived as a response to within-host adverse conditions by the parasite to maximize transmission, mild in-host conditions such as low doses of antimalarial drugs and RBC resource limitation are thought to influence the parasite to restrain from reproduction (Reece et al., 2010) hence improving prospects of host survival and opportunities for future transmission.

Peak gametocytaemia follows 7-10 days after the peak of asexual parasites (Thomson, 1911), suggesting a relationship between asexual parasitaemia and gametocyte density. Low parasitaemia in continuous culture is accompanied by lower rates of conversion of gametocytes (Carter and Miller, 1979). In a rodent model study exploring how environmental factors and parasite density shape transmission strategies, an analysis of gametocytogenesis in *Plasmodium chabaudi* suggested that investment in transmission increases as RBCs decline and for some genotypes increases at low parasite densities (Cameron et al., 2013). This kind of terminal investment is likely to be driven by increased parasite immune/drug clearance from the host as the parasite responds to survival cues.

A cross sectional survey in Papua New Guinea to identify *P. falciparum* gametocyte carriage in children reported a higher likelihood to detect gametocytes in samples with high blood stage parasitaemia (Koepfli et al., 2015). They concluded that asexual parasite density was the strongest predictor for gametocyte carriage. Karl and colleagues looking at risk factors for gametocyte carriage in Papua New Guinea observed decreasing *P. falciparum* gametocyte positivity with increasing asexual parasitaemia in children with uncomplicated malaria prior to treatment (Karl et al., 2016). This negative association between gametocyte positivity and asexual parasitaemia has been described before where low blood stage parasitaemia (<1,000 parasites/µl) was shown to be a risk factor for patent gametocytaemia (Price et al., 1999) and high parasite densities (>100,000 parasites/ µl) were less frequently gametocyte carriers (von Seidlein et al., 2001a). These findings do not conclusively show the contributing role of specific parasitaemia levels to transmission. Moreover, most of the studies have been done in children and no studies show the significance of the older age groups to the infectious reservoir.

1.6 Sex ratio

In addition to gametocyte prevalence and the duration of carriage, mosquito infectivity is dependent on gametocyte sex ratio. In natural populations, gametocyte sex ratio is usually female-biased since male gametocytes can produce up to eight microgametes each derived from a single male gametocyte and therefore fertilize several female gametes (Paul et al., 2002). However, there are variations in sex ratio during the course of infection or at lower gametocyte densities whereby a more male-biased ratio becomes important for transmission success (Bousema and Drakeley, 2011). Gametocyte sex ratio measured in children enrolled in

a number of antimalarial drug efficacy studies for an 8-year period (Sowunmi et al., 2009b) shows a female biased sex ratio at enrollment with an overall increase over the course of infection. In this high-powered study, non-artemisinin monotherapy was positively associated with a male-biased sex ratio contrary to artesunate or ACTs which significantly reduced sex ratio (a female biased sex ratio) considering that male gametocytes are most sensitive to antimalarial drugs (Delves et al., 2013).

A critical point arises as to whether observed female sex ratio is due to cyto-adherence and selective retention of male gametocytes in deep tissue accompanied with preferential release of female gametocytes into peripheral blood or a selective killing effect on male gametocytes (Sowunmi et al., 2009). It is, therefore, possible that partner drugs in ACTs may modify their effects to produce a female-biased sex ratio (Sowunmi et al., 2009). An additional argument is that the sex ratio of first-appearing gametocytes as opposed to sex ratio of all gametocytes present in circulation would be a more sensitive indicator of the effects of antimalarial drugs (Sowunmi et al. 2009).

1.7 Quantification of gametocytes

Detailed monitoring of parasite clearance dynamics after drug treatment is required to determine whether parasite responsiveness to antimalarial drug therapies is changing. Light microscopy has been used over the years to detect and quantify parasites after drug treatment. However, very low parasite densities can be missed when using standard light microscopy due to limitations in sensitivity thus more sensitive and robust molecular methods are required to detect and quantify sub-microscopic gametocyte densities.

The expression profiles of *18S* ribosomal RNA, Pfs16, Pfg27 and Pfs25 genes have been determined in studies that aimed to identify potential drug targets (Berry et al., 2009) and this knowledge has been applied in the development of quantification assays based on the expression of these stage-specific transcripts. This is also based on previous studies on the expression of Pfs25 gene which was reported to be continuously expressed in mature gametocytes (Babiker et al., 1999) and during ookinete development. To facilitate studies on gametocyte carriage and sex ratio, a female-specific reverse transcriptase polymerase chain reaction (RT-PCR) assay which targets the Pfs25 gene and a male-specific RT-PCR assay which targets the Pfs230p gene were developed (Schneider et al. 2015). These assays require

known concentrations of gametocytes for determination of sample gametocyte densities. We employed a RT-PCR assay targeting Pfs25 and Pfs230p genes to detect female and male gametocytes, respectively. The detection of these sex-specific genes was in turn used to determine gametocyte sex ratio. This was done using samples from a randomized controlled trial set out to assess the efficiency of three anti-malarial drug treatments to clear asexual parasitaemia.

1.8 Justification

Gametocytes are responsible for malaria transmission maintaining high infection rates in regions where gametocyte carriage and prevalence remain high. Even at low densities, gametocyte carriage has been shown to be infectious to mosquitoes. Although other factors such as seasonality, age and genetic variation affect gametocyte carriage, a significant impact is influenced by the choice of antimalarial drugs used especially in endemic areas. Meaning that gametocyte prevalence, density and circulation time can be lowered to minimal levels even with the current drug regimens provided there is evidence for the best drug combinations. To identify these combinations, it is important to determine time trends regarding clearance and appearance of gametocytes after asexual parasite drug treatment. Asexual parasites are the prerequisite stages to sexual stages and changes in parasitaemia levels within the host will influence gametocyte carriage. However, there are studies that show conflicting associations between asexual parasitaemia and gametocyte positivity. Malaria transmission is known to rely on the transfer of the sexual stages of *P. falciparum* to the mosquito vector; therefore sex ratio is a key determinant to infectivity success. However, male and female gametocytes have different drug sensitivities and this could alter transmission potential and mosquito infectivity. This study, therefore, sought to determine the contributing role of different parasitaemia levels to gametocyte carriage and to determine the clearance and emergence of gametocytes post-asexual drug treatment with three drug regimens: i) Atovaquone-proguanil and Artesunate, ii) Artesunate, iii) Sulphadoxine-pyrimethamine and Artesunate and Primaquine. Sex ratio of first appearing gametocytes were assessed and how the ratio changed over time.

1.9 Hypothesis

Individuals with low level asexual parasitaemia post-treatment are more likely to present with gametocytaemia.

1.10 Study objectives

1.10.1 Main objective

To determine gametocyte dynamics in individuals presenting with low level parasitaemia post-asexual drug treatment.

1.10.2 Specific objectives

1) To establish the relationship between parasite density and gametocyte carriage
2) To determine the effects of drug treatment on gametocyte clearance
3) To determine the effects of drug treatment on gametocyte appearance
4) To assess drug induced changes in gametocyte sex ratio

CHAPTER 2 : METHODOLOGY

2.1 Ethics and consent

This study was approved by the ethical review committees in Kenya and the United Kingdom (SERU Protocol Number 2565). Written informed consent (pertaining the nature of the trial and potential risks as well as their obligations) was obtained from all volunteers before they were included in the trial (See Appendix 1). The study was conducted as per the Declaration of Helsinki("World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.," 2013).

2.2 Study area

This study was carried out in Junju, a village located in the southern parts of Kilifi County along the Kenyan coast. The Kenyan coast has previously been described as a malaria endemic region with intermittent spots of both low and high transmission intensities (Bejon et al., 2010). Kilifi County experiences hot and humid weather and has diverse ecological zones. The northern part of Kilifi County is a low transmission area with entomologic inoculation rate (EIR) of 10 infective bites/person/year (Mbogo et al., 1995) while the southern part is generally a moderate to high transmission area with estimates of 22-53 infective bites/person/year (Mbogo et al., 2003). Long rains occur from March-June whereas short rains start in October through to December and transmission increases just after the start of the rainy seasons. During the dry season, transmission is maintained at a low level. In comparison to other regions in Africa where up to EIR values of 667 were reported (Hay et al., 2000, Kilama et al., 2014), Junju which is located in the southern part of Kilifi county qualifies as an area of moderate transmission.

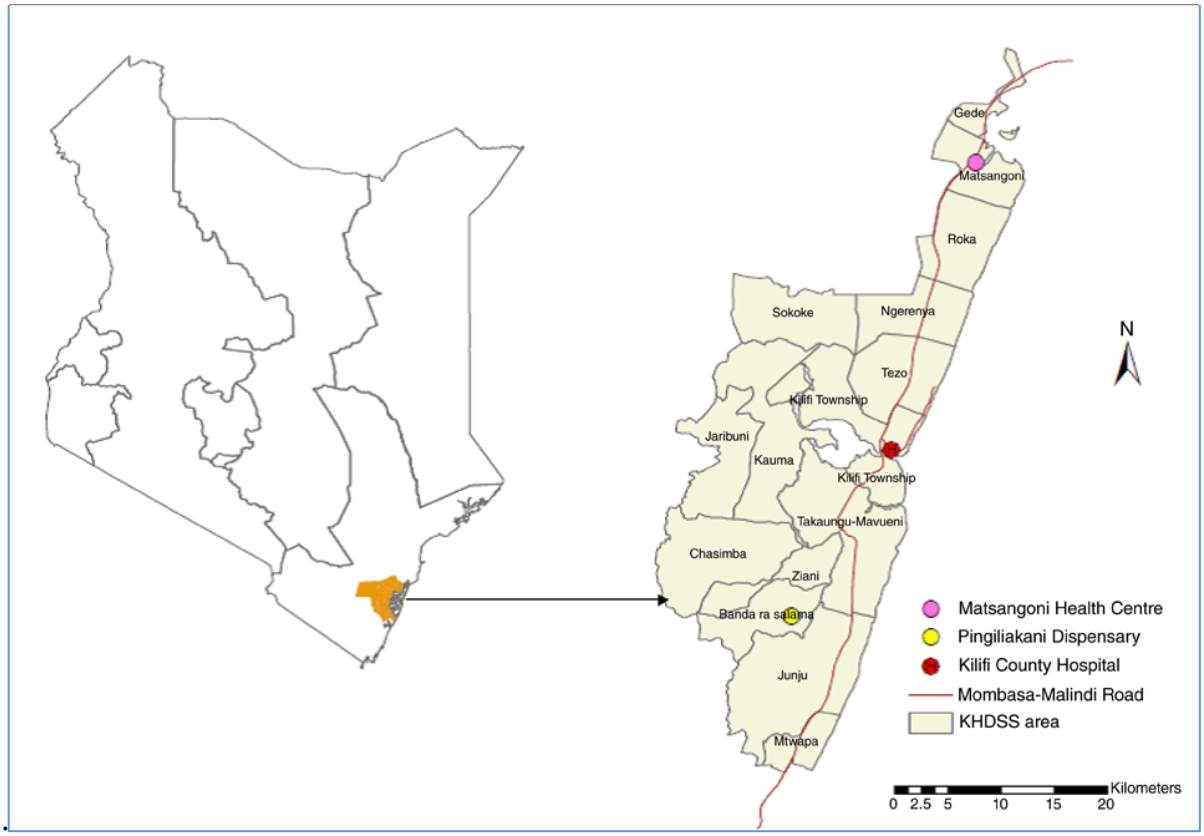

Figure 2.1: Map of Kenya showing the study area in Kilifi County

Anopheles mosquitoes are common in Kilifi with *Anopheles arabiensis* being more prevalent in the north of Kilifi while *Anopheles gambiae sensu lato* and *Anopheles funestus* are the dominant malaria vectors in the southern part (Mbogo et al., 2003). ITN use in the study area has increased from 55.9% in 2009 to a peak of 82.6% in 2013 (Mogeni et al., 2016) partly due to a mass distribution of ITNs in the same year.

2.3 Study design

This study was nested within a larger study on Malaria Parasite Clearance (MALPAC) carried out in Junju, Kilifi County from November 2013 to February 2014. The main aim was to identify which drug combination(s) effectively clear asexual parasites without subsequent inhibition of parasite growth. This information would feed into optimization of future Phase IIb vaccine trials prior to vaccine administration. In this study, blood samples were taken from 90 healthy adults.

2.3.1 Inclusion criteria

1) Consenting adults aged 18 – 50 years in good health.
2) Remain resident in the study area for the study duration.
3) Informed Consent

2.3.2 Exclusion criteria

1) Any significant medical disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.
2) Hemoglobin less than 11.3 g/dl for men and less than 10g/dl for women, where judged to be clinically significant in the opinion of the investigator.
3) Blood transfusion within the month preceding enrolment.
4) Current participation in another clinical trial or recent participation within 12 weeks of this study.
5) Any other finding which in the opinion of the investigators would increase the risk of an adverse outcome from participation in the trial.
6) Pregnant or lactating women.
7) Women unwilling to use contraception for the duration of drug treatment (to prevent chances of conception).
8) Likelihood of travel away from the study area.

Simple randomization of participants into 3 drug arms was done by an independent statistician with an allocation ratio of 1:1:1. Volunteers were randomized to receive 1) Atovaquone-Proguanil and Artesunate (for 3 days); 2) Artesunate (for 7 days); or 3) Sulphadoxine-Pyrimethamine, Artesunate and Primaquine (1 dose/ 3 days/ 1 dose). However, individuals with G6PD deficiency were only randomized between groups 1 and 2 to avoid hemolytic anaemia in the event that such volunteers are given primaquine. Participants with normal G6PD levels were randomized between all three drug arms.

Drug administration was initiated on day 0. Monitoring and sampling regimens were identical for all participants and began from day 0 up to day 84 at intervals of 3 times per week for the first 4 weeks, then subsequently twice per week for 3 weeks and finally once per week for 6 weeks.

All 90 participants completed the 84-day follow up and a total of 2,250 samples collected covering 25 time points for each subject. However, only 20 time points were available for this study. Blood samples of 0.1ml were stored in trizol and kept at −80 °C until analyzed.

2.4 Laboratory assays

There were a total 20 time points representing asexual parasite sampling time frames. Data on asexual parasitaemia had been previously determined (Unpublished work) by quantitative polymerase chain reaction (PCR) targeting 18s ribosomal DNA for each time point was available and was used to generate a sample set to answer objective 1 (establish the relationship between parasite density and gametocyte carriage). Thus 474 samples (26% of total samples representing 32 individuals) were selected for RNA extraction and cDNA synthesis with the following criteria of selection: (i) time points with low parasitaemia ≤100 parasites/µl; (ii) time points with the highest and lowest parasitaemia; (iii) day 0 time point positive for *P. falciparum*; and (iv) at least one-time point negative for asexual parasites. An additional sample set (n = 535) was analyzed to include all individuals with more than two time points positive for asexual parasites irrespective of a day 0 positivity.

2.4.1 RNA Extraction and cDNA Synthesis

RNeasy mini extraction kit (QIAGEN®) was used to extract total RNA from the samples per manufacturer's protocol with modifications. Briefly, 100µl whole blood stored in trizol was divided into two equal volumes and lysed in 600µl RLT lysis buffer. The lysate was transferred into DNA separation columns, centrifuged for 3 minutes at maximum speed. One volume (600µl) of 70% molecular grade ethanol was added to the supernatant and mixed well by pipetting to precipitate total RNA. Up to 700µlof sample was transferred to an RNeasy Mini spin column placed in a 2ml collection tube and centrifuged for 30 seconds at 8000 x g. The flow-through was discarded, 700µl Buffer RW1 added to the spin column and span for 30 seconds at 8000 x g. The flow-through was discarded, 500µl Buffer RPE added to the spin column and centrifuged for 30 seconds at 8000 x g. A second wash with Buffer RPE was

done for 2 minutes at 8000 x g. RNeasy spin columns were placed in a new 2ml collection tube and centrifuged at full speed for 1 minute to dry the silica membrane. The RNeasy spin columns were placed in new 1.5ml collection tube and 40µl of RNase-free water added directly to the membrane. Elution of RNA was done for 1 minute at 8000 x g. Complementary DNA (cDNA) synthesis was performed using Invitrogen SuperScript VILO cDNA synthesis kit per manufacturer's protocol. A total reaction volume of 20µl comprised of 4µl of master mix, 10µl of RNA and 6µl of nuclease-free water for each sample. Reverse Transcription conditions were set to run at, 25°C for 10 minutes, 42°C for 90 minutes, 85°C for 5 minutes and cooling at 15°C for 10 minutes.

For a subset of samples (n = 128) cDNA synthesis was done using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems™) after running out of the Invitrogen SuperScript VILO cDNA synthesis kit. A similar amount of RNA (10µl) was used to synthesize single-stranded cDNA from total RNA with master mix volumes reconstituted as described by the manufacturer. Briefly, to prepare 2× Reverse Transcription master mix, the kit components were allowed to thaw on ice and mixed in a total of 10µl per reaction as shown:

10× RT Buffer - 2.0µl

25× dNTP Mix (100 mM) - 0.8µl

10× RT Random Primers - 2.0µl

Reverse Transcriptase - 1.0µl

Nuclease-free H2O - 4.2µl

The reaction conditions for Reverse Transcription were set to run at, 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes and 4°C for 15 minute. All cDNA synthesis reactions were run using an Applied Biosystems®Veriti®96-Well Thermal Cycler.

Ten microliters of cDNA was diluted in 390ul of nuclease-free water to obtain a dilution of 1:40 and stored at -20 until the time of analysis. Diluted cDNA was used as template to assay for gametocyte positivity.

2.4.2 Gametocyte detection – RT-PCR

To detect gametocytes 2X SYBR® Green Master Mix (Applied Biosystems™) was used to amplify target sequences by reverse transcriptase PCR. Primers targeting Pfs25 (female-specific) and Pfs230p (male-specific) transcripts were as shown (Wampfler et al., 2013). To normalize expression data, samples were assayed alongside ubiquitin specific primers as a housekeeping gene.

Table 2.1: Primer sequences for target proteins.

Primer Name	Sequence
Pfs25 forward	TAATGCGAAAGTTACCGTGG
Pfs25 reverse	TCCATCAACAGCTTTACAGG
Pfs230p	CCCAACTAATCGAAGGGATGAA
Pfs230p reverse	AGTACGTTTAGGAGCATTTTTTGGTAA
PF08_0085 forward	ACCAGCTGATACTCCATGGG
PF08_0085 reverse	GCTGTTAGGGTTTGGGTCA

However, after running male-specific gametocyte detection assays on 373 samples there were sensitivity limitations of the Pfs230p mRNA positivity rates. Proceeding experiments thereafter only targeted the female gametocytes using Pfs25.

Primer mix preparations were obtained by combining forward primer, reverse primer and nuclease-free water at a concentration of 10mM in the ratio 2:2:1 making a total volume of 5µl. All samples and reagents were left to thaw on ice before use and reaction plates were set up on ice to maintain integrity of template and reagents.

Each well had a total reaction volume of 20µl: 10µl of 2X SYBR® Green Master mix, 5µl of primer (either target or housekeeping) and 5µl of cDNA sample. Samples were run in duplicate for both target and housekeeping genes. Cycling conditions used for this assays were: 95°C for 15 minutes and then 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds and 68°C for 30 seconds. All RT-PCR reactions were run on an ABI 7500 Real-Time PCR system (Applied Biosystems™).

Positive and negative control samples were included in all PCR reactions. For positive control, asexual parasites of 3D7 strain were cultured up to gametocyte stages as described elsewhere (Ifediba and Vanderberg, 1981). RNA was extracted from day 14 mature gametocytes and cDNA synthesized using the above mentioned kits and included in the gametocyte detection assays. Uninfected red blood cells were also taken through the same process and used as a negative control. Non-template control was also included as a control and was dH_2O.

Expression levels between the target and housekeeping genes were determined using the following equation $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001) (a log fold change between expression of the house keeping gene and the target gene). Samples were recorded as positive for gametocytes if they had a clear amplification curve above threshold and a -Delta Delta C_T value greater than 0.

2.5 Data analysis

Statistical analysis were done using Graph Pad Prism software version 7.03 (San Diego, CA, USA) and R software (version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria).

Gametocyte carriage was assessed at every time point for the entire sample set (N = 879 representing N = 44 participants) and expressed as the proportion of participants with patent gametocytaemia. Time points with no asexual parasitaemia were adjusted to half the lower detection limit of the RT-PCR assay (0.01parasites/µl) to allow for log transformation. Statistical significance of asexual parasitaemia for non-parametric data was determined by Mann-Whitney U test using unpaired ranks to compare between gametocyte positive and negative samples. Kaplan Meier survival analysis was used to show gametocyte clearance in individuals with patent gametocytaemia. Initial appearance of gametocyte was compared with gametocyte reappearance using the Kaplan Meier analysis. Statistical significance of any differences observed was determined by Log-rank (Mantel-Cox) test. Samples with missing asexual parasitaemia or gametocyte positivity data were not included in the analysis.

CHAPTER 3 : RESULTS

3.1 Study population

All 90 participants completed the 84-day follow up and a total of 2,250 samples collected covering 25 time points for each subject. However, only 20 time points were available for this study. The participants were randomized to each drug arm in a 1:1:1 ratio to receive AP + AS, AS or SP + AS + PQ respectively. About half of the participants (55.6%, 50/90) were parasite negative at enrollment with 21 of these remaining negative throughout the entire study period. Of those negative for asexual parasites at enrollment, 29/50 acquired parasites in at least one time point during follow-up. Analysis for gametocyte prevalence and kinetics was therefore confined to a subset of 45 participants with more than two time points positive during follow up (except for 1 which was not available at the time of analysis thus a total of 44 participants were analyzed).

There was no difference in the age of participants across the three different drug treatment groups (Table 3.1).

Table 3.1: Baseline characteristics for study participants.

	AP + AS	**AS**	**SP + AS + PQ**
Age in years			
Median (range)	29(20-50)	29(18-49)	29.5(18-48)
Mean (SD)	31(8)	31(8)	31(8)
Gender (%, (n/N))			
Female	53(16/30)	57(17/30)	37(11/30)
Asexual parasite density (Day 0)			
Median (range)	69(0-36,552)	0(0-2,418,101)	0(0-20,250)
Mean (SD)	4,959(10741)	85,349(441099)	1,590(4655)

AP; Atovaquone-Proguanil, AS; Artesunate, SP; Sulphadoxine and PQ; Primaquine. SD is the standard deviation around the sample mean.

The median age at enrollment for the subset of individuals analyzed in this study was 30 (range, 20-50), 29 (range, 18-49) and 34 (range, 23-47) for AP + AS, AS and SP + AS + PQ respectively (Table 3.2). There was a significant difference in asexual parasitaemia at enrollment for all drug arms (P= 0.0440, Kruskal-Wallis test).

Table 3.2: Description of the analyzed subset of participants.

	AP + AS (N = 17)	**AS** (N = 13)	**SP + AS + PQ** (N = 14)
Age in years			
Median (range)	30 (20-50)	29 (18-49)	34 (23-47)
Mean (SD)	31(9)	32(10)	33(8)
Gender			
Female	41% (7/17)	54% (7/13)	36% (5/14)
Asexual parasite density (Day 0)			
Median (range)	839 (0-36,552)	749 (0-2,418,101)	28 (0-14,527.13)
Mean (SD)	8,742 (13191)	196,958 (668127)	1,957 (4300)
Gametocyte prevalence			
at enrollment	29% (5/17)	23% (3/13)	21% (3/14)

AP; Atovaquone Proguanil, AS; Artesunate, SP; Sulphadoxine and PQ; Primaquine. SD is the standard deviation around the mean.

3.2 Gametocyte carriage

Gametocyte prevalence was 25% (11/44) at enrollment and varied with time and was similar to asexual blood stage proportions irrespective of drug regimen (Figure 3.1). Proportion of gametocytaemic individuals decreased to 6.8% (3/44) by day 16 and remained relatively low up to day 38 where prevalence increased to a peak of 29.5% (13/44) on day 56. This was followed by a gradual decline until day 84. Similarly, there was a fall in asexual parasite prevalence from 79.5% (35/44) at enrollment to 20.5% (9/44) on day 16 as a result of drug clearance. However, after this, participants presenting with parasitaemia increased to73% (32/44) on day 56 and plateaued until day 84.

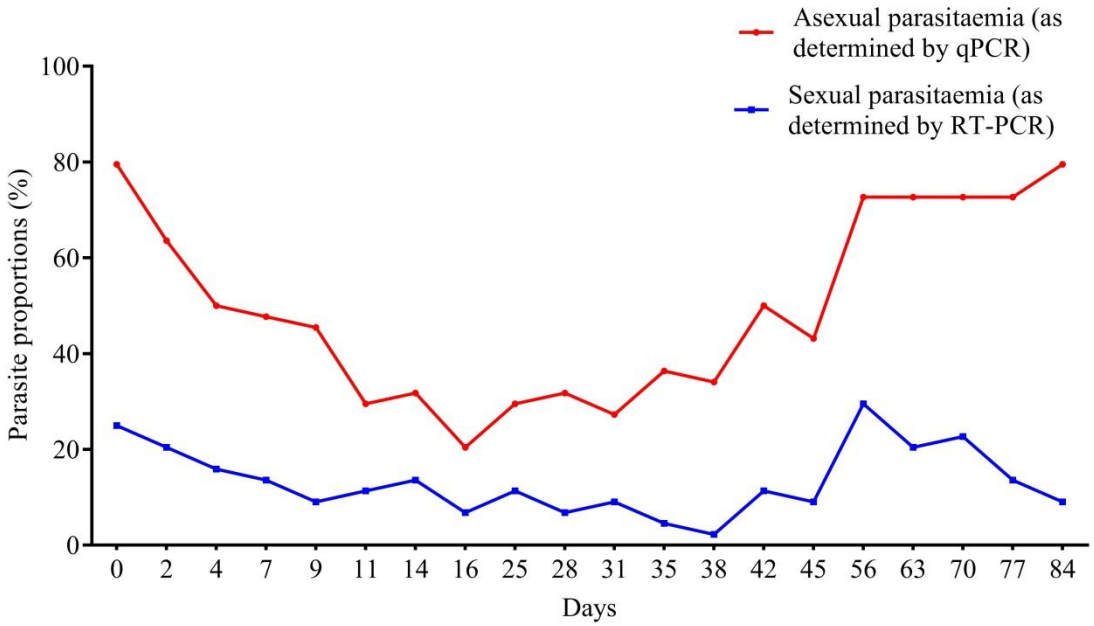

Figure 3.1: Proportion of asexual parasitaemia (red line) and sexual parasitaemia (blue line) over time throughout the study period (day 0-84).

Wary of the sample size limitations, we sought to determine gametocyte carriage in different drug arms for select time points, of 14 day-interval which is ideally the time it takes for mature gametocytes to be detected in peripheral circulation (Figure 3.2). Gametocyte prevalence in AP+AS group declined gradually to reach a low of 5.9% after 4 weeks while in AS group gametocytes were rapidly cleared by the second day. Prevalence in SP + AS + PQ group increased from 21.4% to 35.7% on day 2 before declining to 7.1% on day 42. However, the number of gametocyte positive individuals increased in the AS and SP+AS+PQ drug arms after day 42 as compared to AP + AS group.

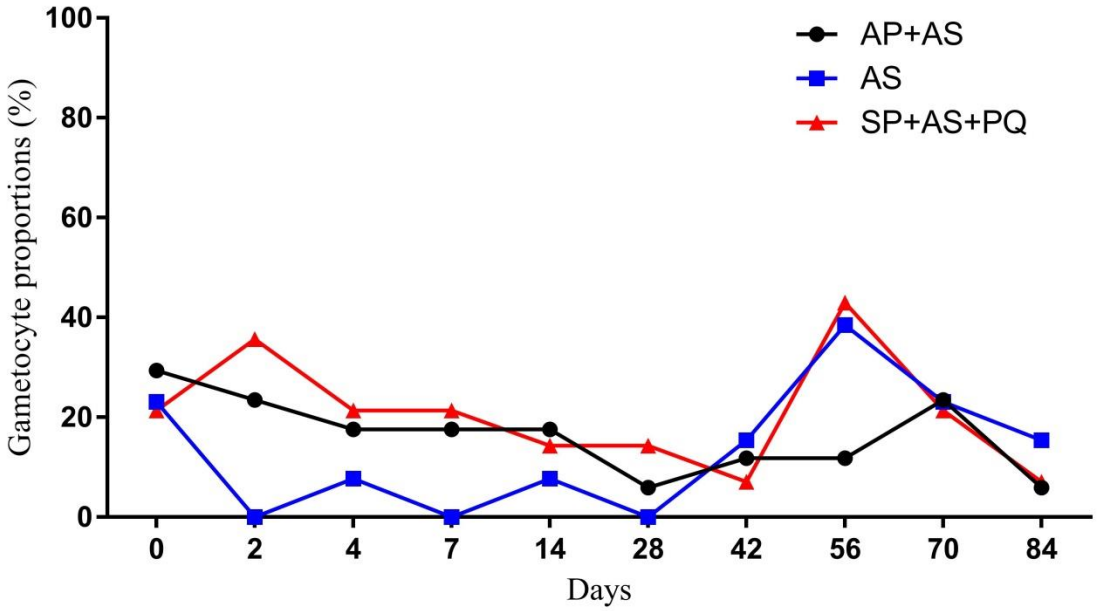

Figure 3.2: Gametocyte prevalence of select time points per drug arm over the entire study period.

Proportion of participants with gametocytes shortly after treatment (7 days) and during follow up (day 9 – day 84) was assessed to determine differences in gametocyte carriage. The proportion of volunteers positive for gametocytes shortly after treatment (7 days post-treatment) was 45.5% (20/44) and increased to 77.3% (34/44) during follow up (84 days post-treatment, p= 0.0022 two-tailed t-test, Figure 3.3) which might be due to recrudescence or new infections although parasite genotyping data to determine this was not analyzed.

Further analysis by specific drug treatment (Figure 3.3) for all participants regardless of carriage at enrollment, shows the lowest risk of gametocytaemia was in AS treatment arm (23.1% (3/13)) within the first week but highest (76.9% (10/13) p=0.006) in the subsequent follow up (84 days). A similar trend was observed in AP + AS group although with modest variations (35% (6/17) to 59% (10/17) p=0.17 Figure 3.3). Prevalence in SP+AS+PQ group remained unchanged at 64% (9/14).

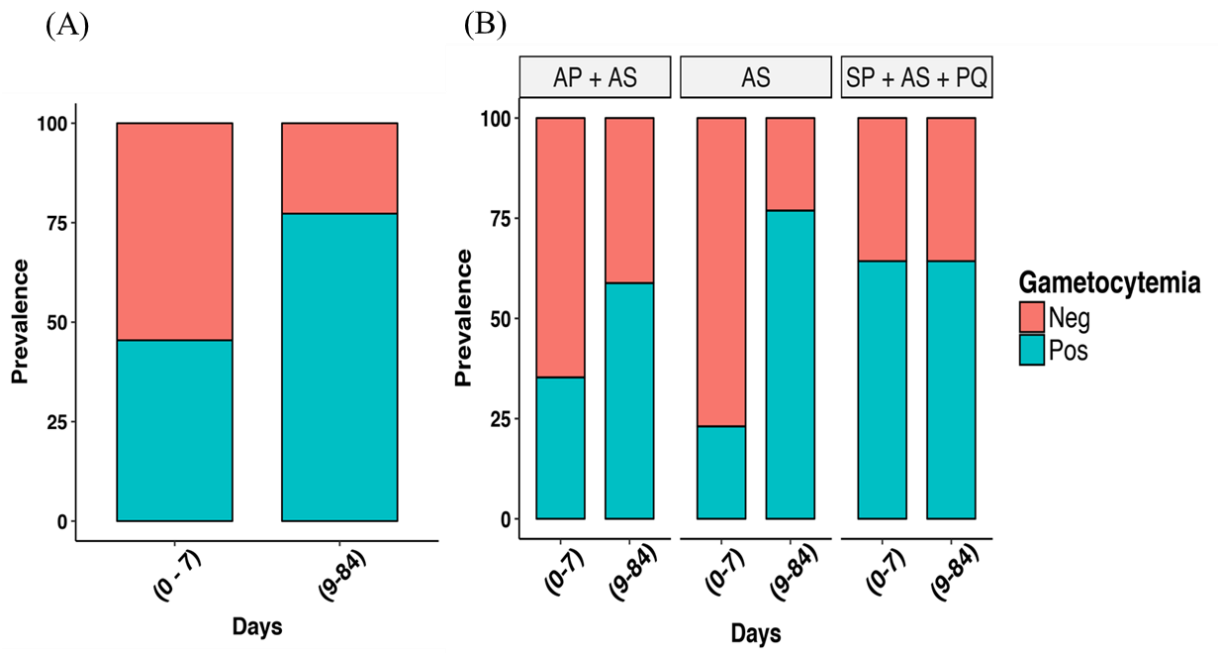

Figure 3.3: Bar graphs of the proportion of participants with gametocytaemia within the first week of treatment (0-7) and during follow up (9-84)

(A); and proportion of participants with gametocytaemia per drug arm within the first week of treatment (0-7) and during follow up (9-84) (B). Pos: gametocyte positive, Neg: gametocyte negative. AP: atovaquone-proguanil, AS: Artesunate, SP: sulphadoxine-pyrimethamine and PQ: Primaquine.

3.3 Association between asexual parasite density and gametocyte positivity

We compared asexual parasitaemia between gametocyte positive and gametocyte negative samples to ascertain whether low parasitaemia was more likely to present with gametocytaemia. High parasite densities were more likely to be gametocytaemic (Figure 3.4, p=0.0001 Mann-Whitney test) while low parasitaemia was more likely to present with no gametocytes.

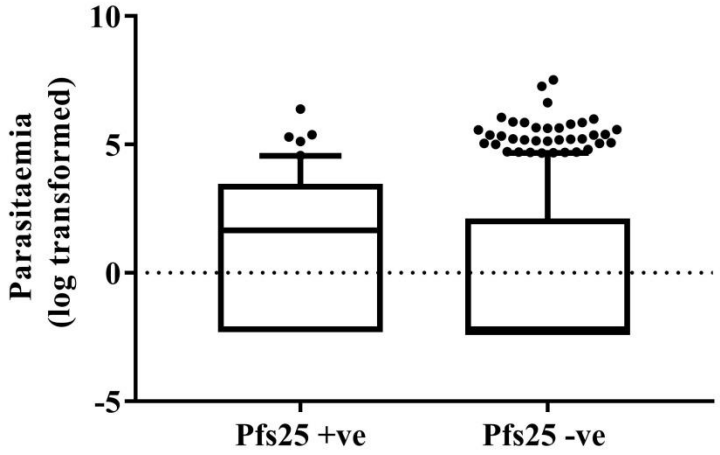

Figure 3.4: A box plot of log transformed parasitaemia for both Pfs25 positive and negative samples.

The box covers 50% of data with a line showing the median and whiskers extending to cover lower and upper limits of 95% confidence intervals.

Asexual parasitaemia were grouped as shown in Table 3.3 to determine which densities highly contribute to gametocyte carriage. Gametocyte proportions increased with increasing densities although there was a remarkable decline in prevalence of gametocytaemia in parasite densities above 50,000 parasites/µl. 9.9% of samples positive for gametocytes had no parasitaemia.

Table 3.3: An illustration of the contribution of different asexual parasite densities to gametocyte positivity.

Asexual parasitaemia	**Gametocyte positive**
0	9.9% (44/444)
>0-100	11.5% (20/174)
>100-1000	14.2% (17/120)
>1000-10000	25.7% (17/66)
>10000-50000	39.5% (15/38)
>50000	10.8% (4/37)

All time points positive for gametocytes were then categorized based on parasite densities, <100 parasites/µl and >100 parasites/µl. Samples with low parasitaemia (<100 parasites/µl) were more likely to test positive for sexual stages than samples with parasitaemia above 100 parasites/µl with borderline significance (p=0.1557, Figure 3.5 Wald method ("Agresti, A., and Coull, B. A. (1998)).

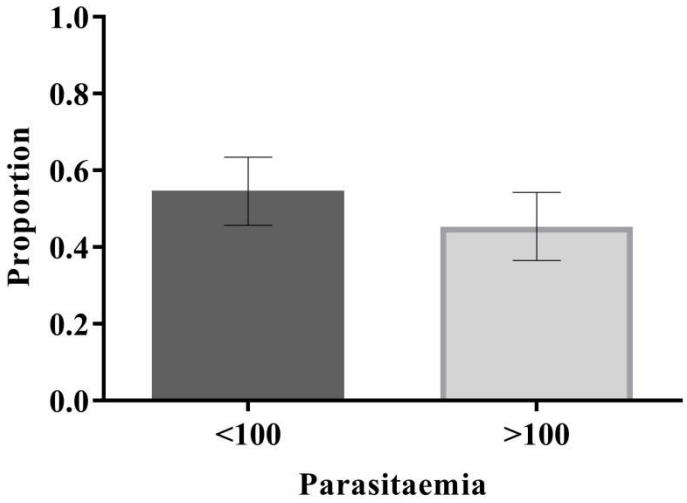

Figure 3.5: A bar graph comparing proportions of gametocyte positive time-points with parasitaemia either <100 parasites/µl or >100 parasites/µl. 95% CI for parasitaemia <100 = (0.4567-0.6343) and (0.3657-0.5433) for parasitaemia >100. Parasitaemia was measured in parasites/ µl

3.4 Gametocyte clearance and appearance

Kinetics of gametocyte clearance was generated from a subset of 11 (25%) participants (out of total 44 subjects analyzed) who were positive for gametocytes at enrollment. Kaplan-Meier survival analysis showed that almost 50% of these individuals cleared gametocytes in 2 days (Figure 3.6 A) while the rest of subjects were gametocyte free by day 9 (median gametocyte clearance time was 3 days (range 2-9 days)) irrespective of the drug administered. This is partly due to the low number of participants used in this analysis. A close comparison with asexual parasite clearance in participants positive at enrollment 35/44 (79.5%) shows a similar trend where 50% of participants had cleared parasites by day 7 (Figure 3.6 B).

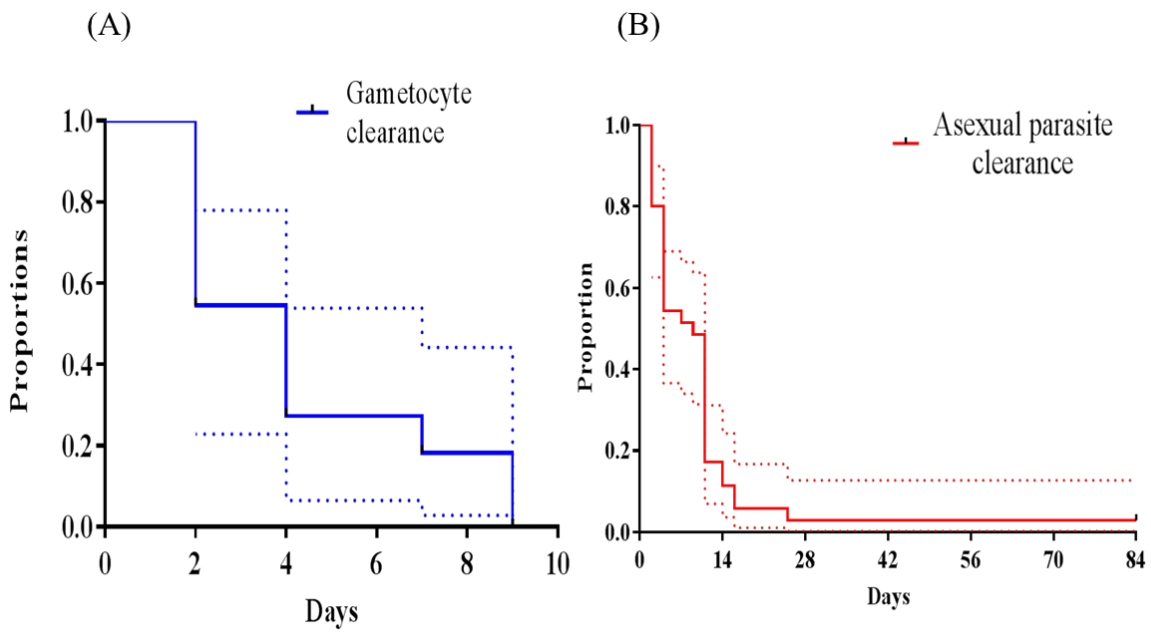

Figure 3.6: shows gametocyte survival analysis for individuals with gametocytaemia at enrollment (A); and parasite clearance curve for individuals with asexual parasites at enrollment (B). The dotted lines show 95% confidence intervals around the survival curves.

Participants negative for gametocytes at enrollment, 72.7% (32/44), were used to determine the kinetics of gametocyte appearance. The estimate of risk of appearance for gametocytes within the first two weeks was almost 50% (Figure 3.7 A) and 81.3% (26/32) of participants developed gametocytes during follow up.

Individuals positive at enrollment cleared gametocytes by day 9.On follow up, these participants were observed to develop gametocytes which we have defined here as gametocyte reappearance. A comparison between the kinetics of gametocyte appearance in individuals negative at enrollment and reappearance patterns in those who had cleared is suggestive of a faster gametocyte acquisition rate in those who had already cleared sexual parasites than in those who had no gametocytaemia at enrollment although the difference was not significant (Figure 3.8, p= 0.2852 Log-rank (Mantel-Cox) test). Appearance of asexual parasites in individuals negative at enrollment is presented in Figure 3.7 B and a comparison with gametocyte appearance curve not possible due to wide confidence intervals.

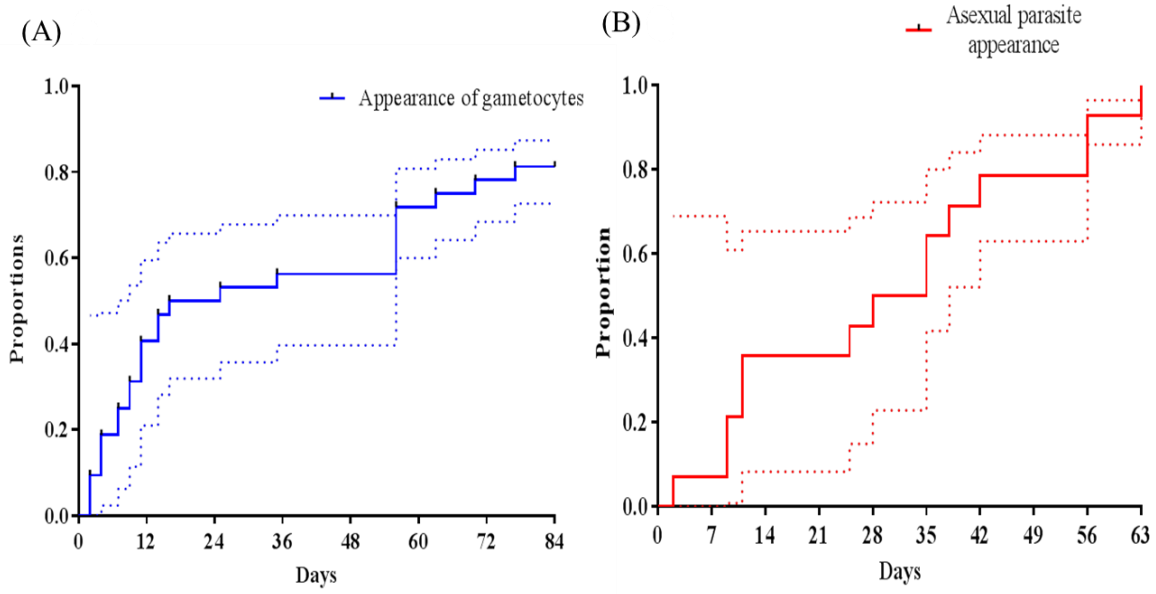

Figure 3.7: Gametocyte appearance (A); and asexual parasite appearance (B) on follow up with the dotted lines showing upper and lower limits of 95% CI.

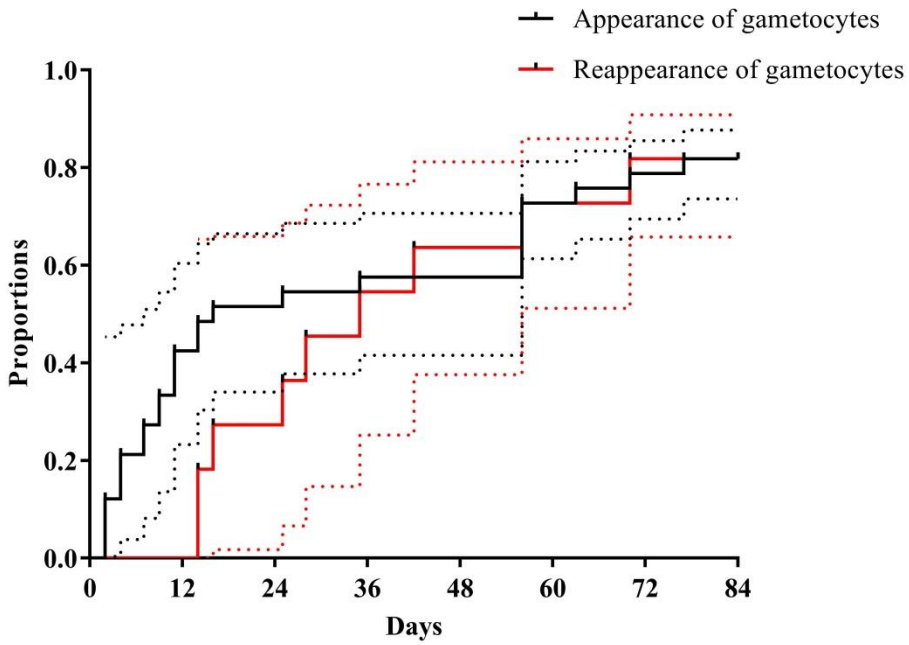

Figure 3.8: Initial gametocyte appearance and gametocyte reappearance curves with the dotted lines showing upper and lower limits

3.5 3.5 Changes in gametocyte sex ratio

Sex ratio was defined as the proportion of gametocytes in peripheral blood that were male (Pickering et al., 2000). Detection of male gametocytes (targeting Pfs230p) was done in a subset of 373 samples representing 43 individuals from the study. Samples positive for male gametocytes were 5/373 representing 3/43 individuals. Comparing this with 54 samples positive for Pfs25 (female specific) from the same sample set depicted an extremely female biased sex ratio (1:10.8). As a result of these low male positives likely due to elevated drug clearance of male gametocytes or sensitivity limitations of the Pfs230p gene, temporal differences in sex ratio was not possible.

CHAPTER 4 : DISCUSSION

The presence of mature gametocytes in peripheral blood is central to transmission of parasites to the mosquito vector. Asymptomatic *falciparum* infections defined as the presence of malaria parasites in the blood in the absence of clinical symptoms (such as fever) are common and result in transmissible parasite forms (Bousema et al., 2004). Children especially below 5 years have reduced exposure to malaria parasites and they therefore have low or no immune protection. With increasing age, acquired immunity is boosted and asymptomatic infections become more prevalent in young adults. This population is not likely to seek medical treatment since they lack clinical symptoms. Gametocytes attributable to immune pressure among other factors emerge maintaining continuous transmission within the population. We provide gametocyte prevalence for 84 days. In this study where adults were recruited, 79.5% of participants had asymptomatic infections with 25% gametocyte prevalence at enrollment. These high proportions underscore the importance of asymptomatic infections since most studies have only focused on gametocyte prevalence in patients after treatment. We noted that asexual parasite prevalence on day 56 was restored and maintained until the end of the study to levels observed at enrollment, suggesting a stable reservoir of asymptomatic individuals after drug clearance. This is partially as a result of similar exposure to parasite infections which generate sustained immune pressure. Gametocyte proportions were observed to follow a similar trend to the asexual parasite prevalence after treatment until day 56 where gametocyte carriage declined despite stable asexual parasite proportions. This implies that other factors not investigated in this study influence gametocyte dynamics besides asexual parasite prevalence. Possible interaction of multiple strains might be in play and an analysis of genotyping data might reveal this.

Gametocyte dynamics after treatment are drug dependent since some drugs have gametocytocidal properties while others induce gametocytogenesis. We demonstrate that gametocyte proportions in AS group were lowest shortly after treatment (7days) while SP+AS+PQ group recorded the highest proportions within the first week of treatment. The effective clearance of immature gametocytes and extended dosage duration of 7 days for the AS group likely caused the reduced proportions and this has been observed in other studies (Pukrittayakamee et al., 2004, Schneider et al., 2006). The synergistic effect of both artesunate and primaquine to inhibit immature gametocytes and mature gametocytes

respectively might have been cancelled by a characteristic effect of SP to increase carriage (Robert et al., 2000, von Seidlein et al., 2001b) . Sexual stage carriage on follow up (9-84 days) increased in all drug arms but was highest in AS group owing to its short half-life. The interpretation of gametocyte prevalence on follow up for the different drug arms are to be taken with caution since gametocyte positivity does not always result in gametocyte infectivity as evident in membrane feeding experiments (Kone et al., 2010). Nevertheless, we have taken gametocyte positivity as a crude measure of infectivity.

The asexual parasite stages are a prerequisite to the sexual stages of *P. falciparum* but the association between parasite density and gametocyte density is highly controversial with studies reporting both positive and negative associations (Karl et al., 2016, Koepfli et al., 2015, Carter and Miller, 1979, von Seidlein et al., 2001a). Since only a small proportion of the asexual parasites commit to gametocytes one would expect a positive association between blood stage parasites and the sexual stages. However, the correlation between the two is not an obvious one and neither can its linearity be assumed. A recent systematic review found varying associations between asexual density and gametocytaemia in the Asian and African setting (WWARN Gametocyte Study Group, 2016). While a gradual negative association between asexual density and gametocytaemia was observed in the Asian context, African studies showed a positive association up to 5,000 parasites/µl beyond which a negative association was seen. Despite the influence of age and other host factors on gametocytaemia, this association still remains inconclusive and highly site dependent.

By comparing asexual parasitaemia between gametocyte positive and gametocyte negative samples, we showed that individuals with high parasite densities are more likely to present with gametocytes than those with low parasite densities. Since only a small fraction of asexual parasites commit to gametocytes, it is rather logical that individuals with high parasite densities will present with gametocytes, an observation made previously in culture (Bruce et al., 1990) and in field isolates (Koepfli et al., 2015). We report a negative association between high asexual parasite density and gametocyte carriage in samples beyond 50,000 parasites/µl. This trend is probably driven by preferential investment of the parasite towards asexual multiplication to increase period of infection as a response to optimal within-host environment that favors survival.

Asymptomatic infections of low parasitaemia (≤100 parasites/µl) are common in the population. We provide the first evidence on the contributing role of parasitaemia below 100 parasites/µl on possible malaria transmission. Despite a high significance value, these findings are suggestive of possible evidence since we expected individuals with parasitaemia >100 parasites/µl to have higher proportions of gametocytes than those with densities of 100 parasites/µl and below (Figure 3.5). Therefore these findings need not be dismissed as mere chance or random variation. These low numbers could imply unfavorable in-host conditions originating from immune/drug clearance causing the parasite to switch to sexual stages to maximize probabilities of human-mosquito transmission. A major caveat in this interpretation is the use of a single time-point of asexual parasitaemia to infer gametocyte dynamics. Cognizant of the fact that gametocytogenesis takes about 10-14 days (Baker, 2010) to have mature forms in peripheral blood, definition of sample characteristics cannot be overemphasized especially in studies that attempt to draw conclusions on the relationship between asexual parasitaemia and gametocyte carriage. Clearly, this explains the number of gametocyte positive samples that had no blood stage parasites (Table 3.3). However, in a total of 872 samples analyzed, 611 samples had asexual parasitaemia ≤100 parasites/µl, which uniquely identifies this as a population with low level parasitaemia.

Differential effects of antimalarial drugs on gametocyte clearance have been reported previously owing to artemisinin dosing and the activity of the non-artemisinin partner drugs (WWARN Gametocyte Study Group, 2016). More significantly, is the increased clearance of gametocytes following addition of PQ to ACTs (Shekalaghe et al., 2007). We could not separately analyze gametocyte clearance in each drug regimen due to sample size limitations. Thus we report clearance kinetics for all subjects positive at enrollment irrespective of drug arm. In this study, the median gametocyte clearance time was 3 days (range2-9) with half of the participants analyzed clearing gametocytes in 2 days. This presents increased clearance rate than in previous studies on patients with uncomplicated *falciparum* malaria where median gametocyte clearance time of 6.8 (0.5-33.6) days was reported (Piyaphanee et al., 2006). Furthermore, the asymptomatic carriage of participants and a generally lower parasite load in this study explains the reduced clearance time. It is probable that PQ contributed significantly to gametocyte clearance due to its gametocytocidal effects (Vale et al., 2009). Artemisinin derivatives are highly efficacious on immature gametocytes and their effect on clearance

cannot be underestimated. Therefore, the combined effect by artemisinin derivatives on asexual parasite and immature gametocyte contributed substantially to the clearance pattern observed in the first 2 days. However, we do not intend to describe drug specific clearance effects rather we maintain that these results suggest that ACTs in general will clear gametocytes in an asymptomatic population in about 9 days.

Appearance of gametocytes is dependent on circulating asexual parasites or release of sequestered immature gametocytes in the presence or absence of blood stages. Similar to gametocyte clearance, a number of factors affect appearance of sexual stages. Data from 26 trials carried out in sub-Saharan Africa looking at individual patient data shows that gametocyte presence on admission was related to young age and low asexual parasitaemia (Zwang et al., 2009). Antimalarial drugs with an effect on sequestered immature gametocytes influence time to gametocyte appearance as shown in a previous study where artesunate was observed to predominantly inhibit gametocyte development (Pukrittayakamee et al., 2004b). Commitment to sexual stages is a key determinant to appearance and any intrinsic or extrinsic factors that either activate this pathway or interfere with gametocyte development and release to the peripheral blood hugely affect the dynamics of gametocyte appearance. However, little information is known regarding the mechanism of sexual commitment. In the present study, we neither address the commitment question nor normalize for all the factors that have been associated with gametocyte formation and development including individual genetic variations. Aware of this gap, we present results from a generally semi-immune population where any variations between individuals represent those expected in a natural set up. Following asexual drug treatment, 81.3% (26/32) of participants negative for gametocytes at enrollment developed gametocytes within the 84 day follow up. In the first 2 weeks, 50% of the participants were gametocytaemic with the rest developing gametocytes after day 56 emerging possibly from reinfections. This findings of increased development of transmissible forms of malaria after treatment underscore the magnitude of the undesirable effects of routine antimalarial drugs. Therefore drugs with proven gametocytocidal effect will be important for adoption to routine treatment of malaria. While gametocytes appear like an inevitable stage of the *Plasmodium* parasite, the clearance kinetics of antimalarial drugs will determine the circulation time of gametocytes and by extension how long an individual remains infectious to mosquitoes. Reappearance of gametocytes in subjects who had already cleared was observed

to be at a faster rate, though not significant, than in individuals who were negative at enrollment. Since reappearance started after 9 days, we cannot associate this trend to treatment. Furthermore, artemisinin derivatives have a very short half-life and any non-artemisinin compounds would be at a lower concentration than at the start or during treatment. This data suggests a possible increase in gametocyte acquisition within a population after gametocyte drug clearance. However, to test this hypothesis a larger sample size will be required so as to have narrow confidence intervals around which a conclusion can be drawn.

We report an extreme female biased sex ratio (1:10.8) as shown by the male specific Pfs230p transcript. This is probably due to male gametocyte-specific drug clearance or sensitivity limitations of the Pfs230p gene, which was also observed in a recent trial (Schneider P et al., 2006). A new assay based on a more abundant gene transcript named male gametocyte-enriched transcript (*PfMGET*) has been developed and validated (Stone et al., 2017), thus analysis of Pfs230p is a limitation to providing any conclusion on sex-ratio in this study.

CHAPTER 5 : CONCLUSION AND RECOMMENDATIONS

Asymptomatic infections are common especially in endemic settings. These infections are generally characterized by low asexual parasitaemia and are more likely to be gametocytaemic contributing to the infectious reservoir. Even after treatment with ACTs, there is 81.3% chance that individuals negative at baseline will develop gametocytes.

We recommend that future studies with larger sample size compare clearance and appearance kinetics between groups and that membrane feeding assays be incorporated to account for infectivity and not the mere assumption of gametocyte carriage. Further, we strongly advise that studies on gametocyte sex ratio should set up assays that target *PfMGET* gene to provide a more sensitive male gametocyte readout.

REFERENCES

Abdelraheem, M.H., Albsheer, M.M.A., Mohamed, H.S., Amin, M., Mahdi Abdel Hamid, M., 2016. Transmission of Plasmodium vivax in Duffy-negative individuals in central Sudan. Trans. R. Soc. Trop. Med. Hyg. 110, 258–260. https://doi.org/10.1093/trstmh/trw014

Agresti, A., and Coull, B. A. (1998), Approximate is Better than "exact" for interval estimation of binomial proportions, The American Statistician, 52: 119-126 - Google Search [WWW Document], n.d. URL https://www.google.com/search?q=Agresti%2C+A.%2C+and+Coull%2C+B.+A.+%281998%29%2C+Approximate+is+Better+than+%22exact%22+for+interval+estimation+of+binomial+proportions%2C+The+American+Statistician%2C+52%3A+119-126&ie=utf-8&oe=utf-8&client=firefox-b-ab (accessed 1.10.18).

Alonso, P.L., Smith, T., Schellenberg, J.R., Masanja, H., Mwankusye, S., Urassa, H., Bastos de Azevedo, I., Chongela, J., Kobero, S., Menendez, C., 1994. Randomised trial of efficacy of SPf66 vaccine against Plasmodium falciparum malaria in children in southern Tanzania. Lancet Lond. Engl. 344, 1175–1181.

Asante, K.P., Abdulla, S., Agnandji, S., Lyimo, J., Vekemans, J., Soulanoudjingar, S., Owusu, R., Shomari, M., Leach, A., Jongert, E., Salim, N., Fernandes, J.F., Dosoo, D., Chikawe, M., Issifou, S., Osei-Kwakye, K., Lievens, M., Paricek, M., Möller, T., Apanga, S., Mwangoka, G., Dubois, M.-C., Madi, T., Kwara, E., Minja, R., Hounkpatin, A.B., Boahen, O., Kayan, K., Adjei, G., Chandramohan, D., Carter, T., Vansadia, P., Sillman, M., Savarese, B., Loucq, C., Lapierre, D., Greenwood, B., Cohen, J., Kremsner, P., Owusu-Agyei, S., Tanner, M., Lell, B., 2011. Safety and efficacy of the RTS,S/AS01E candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial. Lancet Infect. Dis. 11, 741–749. https://doi.org/10.1016/S1473-3099(11)70100-1

Babiker, H.A., Abdel-Wahab, A., Ahmed, S., Suleiman, S., Ranford-Cartwright, L., Carter, R., Walliker, D., 1999. Detection of low level Plasmodium falciparum gametocytes using reverse transcriptase polymerase chain reaction. Mol. Biochem. Parasitol. 99, 143–148.

Baker, D.A., 2010. Malaria gametocytogenesis. Mol. Biochem. Parasitol. 172, 57–65. https://doi.org/10.1016/j.molbiopara.2010.03.019

Bejon, P., Williams, T.N., Liljander, A., Noor, A.M., Wambua, J., Ogada, E., Olotu, A., Osier, F.H.A., Hay, S.I., Färnert, A., Marsh, K., 2010. Stable and Unstable Malaria Hotspots in Longitudinal Cohort Studies in Kenya. PLOS Med. 7, e1000304. https://doi.org/10.1371/journal.pmed.1000304

Berry, A., Deymier, C., Sertorio, M., Witkowski, B., Benoit-Vical, F., 2009. Pfs 16 pivotal role in Plasmodium falciparum gametocytogenesis: a potential antiplasmodial drug target. Exp. Parasitol. 121, 189–192. https://doi.org/10.1016/j.exppara.2008.10.010

Bhatt, S., Weiss, D.J., Mappin, B., Dalrymple, U., Cameron, E., Bisanzio, D., Smith, D.L., Moyes, C.L., Tatem, A.J., Lynch, M., Fergus, C.A., Yukich, J., Bennett, A., Eisele, T.P., Kolaczinski, J., Cibulskis, R.E., Hay, S.I., Gething, P.W., 2015. Coverage and system efficiencies of insecticide-treated nets in Africa from 2000 to 2017. eLife 4. https://doi.org/10.7554/eLife.09672

Bousema, J.T., Gouagna, L.C., Drakeley, C.J., Meutstege, A.M., Okech, B.A., Akim, I.N., Beier, J.C., Githure, J.I., Sauerwein, R.W., 2004. Plasmodium falciparum gametocyte carriage in asymptomatic children in western Kenya. Malar. J. 3, 18. https://doi.org/10.1186/1475-2875-3-18

Bousema, T., Drakeley, C., 2011. Epidemiology and Infectivity of Plasmodium falciparum and Plasmodium vivax Gametocytes in Relation to Malaria Control and Elimination. Clin. Microbiol. Rev. 24, 377–410. https://doi.org/10.1128/CMR.00051-10

Bousema, T., Okell, L., Shekalaghe, S., Griffin, J.T., Omar, S., Sawa, P., Sutherland, C., Sauerwein, R., Ghani, A.C., Drakeley, C., 2010. Revisiting the circulation time of Plasmodium falciparum gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. Malar. J. 9, 136. https://doi.org/10.1186/1475-2875-9-136

Bruce, M.C., Alano, P., Duthie, S., Carter, R., 1990. Commitment of the malaria parasite Plasmodium falciparum to sexual and asexual development. Parasitology 100 Pt 2, 191–200.

Cameron, A., Reece, S.E., Drew, D.R., Haydon, D.T., Yates, A.J., 2013. Plasticity in transmission strategies of the malaria parasite, Plasmodium chabaudi: environmental and genetic effects. Evol. Appl. 6, 365–376. https://doi.org/10.1111/eva.12005

Carter, R., Miller, L.H., 1979. Recent developments in production and purification of malaria antigens: Evidence for environmental modulation of gametocytogenesis in Plasmodium falciparum in continuous culture. Bull. World Health Organ. 57, 37–52.

Clyde, D.F., McCarthy, V.C., Miller, R.M., Hornick, R.B., 1973. Specificity of protection of man immunized against sporozoite-induced falciparum malaria. Am. J. Med. Sci. 266, 398–403.

Cui, L., Mharakurwa, S., Ndiaye, D., Rathod, P.K., Rosenthal, P.J., 2015. Antimalarial Drug Resistance: Literature Review and Activities and Findings of the ICEMR Network. Am. J. Trop. Med. Hyg. 93, 57–68. https://doi.org/10.4269/ajtmh.15-0007

Delves, M.J., Ruecker, A., Straschil, U., Lelièvre, J., Marques, S., López-Barragán, M.J., Herreros, E., Sinden, R.E., 2013. Male and Female Plasmodium falciparum Mature Gametocytes Show Different Responses to Antimalarial Drugs. Antimicrob. Agents Chemother. 57, 3268–3274. https://doi.org/10.1128/AAC.00325-13

Doctor, S.M., Liu, Y., Anderson, O.G., Whitesell, A.N., Mwandagalirwa, M.K., Muwonga, J., Keeler, C., Emch, M., Likwela, J.L., Tshefu, A., Meshnick, S.R., 2016. Low prevalence of Plasmodium malariae and Plasmodium ovale mono-infections among children in the Democratic Republic of the Congo: a population-based, cross-sectional study. Malar. J. 15, 350. https://doi.org/10.1186/s12936-016-1409-0

Eckstein-Ludwig, U., Webb, R.J., Van Goethem, I.D.A., East, J.M., Lee, A.G., Kimura, M., O'Neill, P.M., Bray, P.G., Ward, S.A., Krishna, S., 2003. Artemisinins target the SERCA of Plasmodium falciparum. Nature 424, 957–961. https://doi.org/10.1038/nature01813

Hay, S.I., Rogers, D.J., Toomer, J.F., Snow, R.W., 2000. Annual Plasmodium falciparum entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. Trans. R. Soc. Trop. Med. Hyg. 94, 113–127.

Howes, R.E., Patil, A.P., Piel, F.B., Nyangiri, O.A., Kabaria, C.W., Gething, P.W., Zimmerman, P.A., Barnadas, C., Beall, C.M., Gebremedhin, A., Ménard, D., Williams,

T.N., Weatherall, D.J., Hay, S.I., 2011. The global distribution of the Duffy blood group. Nat. Commun. 2, 266. https://doi.org/10.1038/ncomms1265

Hyde, J.E., 2005. Exploring the folate pathway in Plasmodium falciparum. Acta Trop. 94, 191–206. https://doi.org/10.1016/j.actatropica.2005.04.002

Ifediba, T., Vanderberg, J.P., 1981. Complete in vitro maturation of Plasmodium falciparum gametocytes. Nature 294, 364–366.

Karl, S., Laman, M., Moore, B.R., Benjamin, J.M., Salib, M., Lorry, L., Maripal, S., Siba, P., Robinson, L.J., Mueller, I., Davis, T.M.E., 2016. Risk factors for Plasmodium falciparum and Plasmodium vivax gametocyte carriage in Papua New Guinean children with uncomplicated malaria. Acta Trop. 160, 1–8. https://doi.org/10.1016/j.actatropica.2016.04.002

Kilama, M., Smith, D.L., Hutchinson, R., Kigozi, R., Yeka, A., Lavoy, G., Kamya, M.R., Staedke, S.G., Donnelly, M.J., Drakeley, C., Greenhouse, B., Dorsey, G., Lindsay, S.W., 2014. Estimating the annual entomological inoculation rate for Plasmodium falciparum transmitted by Anopheles gambiae s.l. using three sampling methods in three sites in Uganda. Malar. J. 13, 111. https://doi.org/10.1186/1475-2875-13-111

Koepfli, C., Robinson, L.J., Rarau, P., Salib, M., Sambale, N., Wampfler, R., Betuela, I., Nuitragool, W., Barry, A.E., Siba, P., Felger, I., Mueller, I., 2015. Blood-Stage Parasitaemia and Age Determine Plasmodium falciparum and P. vivax Gametocytaemia in Papua New Guinea. PloS One 10, e0126747. https://doi.org/10.1371/journal.pone.0126747

Kone, A., van de Vegte-Bolmer, M., Siebelink-Stoter, R., van Gemert, G.-J., Dara, A., Niangaly, H., Luty, A., Doumbo, O.K., Sauerwein, R., Djimde, A.A., 2010. Sulfadoxine-pyrimethamine impairs Plasmodium falciparum gametocyte infectivity and Anopheles mosquito survival. Int. J. Parasitol. 40, 1221–1228. https://doi.org/10.1016/j.ijpara.2010.05.004

Lefèvre, G., Looareesuwan, S., Treeprasertsuk, S., Krudsood, S., Silachamroon, U., Gathmann, I., Mull, R., Bakshi, R., 2001. A clinical and pharmacokinetic trial of six doses of artemether-lumefantrine for multidrug-resistant Plasmodium falciparum malaria in Thailand. Am. J. Trop. Med. Hyg. 64, 247–256.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262

Makanga, M., 2014. A review of the effects of artemether-lumefantrine on gametocyte carriage and disease transmission. Malar. J. 13, 291. https://doi.org/10.1186/1475-2875-13-291

Mbogo, C.M., Mwangangi, J.M., Nzovu, J., Gu, W., Yan, G., Gunter, J.T., Swalm, C., Keating, J., Regens, J.L., Shililu, J.I., Githure, J.I., Beier, J.C., 2003. Spatial and temporal heterogeneity of Anopheles mosquitoes and Plasmodium falciparum transmission along the Kenyan coast. Am. J. Trop. Med. Hyg. 68, 734–742.

Mbogo, C.N.M., Snow, R.W., Khamala, C.P.M., Kabiru, E.W., Ouma, J.H., Githure, J.I., Marsh, K., Beier, J.C., 1995. Relationships between Plasmodium Falciparum Transmission by Vector Populations and the Incidence of Severe Disease at Nine Sites on the Kenyan Coast. Am. J. Trop. Med. Hyg. 52, 201–206. https://doi.org/10.4269/ajtmh.1995.52.201

McKeage, K., Scott, L., 2003. Atovaquone/proguanil: a review of its use for the prophylaxis of Plasmodium falciparum malaria. Drugs 63, 597–623.

Medicine, E.M.S.A.P. of P.U. of N.C. of, School, W.B.P.P. of P.U. of M.M., 2000. The Antimicrobial Drugs. Oxford University Press, USA.

Mogeni, P., Williams, T.N., Fegan, G., Nyundo, C., Bauni, E., Mwai, K., Omedo, I., Njuguna, P., Newton, C.R., Osier, F., Berkley, J.A., Hammitt, L.L., Lowe, B., Mwambingu, G., Awuondo, K., Mturi, N., Peshu, N., Snow, R.W., Noor, A., Marsh, K., Bejon, P., 2016. Age, Spatial, and Temporal Variations in Hospital Admissions with Malaria in Kilifi County, Kenya: A 25-Year Longitudinal Observational Study. PLOS Med. 13, e1002047. https://doi.org/10.1371/journal.pmed.1002047

Olotu, A., Fegan, G., Wambua, J., Nyangweso, G., Leach, A., Lievens, M., Kaslow, D.C., Njuguna, P., Marsh, K., Bejon, P., 2016. Seven-Year Efficacy of RTS,S/AS01 Malaria Vaccine among Young African Children. N. Engl. J. Med. 374, 2519–2529. https://doi.org/10.1056/NEJMoa1515257

Organization, W.H., 2006. Guidelines for the Treatment of Malaria. World Health Organization.

Patarroyo, G., Franco, L., Amador, R., Murillo, L.A., Rocha, C.L., Rojas, M., Patarroyo, M.E., 1992. Study of the safety and immunogenicity of the synthetic malaria SPf66 vaccine in children aged 1-14 years. Vaccine 10, 175–178.

PATH and GSK welcome progress toward RTS,S malaria vaccine pilot implementation with selection of countries [WWW Document], 2017. . Malar. Vaccine Initiat. URL http://www.malariavaccine.org/news-events/news/path-and-gsk-welcome-progress-toward-rtss-malaria-vaccine-pilot-implementation (accessed 1.7.18).

Paul, R.E.L., Brey, P.T., Robert, V., 2002. Plasmodium sex determination and transmission to mosquitoes. Trends Parasitol. 18, 32–38.

Piyaphanee, W., Krudsood, S., Tangpukdee, N., Thanachartwet, W., Silachamroon, U., Phophak, N., Duangdee, C., Haoharn, O., Faithong, S., Wilairatana, P., Leowattana, W., Looareesuwan, S., 2006. Emergence and clearance of gametocytes in uncomplicated Plasmodium falciparum malaria. Am. J. Trop. Med. Hyg. 74, 432–435.

Price, R., Nosten, F., Simpson, J.A., Luxemburger, C., Phaipun, L., Ter Kuile, F., Van Vugt, M., Chongsuphajaisiddhi, T., White, N.J., 1999. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. Am. J. Trop. Med. Hyg. 60, 1019–1023.

Pukrittayakamee, S., Chotivanich, K., Chantra, A., Clemens, R., Looareesuwan, S., White, N.J., 2004a. Activities of Artesunate and Primaquine against Asexual- and Sexual-Stage Parasites in Falciparum Malaria. Antimicrob. Agents Chemother. 48, 1329–1334. https://doi.org/10.1128/AAC.48.4.1329-1334.2004

Pukrittayakamee, S., Chotivanich, K., Chantra, A., Clemens, R., Looareesuwan, S., White, N.J., 2004b. Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. Antimicrob. Agents Chemother. 48, 1329–1334.

Pulford, J., Hetzel, M.W., Bryant, M., Siba, P.M., Mueller, I., 2011. Reported reasons for not using a mosquito net when one is available: a review of the published literature. Malar. J. 10, 83. https://doi.org/10.1186/1475-2875-10-83

Reece, S.E., Ali, E., Schneider, P., Babiker, H.A., 2010. Stress, drugs and the evolution of reproductive restraint in malaria parasites. Proc. Biol. Sci. 277, 3123–3129. https://doi.org/10.1098/rspb.2010.0564

Reece, S.E., Ramiro, R.S., Nussey, D.H., 2009. Plastic parasites: sophisticated strategies for survival and reproduction? Evol. Appl. 2, 11–23. https://doi.org/10.1111/j.1752-4571.2008.00060.x

Robert, V., Awono-Ambene, H.P., Le Hesran, J.Y., Trape, J.F., 2000. Gametocytemia and infectivity to mosquitoes of patients with uncomplicated Plasmodium falciparum malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine. Am. J. Trop. Med. Hyg. 62, 210–216.

RTS,S Clinical Trials Partnership, 2014. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. PLoS Med. 11, e1001685. https://doi.org/10.1371/journal.pmed.1001685

Schneider, P., Bousema, T., Omar, S., Gouagna, L., Sawa, P., Schallig, H., Sauerwein, R., 2006. (Sub)microscopic Plasmodium falciparum gametocytaemia in Kenyan children after treatment with sulphadoxine-pyrimethamine monotherapy or in combination with artesunate. Int. J. Parasitol. 36, 403–408. https://doi.org/10.1016/j.ijpara.2006.01.002

Schneider, P., Reece, S.E., van Schaijk, B.C.L., Bousema, T., Lanke, K.H.W., Meaden, C.S.J., Gadalla, A., Ranford-Cartwright, L.C., Babiker, H.A., 2015. Quantification of female and male Plasmodium falciparum gametocytes by reverse transcriptase quantitative PCR. Mol. Biochem. Parasitol. 199, 29–33. https://doi.org/10.1016/j.molbiopara.2015.03.006

Shekalaghe, S., Drakeley, C., Gosling, R., Ndaro, A., Meegeren, M. van, Enevold, A., Alifrangis, M., Mosha, F., Sauerwein, R., Bousema, T., 2007. Primaquine Clears Submicroscopic Plasmodium falciparum Gametocytes that Persist after Treatment with Sulphadoxine-Pyrimethamine and Artesunate. PLOS ONE 2, e1023. https://doi.org/10.1371/journal.pone.0001023

Singh, B., Kim Sung, L., Matusop, A., Radhakrishnan, A., Shamsul, S.S.G., Cox-Singh, J., Thomas, A., Conway, D.J., 2004. A large focus of naturally acquired Plasmodium knowlesi infections in human beings. Lancet Lond. Engl. 363, 1017–1024. https://doi.org/10.1016/S0140-6736(04)15836-4

Sinka, M.E., Bangs, M.J., Manguin, S., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Okara, R.M., Van Boeckel, T.,

Godfray, H.C.J., Harbach, R.E., Hay, S.I., 2010. The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasit. Vectors 3, 117. https://doi.org/10.1186/1756-3305-3-117

Sinka, M.E., Bangs, M.J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Burkot, T.R., Harbach, R.E., Hay, S.I., 2012. A global map of dominant malaria vectors. Parasit. Vectors 5, 69. https://doi.org/10.1186/1756-3305-5-69

Sowunmi, A., Balogun, S.T., Gbotosho, G.O., Happi, C.T., 2009a. Effects of amodiaquine, artesunate, and artesunate–amodiaquine on Plasmodium falciparum malaria-associated anaemia in children. Acta Trop. 109, 55–60. https://doi.org/10.1016/j.actatropica.2008.09.022

Sowunmi, A., Gbotosho, G.O., Happi, C.T., Folarin, O.A., Balogun, S.T., 2009b. Population structure of Plasmodium falciparum gametocyte sex ratios in malarious children in an endemic area. Parasitol. Int. 58, 438–443. https://doi.org/10.1016/j.parint.2009.08.007

Sowunmi, A., Nkogho, O.O., Okuboyejo, T.M., Gbotosho, G.O., Happi, C.T., Adewoye, E.O., 2009c. Effects of mefloquine and artesunate mefloquine on the emergence, clearance and sex ratio of Plasmodium falciparum gametocytes in malarious children. Malar. J. 8, 297. https://doi.org/10.1186/1475-2875-8-297

Sowunmi, A., Nkogho, O.O., Okuboyejo, T.M., Gbotosho, G.O., Happi, C.T., Adewoye, E.O., 2009d. Effects of mefloquine and artesunate mefloquine on the emergence, clearance and sex ratio of Plasmodium falciparum gametocytes in malarious children. Malar. J. 8, 297. https://doi.org/10.1186/1475-2875-8-297

Srivastava, I.K., Vaidya, A.B., 1999. A Mechanism for the Synergistic Antimalarial Action of Atovaquone and Proguanil. Antimicrob. Agents Chemother. 43, 1334–1339.

Stone, W., Sawa, P., Lanke, K., Rijpma, S., Oriango, R., Nyaurah, M., Osodo, P., Osoti, V., Mahamar, A., Diawara, H., Woestenenk, R., Graumans, W., van de Vegte-Bolmer, M., Bradley, J., Chen, I., Brown, J., Siciliano, G., Alano, P., Gosling, R., Dicko, A., Drakeley, C., Bousema, T., 2017. A Molecular Assay to Quantify Male and Female Plasmodium falciparum Gametocytes: Results From 2 Randomized Controlled Trials

Using Primaquine for Gametocyte Clearance. J. Infect. Dis. 216, 457–467. https://doi.org/10.1093/infdis/jix237

Thomson, D., 1911. I.—A Research into the Production, Life and Death of Crescents in Malignant Tertian Malaria, in Treated and Untreated Cases, by an Enumerative Method. Ann. Trop. Med. Parasitol. 5, 57–81. https://doi.org/10.1080/00034983.1911.11686341

Tukei, B.B., Beke, A., Lamadrid-Figueroa, H., 2017. Assessing the effect of indoor residual spraying (IRS) on malaria morbidity in Northern Uganda: a before and after study. Malar. J. 16, 4. https://doi.org/10.1186/s12936-016-1652-4

Vale, N., Moreira, R., Gomes, P., 2009. Primaquine revisited six decades after its discovery. Eur. J. Med. Chem. 44, 937–953. https://doi.org/10.1016/j.ejmech.2008.08.011

von Seidlein, L., Drakeley, C., Greenwood, B., Walraven, G., Targett, G., 2001a. Risk factors for gametocyte carriage in Gambian children. Am. J. Trop. Med. Hyg. 65, 523–527.

von Seidlein, L., Drakeley, C., Greenwood, B., Walraven, G., Targett, G., 2001b. Risk factors for gametocyte carriage in Gambian children. Am. J. Trop. Med. Hyg. 65, 523–527.

Wampfler, R., Mwingira, F., Javati, S., Robinson, L., Betuela, I., Siba, P., Beck, H.-P., Mueller, I., Felger, I., 2013. Strategies for Detection of Plasmodium species Gametocytes. PLOS ONE 8, e76316. https://doi.org/10.1371/journal.pone.0076316

White, N., 2011. The parasite clearance curve. Malar. J. 10, 278. https://doi.org/10.1186/1475-2875-10-278

White, N.J., 2008. The role of anti-malarial drugs in eliminating malaria. Malar. J. 7, S8. https://doi.org/10.1186/1475-2875-7-S1-S8

White, N.J., 2004. Antimalarial drug resistance. J. Clin. Invest. 113, 1084. https://doi.org/10.1172/JCI21682

White, N.J., 1998. Preventing antimalarial drug resistance through combinations. Drug Resist. Updat. Rev. Comment. Antimicrob. Anticancer Chemother. 1, 3–9.

White, N.J., 1994. Clinical pharmacokinetics and pharmacodynamics of artemisinin and derivatives. Trans. R. Soc. Trop. Med. Hyg. 88 Suppl 1, S41-43.

WHO | Updated WHO policy recommendation: Single dose primaquine as a gametocytocide in Plasmodium falciparum malaria [WWW Document], n.d. . WHO. URL

http://www.who.int/malaria/publications/atoz/who_pq_policy_recommendation/en/ (accessed 10.9.16).

WHO | World malaria report 2017 [WWW Document], n.d. . WHO. URL http://www.who.int/malaria/publications/world-malaria-report-2017/en/ (accessed 1.7.18).

Woldearegai, T.G., Kremsner, P.G., Kun, J.F.J., Mordmüller, B., 2013. Plasmodium vivax malaria in Duffy-negative individuals from Ethiopia. Trans. R. Soc. Trop. Med. Hyg. 107, 328–331. https://doi.org/10.1093/trstmh/trt016

World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects., 2013. . JAMA 310, 2191–2194. https://doi.org/10.1001/jama.2013.281053

WWARN Gametocyte Study Group, 2016. Gametocyte carriage in uncomplicated Plasmodium falciparum malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. BMC Med. 14. https://doi.org/10.1186/s12916-016-0621-7

Zwang, J., Olliaro, P., Barennes, H., Bonnet, M., Brasseur, P., Bukirwa, H., Cohuet, S., D'Alessandro, U., Djimdé, A., Karema, C., Guthmann, J.-P., Hamour, S., Ndiaye, J.-L., Mårtensson, A., Rwagacondo, C., Sagara, I., Same-Ekobo, A., Sirima, S.B., van den Broek, I., Yeka, A., Taylor, W.R., Dorsey, G., Randrianarivelojosia, M., 2009. Efficacy of artesunate-amodiaquine for treating uncomplicated falciparum malaria in sub-Saharan Africa: a multi-centre analysis. Malar. J. 8, 203. https://doi.org/10.1186/1475-2875-8-203

APPENDIX: INFORMED CONSENT IN GIRIAMA, SWAHILI AND ENGLISH

Consent form in Giriama

INVESTIGATORS	INSTITUTIONS
Caroline Ogwang (PI), Francis Ndungu(PI), Britta Urban, Domtila Kimani, Jedidah Mwacharo, Jimmy Shangala, Philip Bejon, Patricia Njuguna, Ken Awuondo	KEMRI CGMRC(Coast)
Faith Osier	Heidelberg University Hospital

KEMRI-Wellcome Trust Programme Fomu Va habari na idhini kwa ahusika va kulinganisha dawa va kumarigiza vidudu kahiza kipindi cha hiri lib va majaribio baada va peR kulola maambukizi ga plasmodium falciparum. | KITSWA: Muradhi wa kulinganisha |dawa tahu za malaria viryahu zirizho zinadima kumarigiza vidudu zha malaria kahiza damu.

Je KEMRI ni noni? KEMRI ni shirika ra serikali ririro tSini ya wizara ya afya, ambaro rinahenda utafiti wa matibabu.Utafiti ni tofauti na matibabu ga kawaida kwa sababu unalenga kupata ngira mbidzo za kuchinga na kuzikiha makongo kwa siku zidzazo kwa man'ufaa ga kila mtu.

Je utafiti uno unahusu noni? ı Malaria ni ukongo wa kawaida na hathari kwa ahoho athithe ambao unasababisha vifo vinji kwa ahoho. Japokuwa malaria idzakala inapatik~na kwa uchache kahika sehemu nyingine za afrika kwa sababu neti na dawa za malaria zinapatikana kwa Jnji, chanjo bado zinahenzekana iii zizulie malaria kwa sababu ichere ni shida bomu. Kila hatu kahiza urimw~ngu mzima, kala dawa mbisha hedu chanjo zinagunduliwa kwa ahoho, utafiti kwanza unahenderwa athumia achache enye afya mbidzo,alafu kwa athumia anji mario na afya mbidzo kabila ahoho kamadzahusisMwa.

Kwa athumia,inadimikika makakala ina vidudu zha malaria kahiza damu za kwao bila kukala na dalili za malaria.Kwa sababu ii,wakathi wa ut~fiti wa chanjo ya malaria, anasayanisi nikukala manaapa ahusika dawa zamalaria iii mamarigize vidudu Zh?Si zha malaria virizho kahiza damu zao, hatha chamba si akongo.Ni muhimu kukala dawa iryahu mandi~opewa indahenda kazi kahiza kipindi kichache,ili uhendi wa kazi wa kwakwe usidime kuhenda ikakala viRomu kulola kala chanjo inahenda kazi kihizho.Kahiza wakathi udziokira, kudzakala na mabadiliko kuhusu uh~ndi wa kazi wa dawa tofauti tofauti za malaria,na vivi kaimanyikana ni dawa hiyo iriyoinahenda kazi tototo na inahenda kazi kwa wakathi much ache ambayo niitumiwe wakathi wa jaribio ra chanjo ya malaria. !

Muradhi uu unalenga kulola ni dawa|hiyo ya malaria ambayo niitumiwe siku zidzazo kala miradhi ya jaribio ra chanjo ya malaria kala unaanza Si~U zidzazo. Fundahenda vivi kwa kuapa atu mario na afya zao, mario mandakubali kuhusika, dawa mwenga kahiza dawa tahu tafauti za malaria na fuathuwirize kwa wiki kumi na tahu,ambao wakathi uu funda apim~ mulatso wao mara kadhaa iii fudime kulola kala vidudu zha malaria vichereko. Dawa zosi tahu za malarra zindizo tumiwa kahiza muradhi uu zaidhinishwa kare kuhumirwa haha Kenya kutibu malariaJundaamua ~i dawa hiyo futumie kumupa mtu hiye kutumia mutindo wa bahathi nasibu,ili kila mtu akale na nafasi !sawa ya kukala kahiza kundi rorosLFunanuia kuhusisha atu mirongo chenda(90) enye afya mbidzo kwa| ujumula (alume na ache) kahiza muradhi uu. Na baada ya muradhi fundalinganisha majibu kumbola kwa 0 atu mario manahumira zo dawa tofauti fulole ni dawa hizo zahenda kazi tototo na kwa haraka zaidhL rNJi muhimu kumanya

kukala muradhi uu si wa chanjo ya malaria ama muradhi wa matibabu.

Je indahusisha noni kwangu?

Herufi za kwanza za muhusika----------L ---Kigiriama PIS na Habari za Kumboza Ruhusa Nakala 1.1 tarehe 29 mwezi wane 2013

Ukikubali kuhusika kahiza muradhi u1J,gathuwirizago gandahendeka:

1. Uchunguzi wa afya wa kw~rZa. Oakitari andakulola afya ya kwako Kwa kukuza maswali, akupime alafu ahale 15ml (vijiko vihahu zha chai) sampuli ya mulatso kumbola kwenyJ mukono kwa upimaji.Kala fundakona kukala una shidp yoyosi ya kiafya ama shida yoyosi kwenye mulatsofundakuelezera na fukuhume wende kwa uchunguzi zaidhi ama kwa matibabu gahedzekanago kwa sipit~1i ya serikali isitahiliyo.Kahiza hali ii,fundakwamba usienderere kuhusika kahiza muradhi.

Funamanya kukala asilimia thithe yb atu kamandadima kutumia dawa mwenga yaho ya dawa za malaria fundizotumia, na manadima kupatikkna na anaemia (mulatso kukosa nguvu ya kuthosha) kwa sababu ya madhara ga dawa.Uchunguzi mumJ,enga waho wa mulatso undagundua aryahu mario kamadima kutumia zizi dawa, iii fuhakikishe maQapewc;l mwenga yaho ya zo dawa mbiri nyingine. Fundamwambira yoyosi ariye anadima kudhurika kwa hali ii.

Here dawa nyingine zozosi,dawa riyingine za malaria zinadima kukala si salama kala zipewa atu enye mimba ama ana ache mario man~amwisa. Lakini, ni salama kwa ana ache kuhenda mimba baada ya kumarigiza kutumia dawa za malaria. Kwa vizho funda:

- Kafundahusisha mtu yoyosi ariy.~ analutsunga ama ana amwisa.
- Fundaauza ana ache mario mapahenza kuhusika kahiza muradhi uu mafupe sampuli ya makonzo iii fudime kupima mimba iii fuhakikishe kukala kamana mimba kabila kuhusika.
- Fundaauza ana ache mafambi~e kukala mandahumira ngira za kupanga uzhazi paka mamarigize kuhumira dawa.
- Fundauyira upimaji wa mimba Kabila kulazha dawa ya kwanza ya malaria na kafundamuhusisha mtu yoyosi andiye kala kipimo cha kwakwe kinaonyesa ana lutsunga.
- Fundauyira kupima mimba siku ya fungahe baada ya kuanza dawa iii kuhakikisha kukala ye mtu kahendere mimba wakathi arekala ana humira dawa ya malaria. Kala kipimo kindaonyesa kukala ana

lutsunga,indahenzekana fuenQerere kumuthuwiriza kahiza kipindi chosi cha uja uziho paka andihodzivugula.

2. Kutumia dawa za malaria: y ndauzwa uanze kuhumira dawa za malaria kahiza siku ya kwanza ya kungira kahiza muradhi.Dawa mbiri ya zo dawa tahu zindehumirwa kahiza muradhi nizihumirwe kwa siku tahu, na mwenga niihumirwe kwa siku fungahe.Ni lazima udze ho kiliniki iii udze urye dawa.Kungezera haho, kala ~usikiratototo kwa ngira yoyosi kahiza kipindi cha muradhi,niudze kahiza kiliniki iii udze uonewe na u:tibiwe ni dakitari wa muradhi.lkidimikika,usitumie dawa nyinine yoyos kabila kumwambira dakitari Wa muradhi kwanza.

3. Uthuwirizi na samupli za mlJlatso: Fundakutsemberera kahiza mudzi W8 kwako mara mwenga iii fukuze habari kuhusu uhumiraji wa neti kahizc mudzi wa kwako.Fundakuza udze k~hiza kiliniki kwa jumula ya mara shirini na handahu(26) kahiza kipind cha wiki kumi na tahu (kwa aryahu! mario mandakala manarya dawa ya malaria ya siku tahu) ama marc mirongo miri na chenda(29) kwa! wiki kumi na tahu (kwa aryahu mario manarya dawa ya sikl fungahe).Kahiza mara zizi za uthuw'rizi,dakitari wa muradhi andakulola afya ya kwako na ahale damu here ithuwirizazho:

- Kahiza wikine (4) za kwa~za,fundahala sampuli za mulatso mara tahu kwa wiki.
- Kahiza wiki tahu (3) zithuwirizazo,fundahala sampuli ya damu mara mbiri kwa wiki.
- Kahiza wiki tandahu (6) zlthuwirizazo,fundahala sampuli ya mulatso mara mwenga kwa wiki.

Herufi za kwanza za muhusika----------J -----Kigiriama PIS na Habari za Kumboza Ruhusa Nakala 1.1 tarehe 29 mwezi wane 2013 ; : 1::1

, Kahiza mara nyinji za uthuwirizi wa uhalaji wa mulatso,fundahala 1 ML(tsini ya kijiko cha chai) kumbola labda mukononi ama chalani, kulingana na mahenzogo. Kwa sampuli nne za mlatso ii (Mwenga indiyohalwa kahiza wiki ya hiri,wiki ya tsano,wiki ya nane na wiki ya kumi na tahu) fundahala 5mls(kijiko cha chai) kumbola kwa mukono.

Ahusika osi mandiohusishwa kahiza jaribio riri mandapewa kitambulisho ambacho kiridakala na picha ya kwao.li indasaidhia kuatambua ahusika na kuakumbusha tarehe za kwao za kudza kahiza kiliniki.

Je kuna madhara ama shida yoyosi kwangu nikihusika kahiza muradhi? Kipao mbele cha kwehu kwa kila muhusika ni afya ya kwao. Kunadima kukala na utsungu ama kuvarurwa wakathi kala mulatso unambozwa ambao inahola baada ya siku chache.Kunadima kukala na uwezekano muchache wa maambukizi.Madhara gaga ganahunguzwa kwa kutumia vifaa virizho vi salama na ahendi a kazi mario mafundishwa.

Madhara mangine ganadima kuonekana kumbolerana ma dawa za malaria.li inajumuisha na kurerwarerwa ni moyo na kuhakikisha,kulumwa ni ndani,kitswa kuluma na kukosa hamu ya kurya.

Muradhi uu undahusisha kuhala wakathi wa kwako na unadima kukala na garama za usafiri.Lakini fundakuujira garama za kwako za usafiri na wakathi wa kwako kwa kukuujiza shilingi magana matsano *(5001=)* kwa kila siku ambayo niufike kahiza kiliniki kwa wakathi udzepangwa ufike kiliniki.

Je kuna manufaa gogosi kwa kuhusika? Kala undahusika kahiza muradhi uU,undapata matibabu kwa ukongo wowosi undiokugwira baada ya kungira kahiza muradhi paka mwisho wa muradhi bila mariho, ambaho unadima ukatibiwa kahiza kiliniki hedu sipitali ya wilaya ya kilifi.Matibabu ga makongo sugu hedu makongo garembolerana na ajali ga muda mure ambago kagahusiana na mipangilio ya muradhi kagandarihirwa ni muradhi.Kala undaonekana kukala una ukongo uwo/majeraha,undatibiwa kulingana na mupangilio wa serikali.

Kwa kuhusika kahiza muradhi uu, undafusaidhia kumanya ni dawa hiyo ya malaria iriyoinadima kuhumirwa kala mtu ana jaribu chanjo ya malaria kulola kala inahenda kazi tototo.li baadaye indasaidhia kumboza chanjo ya malaria ambayo indareha faidha za afya

kahiza kizhazi kidzacho.

Je kundakalani kala nindakahala kuhusika? Kuhusika kosi kahiza utafiti ni hiyari.Uhuru kuamua kala unahenza kushiriki au la.Undaenderera kupata huduma za kawaida za afya kahiza kiliniki ambayo muradhi unahendeka hatha kala kundahusika.Kala undakubali kuhusika,funahenza uelewe kukala u huru kubadilisha maazo wakathi wowosi na kumbola kahiza utafiti. Ii kaindaathiri huduma za kwako za afya vivi na hatha siku zidzazo.

Na hangine kala udzambola kahiza muradhi kabila muradhi kugoma,fundakuuza udze kwa uchunguzi wa kiafya kahiza wakathi wa muradhi.

Je kundakalani kwa zo sampuli? Sampuli za mulatso zindaikwa iryahu namba ya kwako ya kukutambulisha kahiza muradhi na kakuna habari za kwako za kibinafsi here dzina ra kwako ama tarehe ya kwako ya kuzhalwa iriyoindaikwa.Ii ni kuhakikisha kukala sampuli zinadima thu kutambulishwa na ahusika ni atu mario manahusika kwa uhehi na utafiti.

Sampuli zindahenderwa upimaji KEMRI,Kilifi, lakini baadhi ya sampuli zindahirikwa ng'ambo kwa sababu athumia ni kukala mana vidudu zha malaria vichache kahiza mulatso wa kwao na funamala fuzhone,funatumia upimaji wa kihakeye (ambazho ni sahihi kamare kushinda kiryahu kipimo cha kawaida kiricho kahiza sipitali) na fundahenza fulole mara mbiri go majibu kwa vizho hundahuma sampuli ngambo na

Herufi za kwanza za muhusika----------Kigiriama PIS na Habari za Kumboza Ruhusa Nakala 1.1 tarehe 29 mwezi

wane 2013 sampuli zindizobaki zindaikwa kahiza maabara ga kwehu ga utafiti KEMRI.Kahiza siku zidzazo,utafiti mush a unadima kuhendwa na sampuli zizi. Utafiti wowosi musha lazima kwanza uidhinishwe ni kamati ya maadili ga utafiti kuhakikisha kukala ahusika masalama na masilahi gao ganarindwa.

Ni hani andiyeona habari kunihusu kahiza utafiti uno?

Habari zehu zosi za utafiti zinaikwa kahiza kabati zenye usalama na kahiza komupyuta zirizo zinaridwa na namba maalum .. Ni atu mario manahusika kwa hehi na utafiti uu ambao manadima kuona habari kumbola kwa ahusika kuhakikisha thu kukala muradhi unahendeka toto na afya ya kila muhusika inarindwa na mandaika habari zizi kwa usiri.

Ni hani adzeruhusu utafiti uno uhendeke?

Utafiti uu waruhusiwa ni kamati za kitaifa zihusishazo kamati za ataalamu kuko Nairobi na kamati ya kilifi kuhakikisha utafiti unahendeka tototo na usalama na haki za ahusika zinaheshimiwa.Malola tototo kazi iii na makikubali kukala utafiti uu ni muhimu kwa Kenya na unathuwa mupangilio wa kitaifa na kimataifa uriokubalika.

Kamati za kitaifa na za kimataifa zindaambirwa kuhusu madhara mabomu gandigombolera.

Je kala nina swali?

Unadima kimuza mumwenga waho yoysi wa ahendi a kazi a kwehu wakathi wowosi. Unadima pia ukawasiliana na aryahu mario manahusika na utafiti uu.

1. Dr Caroline Ogwang: KEMRI-Wellcome Trust, P.O Box 230, Kilifi Kenya namba ya simu:041 7522063
2. Dr. Francis Ndung'u:KEMRI-Welicome Trust,P.O Box, 230, Kilifi, Kenya. Namba ya simu.041 7522063

Kala unamala kumuza mtu yoyosi ariye a huru na utafiti uu tafadhali wasiliana:
The Community Liaison Manager, KEMRI-Kilifi, namba ya simu: 041 7522063, Mobile: 0723 342780

Na Mwandishi -Kamati ya maadili ya utafiti ya KEMRI, S.L.P., 54840-00200, Nairobi, namba ya simu: 020 272 2541 Mobile: 0722205901 or 0733400003

Utafiti uu unasaidhiwa ni chuo kikuu cha Oxford, ambacho mandarihira garama zosi za matibabu ama mariho gogosi ga garama za madhara mabomu gandigombolera here ajali zimboleranazo na jaribio. Herufi za kwanza za muhusika----------Kigiriama PIS na Habari za Kumboza Ruhusa Nakala 1.1 tarehe 29 mwezi wane 2013

t

Kitswa cha muradhi: Kulinganisha madawa ga kumarigiza vidudu kahiza kipindi cha lib kwa majaribio baada ya peR kulola maambukizi ga *Plasmodium falcipararum.*

1. Mimi, Nidzasikira jaribio rikielezerwa kwangu.Nidzaelewa kila kitu nidzichoshoma na nidzasikira vikielezerwa kwangu na maswali gangu gadzajibiwa kikamilifu.Naelewa kukala nadima kubadilisha maazo wakathi wowosi.

D *Tafadhali ika tiki* nakubali kuhusika kahiza utafiti. D *Tafadhali ika* tiki nakubali sampuli ziikwe

Sahihi ya muhusika Tharehe
Saa

Dzina ra muhusika
Sahihi ya Muchunguzi: ---------- Tharehe ----------
Dzi na ra m u c hun9 uzi: ---------- Saa: ----------

kwa utafiti wa siku zidzazo. D *Tafadhali ika tiki* Nakubali sampuli zisafirishwe.

(Tafadhali dhika dzina kwa herufi bomu)

Sahihi ya muhusika Tharehe
Saa

Dzina ra muhusika
Sahihi ya Muchunguzi: ---------- Tharehe ----------
Dzi na ra m u c hun9 uzi: ---------- Saa: ----------
(Tafadhali dhika dzina)

1. Mimi, (Ozina ra muhendi wa kazi wa KEMRI ahalaye idhini) nathibitisha ya kwamba nidzathuwa muongozo wa muradhi uu na nidzaelezera habari za muradhi kw~ muhusika here adziyehanzwa haho dzulu,na adzaelewa wo muradhi zho urizho na umuhimu wa muradhi na adzakubali kuhusika kahiza muradhi.adzapewa nafasi ya kuuza maswali ambago gadzajibiwa kikamilifu.

2. Nathibitisha kukala habari kahiza karathsi ii ya adhini zidzaelezwa tototo,na zidzaeleweka kwa muhusika

na idhini idzambozwa ni muhusika bila kulazimishwa. Sa h i h i ya s h a hid i----------

----T hare h e ----------Ozi n a ra s h a h idi: ----------S a a: ----------

---
(Tafadhali dhika dzina kwa herufi bomu)

**Shahidi ni aka/e ni mtu ariye huru na jaribio ama muhendi* wa *kazi ariye kahusikire kulazwa kuvoya idhini..*

Sahihi ya dzalagumbe ya muhusika adziyehadzwa ho dzulu kala kadima kudhika: _____
MUHUSIKA NIAPEWE KARATHASI YA IDHINIIDZIYONG/ZWA SAHIHI AKAIKE.

Herufi za kwanza za muhusika----------Kigiriama PIS na Habari za Kumboza Ruhusa Nakala 1.1 tarehe 29 mwezi wane 2013

Consent form in Swahili

Fomu ya maelezo na idhini ya Shirika la KEMRI-Wellcome Trust Kwa kulinganisha umalizaji wa vidudu kwa kutumia madawa katika awamu ya IIb ya mipango ya tafiti kabla ya kutumia PCR kwa kufuatilia maambukizi ya Plasmodium Falciparum

KICHWA LA RAHISI: Utafiti wa kulinganisha jinsi dawa tatu tofauti za malaria zinaweza kuondoa vidudu kutoka kwa damu.

WATAFITI NA MASHIRIKA

INVESTIGATORS	INSTITUTIONS
Caroline Ogwang (PI), Francis Ndungu(PI), Britta Urban, Domtila Kimani, Jedidah Mwacharo, Jimmy Shangala, Philip Bejon, Patricia Njuguna, Ken Awuondo	KEMRI CGMRC(Coast)
Faith Osier	Heidelberg University Hospital

KEMRI ni nini?

KEMRI ni shirika la serikali chini ya wizara ya afya, linalofanya utafiti wa matibabu. Utafiti ni tofauti na matibabu ya kawaida kwa sababu utafiti unalenga kutafuta njia bora za kukinga na kutibu magonjwa siku za usoni kwa manufaa ya watu wote.

Utafiti huu nahusu nini?

Malaria ni ugonjwa unaopatikana kwa wingi na huwa hatari hasa kwa watoto wadogo na ambao husababisha vifo vingi. Ijapokuwa malaria inaendelea kupungua katika sehemu zingine za Africa kutokana na kupatikana kwa urahisi kwa neti za mbu na dawa za malaria, chanjo bado zinahitajika kuzuia malaria kwani bado ni tatizo kubwa. Mahali popote duniani, dawa ama chanjo mpya zinapotengenezwa kwa ajili ya watoto, utafiti huanza kufanywa kwa watu wazima wachache wenye afya njema, kisha kwa watu wazima wengi zaidi wenye afya njema kabla kujumuishwa kwa watoto.

Katika watu wazima, inawezekana kuwa na vidudu vya malaria kwenye damu bila ya dalili zozote za maambukizi. Kwa sababu hii, katika utafiti wa chanjo ya malaria, wanasayansi kwa kawaida huwapatia washiriki dawa za malaria kuondoa vidudu vyovyote vilivyo kwenye damu, hata kama wana afya. Pia ni muhimu dawa za malaria zinazopeanwa kwa madhumuni haya ziwe zinaweza kufanya kazi kwa muda mfupi, la sivyo matokeo yake huenda yakafanya iwe vigumu kupima jinsi chanjo inavyofanya kazi. Kwa muda, kumekuwa na mabadiliko ya jinsi dawa za malaria zinavyofanya kazi, na sasa haijulikani kama ni dawa gani ya malaria inayofanya kazi vyema na kwa muda mfupi ili itumike katika tafiti za chanjo ya malaria.

Utafiti huu unalenga kujua ni dawa gani ya malaria ambayo ni bora kutumika katika mwanzo wa tafiti za malaria za siku za usoni. Tutafanya hivi kwa kuwapa watu wenye afya njema watakaokubali kushiriki moja kati ya dawa tatu za malaria na wafuatiliwe kwa wiki 13, wakati ambao tutapima damu zao mara kadhaa ili kuangalia kama kuna vidudu vyovyote vya malaria. Dawa hizi zote 3 za malaria zinazotumika kwenye utafiti huu tayari zimesajiliwa kutumika Kenya kutibu malaria. Tutaamua ni dawa gani tutampatia nani kutumia mbinu ya kuamua bila ya mapendeleo, ili kila mmoja awe na nafasi sawa ya kuwa kwa kundi lolote. Tunanuia kujumuisha watu wazima 90 wenye afya njema (wake kwa waume) katika utafiti huu. Mwisho wa utafiti, tutalinganisha majibu kutoka kwa wale wanaochukua dawa tofauti za malaria kuona ni dawa gani iliyofanya kazi vyema na kwa haraka zaidi. Ni muhimu kujua kwamba utafiti huu si wa chanjo au matibabu ya malaria.

Itahusisha nini kwangu?

Ukikubali kuhusika katika utafiti huu, yafwatayo yatafanyika;

1. Uchunguzi wa kiafya

Daktari ataangalia afya yako kwa jumla kwa kukuuliza maswali, kukuchunguza na kuchukua damu kiasi cha vijiko vitatu vya chai (15mls) kutoka kwa mkono ili ikapimwe. Tukiona kama una matatizo yoyote ya afya au hali isio ya kawaida katika damu iliopimwa, tutakueleza na kukuelekeza kwa kupimwa zaidi au matibabu unayohitaji kwa kituo cha afya cha Serikali. Katika hali hii, hatutakuuliza kuendelea kushiriki katika utafiti huu.

Tunajua ya kwamba asilimia chache ya watu hawawezi kusaga mmoja ya dawa za malaria amabayo tutatumia, kwa hiyo wataweza kuwa na upungufu wa damu kwa sababu ya athari za dawa. Mmoja ya vipimo vya damu kitaweza kugundua mtu asieweza kutumia hii dawa, kwa hivyo tutahakikisha watapewa moja kati dawa mbili. Tutamwelezea yeyote ambaye ataonekana anasumbuliwa katika hali hii.

Kama dawa nyingine ya aina yoyote, baadhi ya dawa za malaria zaweza kuwa hazina usalama kwa kina mama waja wazito au wale wanao nyonyesha. Hata hivyo, ni salama kina mama kuwa na mimba baada ya kumaliza dawa za malaria.

Kwa hivyo:

- Hatutashirikisha mama mja mzito au anayenyonyesha
- Tutauliza kina mama wanaopendelea kushiriki katika majaribio kutupatia mkojo ili kupima mimba kuhakikisha hawana mimba kabla kujiunga
- Tutauliza kina mama kutumia njia za kuzuia mimba mpaka matibabu ya dawa yamalizike
- Kurudia kupima mimba kabla kumuanzisha dawa ya malaria na kusimamisha yoyote atakaye patikana ana mimba.
- Tutarudia kupima mimba siku ya saba baada ya kumuanzisha dawa ya malaria kuthibisha ya kwamba hakuwa na mimba wakati alipotumia dawa ya malaria. Ikiwa kipimo kitaonyesha ana mimba, tutamfuatilia mpaka azae.

2. Kutumia dawa za malaria

Utaulizwa kuanza kutumia dawa za malaria siku ya kwanza ukijiunga na utafiti. Mbili kati ya dawa tatu lazima zitumiwe kwa siku tatu, na moja kati ya hizo lazima itumike kwa siku saba. Ni lazima uhudhurie kiliniki kupewa dawa hizo. Kwa kuongezea,ikiwa hujihisi vizuri kiafya kwa hali yoyote wakati ukiwa katika utafiti, ni lazima uje kliniki uonekane na kutibiwa na daktari wa utafiti. Kwa hali yoyote usitumie dawa yoyote bila ya kumuuliza daktari wa utafiti kwanza.

3. Ufuatiliaji na sampuli za damu

Tutatembelea nyumbani kwako mara moja kukuuliza utumiaji wa chandarua (neti ya kuzuia mbu) tutakuuliza uje kiliniki kwa jumla mara 26 kati ya wiki 13 (kwa wale waliotumia dawa kwa siku 3) na kwa jumla mara 29 kati ya wiki 13(kwa wale waliotumia dawa kwa siku 7). Wakati wa mahudhurio haya, daktari wa utafiti ataangalia afya yako kwa jumla na kuchukua damu kama ifwatavyo:

- Wiki nne za kwanza, tutachukua damu mara 3 kwa wiki
- Wiki tatu zifwatazo, tutachukua damu mara 2 kwa wiki
- Kwa wiki 6 zifwatazo, tutachukua damu mara moja kwa wiki

Mara nyingi katika ufwatilizio wa damu, tutachukuwa damu kiasi cha chini ya nusu kijiko cha chai (1ml) kutoka katika mkono au kidole, italingana na chaguo lako. 4 katika ya hizi sampuli (1 iliochukuliwa wakati ya wiki ya 2, wiki ya 5, wiki ya 8 na wiki ya 13), tutachukua damu kiasi cha kijiko kimoja cha chai (5ml) kutoka kwa mkono wako.

Washiriki wote katika majaribio haya watapewa vitambulisho vitakavyokuwa na picha zao. Hii itasaidia kutambua washiriki na kukumbusha tarehe za kuhudhuria kliniki.

Je kuna athari au usumbufu kwangu kwa kushiriki kwenye utafiti huu?
Afya njema kwa kila mshiriki ndiyo tumeipa kipaumbele. Kuna uwezekano kuwa na maumivu na kupujuka kunakosababishwa na utoaji wa damu utakaoisha baada ya siku chache. Kuna athari ndogo ya kupata maambukizi. Hii imepunguzwa na utumiaji wa vifaa visafi na wafanyikazi waliofunzwa.

Kuna madhara yanayoweza kusababishwa na dawa ya malaria. Hii inajumuisha kichefuchefu na kutapika, maumivu ya tumbo, maumivu ya kichwa na kukosa hamu ya chakula.
Utafiti huu utahusisha utumiaji wa wakati wako na unaweza kuwa na gharama za nauli. Hata hivyo tutakupa fidia kwa muda wako na matumizi ya nauli kwa malipo ya shilingi 500 kwa kila siku ambayo utahudhuria kliniki katika siku zilizopangwa.

Je kuna manufaa yoyote kwa kushiriki?
Unaposhiriki katika utafiti huu, utapokea matibabu kwa magonjwa yoyote mapya utakayokuwa nayo kuanzia kujiunga na utafiti hadi mwisho wa utafiti bila malipo, na haya yanawezatibiwa katika kliniki ama hospitali kuu ya Kilifi. Matibabu ya magonjwa sugu ama majeraha ya muda mrefu yasiyohusiana na taratibu za utafiti hazitalipwa na utafiti. Ukipatikana na maradhi au majeraha ya aina hii, utatibiwa kulingana na mpangilio ulioko wa serikali.

Kwa kushiriki kwenye utafiti huu, utatusaidia kutambua ni dawa gani ya malaria inayoweza kutumika wakati mtu anajaribu chanjo ya malaria kuona kama inafanya kazi vyema. Hii hatimaye itatusaidia kutengeneza chanjo ya malaria itakayoleta manufaa ya kiafya kwa kizazi cha siku za usoni cha watoto.

Je kutafanyika nini nikikataa kushiriki?
Kushiriki kote kwenye utafiti ni hiari. Una uhuru wa kuamua iwapo ungetaka kushiriki au la. Utaendelea kupokea uangalizi uliopendekezwa katika kliniki ya utafiti hata usiposhiriki. Unapokubali kushiriki, tungependa uelewe ya kwamba uko na uhuru wa kubadilisha nia yako wakati wowote na kujitoa kutoka kwa utafiti. Hii haitaathiri uangalizi wa afya yako sasa na siku za usoni.
Ukijitoa kabla utafiti kukamilika, bado tunakusihi uje kwa uangalizi wa afya kwa muda wa utafiti.

Nini kitafanyika kwa sampuli?
Sampuli za damu zitapewa nambari ya kitambulisho ya utafiti na hakuna habari za kibinafsi kama vile jina au tarehe ya kuzaliwa zitakazotumika. Hii ni kuhakikisha kwamba sampuli zitaweza kulinganishwa kwa washiriki na watu wanaohusika na utafiti kwa ukaribu.

Sampuli zitachunguzwa hapa KEMRI Kilifi, ijapokuwa sampuli zingine zaweza kutumwa Oxford na Heidelberg (Ujerumani). Kwa kuwa watu wazima huwa na kiwango kidogo cha vidudu vya malaria katika damu tunavyohitaji kuvitambua, tunatumia vipimo spesheli (sahihi zaidi ya zile za malaria za kawaida zinazopatikana hospitalini) na tunaweza hitaji kuchunguza kwa mara nyingine baadhi ya majibu kwa kutuma sampuli Oxford. Sampuli zozote zitakazobaki zitahifadhiwa katika maabara yetu ya utafiti hapa KEMRI. Siku za usoni, tafiti mpya zinaweza kufanywa kutumia sampuli hizi. Tafiti zozote za siku za usoni lazima kwanza ziidhinishwe na kamati ya maadili ya utafiti ili kuhakikisha kwamba usalama na afya ya washiriki imelindwa.

Ni nani ataweza kuona habari zinazonihusu katika utafiti huu?
Stakabadhi zote zitawekwa kwa usalama katika kabati zilizofungwa na tarakilishi zinazofungwa na nambari maalum za siri. Watu wanaohusika kwa karibu sana na utafiti huu ndio watakaoweza kuona habari hizi kutoka kwa wahusika ili kuhakikisha kwamba utafiti unaendeshwa sawasawa na afya ya kila mhusika inatunzwa vyema na wataweka kila habari kwa njia ya uangalifu

Ni nani ameruhusu utafiti huu ufanyike?
Utafiti huu umeidhinishwa na kamati maalum za hapa nchini na za kimataifa, zikijumuisha kamati za wataalamu za Nairobi na kamati ya Kilifi, kuhakikisha kwamba utafiti unafanywa vyema na kwamba usalama

na haki za wahusika zinaheshimiwa. Wameangalia kazi hii kwa uangalifu na kukubaliana kwamba utafiti huu ni muhimu na wafaa hapa Kenya na unafuata muongozo uliokubalika kitaifa na kimataifa.

Kamati za hapa nchini na za kimataifa zitajulishwa juu ya madhara yoyote makubwa ambayo yataonekana.

Na je kama nina swali lolote?
Unaweza kuuliza swali mfanyikazi wetu yeyote wakati wowote. Pia unaweza kuwasiliana na wale ambao wanahusika na utafiti huu.

1. Daktari Caroline Ogwang: Shirika la KEMRI-Wellcome Trust, Sanduku la barua 230, Kilifi Kenya Simu: 041 7522063
2. Daktari. Francis Ndung'u: Shirika la KEMRI-Wellcome Trust, Sanduku la barua 230, Kilifi, Kenya. Simu. 041 7522 063

Ukitaka kuwasiliana na mtu aliye huru kuhusu utafiti huu, tafadhali wasiliana na

Maneja wa Uhusiano mwema na jamii, KEMRI-Kilifi, Simu: 041 7522 063, Rununu: 0723 342780

Na

Katibu Mkuu – KEMRI/Kamati ya Maadili

Sanduku la barua 54840-00200, Nairobi, Simu: 020 272 2541 Rununu: 0722205901 au 0733400003

Utafiti huu unapata msaada kutoka chuo kikuu cha Oxford, watakaolipia matibabu ama fidia iwapo kumetokea tukio lisilotarajiwa la kuumia kutokana na utafiti huu

Consent form in English

KEMRI – Wellcome Trust Programme Participant Information and Consent Form
For comparing drug-regimens for clearing parasites in Phase IIb trial designs prior to PCR monitoring for *Plasmodium falciparum* infection

INVESTIGATORS	INSTITUTIONS
Caroline Ogwang (PI), Francis Ndungu(PI), Britta Urban, Domtila Kimani, Jedidah Mwacharo, Jimmy Shangala, Philip Bejon, Patricia Njuguna, Ken Awuondo	KEMRI CGMRC(Coast)
Faith Osier	Heidelberg University Hospital

LAY TITLE: A study to compare how three different anti-malarial drug regimens can clear parasites from the blood.

What is KEMRI?
KEMRI is a government organization under the Ministry of Health, which carries out medical research. Research is different from normal treatment because research aims to find better ways of preventing and treating illness in the future for everybody's benefit.

What is this research about?
Malaria is a common and serious disease in young children that results in many childhood deaths. Although malaria is becoming less frequent in some parts of Africa as bed nets and anti-malarial drugs become more available, vaccines are still needed to prevent malaria as it is still a major problem. Everywhere in the world, when new drugs or vaccines are being developed for children, research is first done in a few healthy adults, then in bigger numbers of healthy adults before children can finally be included.

In adults, it is possible for them to have malaria parasites in their blood without any symptoms of malaria. For this reason, during research on malaria vaccines, scientists often give study participants anti-malarial medicines to clear any parasites present in their blood, even though they are healthy. It is also important that the anti-malarial medicines given for this purpose work over a short period, because otherwise their effect would make it difficult to measure how well the vaccine itself was working. Over time, there have been changes in how well different antimalarial medicines work, and it is not now known which is the most effective but short acting antimalarial medicine to use during malaria vaccine trials.

This study aims to find out which is the best antimalarial drug to use at the start of future malaria vaccine trials. We will do this by giving healthy people who agree to participate one of three different antimalarial medicines and following them up for 13 weeks, during which time we will test their blood several times to check if any malaria parasites are present. All three antimalarial medicines used in this study are already registered for use in Kenya to treat malaria. We will decide which medicine to give to which person using a system based on chance, such that everyone has the same chance of being in any group. We aim to include 90 healthy adults in total (men and women) in this study. At the end of the study we will compare the results from those taking the different antimalarial medicines to see which medicine worked best and most quickly. It is important to realize that this study is not a malaria vaccine or treatment study.

What will it involve for me?
If you agree to take part in this study, the following will happen:

1. **Initial health check**

A clinician will check your general health by asking questions, examining you and taking a 15 ml (3 teaspoons) blood sample from your arm for testing. If we find you have any health problems or abnormal blood tests, we will explain this to you and refer you for any further tests or treatment you need at the most appropriate government health facility. In this case, we will not ask you to continue participating in this study.

We know that a small percentage of all people may not be able to breakdown one of the antimalarial medicines that we will use, and that they might develop anaemia (blood not strong enough) because of the effect of the medicine. One of the blood tests will identify anyone not able to take this medicine, so that we make sure they are given one of the other two. We will inform anyone found to be affected in this way.

Just like any other types of medicine, some anti-malarial medicines may not be safe when administered to pregnant or breastfeeding women. However, it is safe for women to become pregnant after completing the anti-malarial drugs. We will therefore:

- not recruit anyone who is pregnant or breast feeding
- ask any women interested in joining this trial to give us a urine specimen for a pregnancy test to make sure they are not pregnant before joining
- ask women to confirm they will use family planning methods until the drug treatment has been completed
- repeat the pregnancy test before we administer the first dose of anti-malarial medicines and withdraw anyone found to have a positive test
- repeat the pregnancy tests on the 7th day after starting the antimalarial medicines to confirm that the person had not been pregnant while taking the anti-malarial drugs. If this test is positive, we will need to continue follow up throughout the pregnancy until delivery

2. **Taking malaria treatment**: You will be asked to start taking an anti-malarial drug on the day of joining the study. Two of the three anti-malarial medicines should be taken for 3 days, and one should be taken for 7 days. You must attend the clinic to take each dose. In addition, if you are unwell in any way during the study, you should come to the clinic where you will be seen and treated by the study doctor. As far as possible, you should not take any other medicines without consulting the study doctor first.

3. **Follow up and blood samples**:

   We will visit your home once to ask for information on any bed nets being used in your home
   We will ask you to come back to clinic a total of either 26 times over 13 weeks (for those who took the 3 day course of anti-malarial medicines) or 29 times over 13 weeks (for those who took the 7 day course). During these visits, the study doctor will check your general health and take blood samples as follows:
   - In the first 4 weeks, we will take blood samples 3 times a week
   - In the next 3 weeks, we will take blood samples 2 times a week
   - In the next 6 weeks, we will take a blood sample once a week

   For most of these follow up blood samples, we will take 1ml (less than half a teaspoon) from either your arm or finger, depending on your choice. For 4 of these samples (one taken during week 2, week 5, week 8 and week 13), we will take 5 ml of blood (1 teaspoon) from your arm.

   All participants enrolled in the trial will be given an identification card with their photo on it. This will help to identify participants and remind them of their clinic appointment dates.

Are there any risks or disadvantages to me for taking part in the study?

Our priority for every participant is their well-being. There may be some pain and bruising associated with blood drawing which will resolve after a few days. There is a small risk of infection. This risk is minimized by use of pre packaged sterile equipment and trained staff.

Some side effects may be seen with the malaria medication. These include nausea and vomiting, abdominal pain, headache and loss of appetite.

This study will involve taking your time and you may have travel costs. However we will compensate for your time and travel expenses with a payment of Ksh 500 for each day that you have to attend the clinic for the scheduled clinic visits.

Are there any benefits for taking part?

If you participate in this study, you will receive medical care for any new illnesses you have from joining the study until the end of the study free of charge, where these can be treated at the clinic or Kilifi district hospital. Treatment of chronic illnesses or long term injuries unrelated to the study procedures will not be paid for by the study. If you are found to have such illnesses/injuries, you will be treated under the existing government programs.

By participating in this study, you will be helping us determine which anti-malarial drugs can be used when one is testing a malaria vaccine to see if it is working well. This will eventually help to develop a malaria vaccine that will bring health benefits to future generations of children.

What will happen if I refuse to participate?
All participation in research is voluntary. You are free to decide if you want to take part or not. You will still receive the recommended standard of care at the study clinic even if you do not take part. If you do agree to participate, we would like you to understand that you are free to change your mind at any time and withdraw from the research. This will not affect your health care now or in the future.

In the event that you leave the study before its completion, we still encourage you to come for health checks during the study period.

What happens to the samples?
The blood samples will be coded with your study identification number and no personal information such as your name or date of birth will be included. This is to ensure that samples can only be linked to the participants by people closely concerned with the research.

Samples will be processed in KEMRI, Kilifi, but some samples will be sent to Oxford and Heidelberg. Because adults often have low levels of malaria parasites in the blood and we need to detect these, we are using specialized tests (more accurate than the usual malaria tests available in hospitals) and we may need to double-check some of our results by sending samples to Oxford. Any remaining samples will be stored at our research laboratories in KEMRI. In the future, new research may be done on these stored samples. Any future research must first be approved by KEMRI Ethics Review Committee to ensure that participants' safety and wellbeing are protected.

Who will have access to information about me in this research?

All our research records are stored securely in locked cabinets and password protected computers. Only the people who are closely concerned with the research will be able to view information from participants just to be sure that the study is being run correctly and the health of every participant is protected and they

will keep the information confidential.

Who has allowed this research to take place?
This study had been approved by local and international committees including expert committees in Nairobi and a committee in Kilifi to make sure the research is conducted properly and that participants' safety and rights are respected. They have looked carefully at this work and agreed that the research is important, relevant to Kenya and follows nationally and internationally agreed guidelines.

The local and international committees will be informed about any serious side effects that are noticed.

What if I have any questions?

You may ask any of our staff questions at any time. You can also contact those who are responsible for this research:
1. Dr Caroline Ogwang: KEMRI-Wellcome Trust, P.O Box 230, Kilifi Kenya Telephone: 041 7522063
2. Dr.Francis Ndung'u: KEMRI-Wellcome Trust, P.O Box, 230, Kilifi, Kenya. Tel. 041 7522 063

If you want to ask someone independent anything about this research please contact:

The Community Liaison Manager, KEMRI – Kilifi, Tel: 041 7522 063, Mobile: 0723 342780 OR
The Secretary - KEMRI Ethics Review Committee, P. O. BOX 54840-00200, Nairobi, Tel number: 020 272 2541 Mobile: 0722205901 or 0733400003

This research is supported by The University of Oxford, who will pay for any treatment or compensation in the unlikely event of any injury resulting from this trial.

Study Title: Comparing drug-regimens for clearing parasites in Phase IIb trial designs prior to PCR monitoring for *Plasmodium falciparum* infection

1. I, _____, have had the trial explained to me. I have understood all that I have read and have had explained to me and had my questions answered satisfactorily. I understand that I can change my mind at any stage.

☐ *Please tick* **I agree to take part in this research**