# MALARIA AND PREGNANCY OUTCOMES
# IN AN AREA OF HIGH HIV AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE
# DEFICIENCY PREVALENCE

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology, Gillings School of Global Public Health

Chapel Hill
2009

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## ABSTRACT

ELLA NKHOMA:  Malaria and Pregnancy Outcomes in an Area of High HIV and Glucose-6- phosphate dehydrogenase deficiency prevalence (Under the direction of Steven Meshnick)

HIV and G6PD deficiency are part of a milieu of population-level biological factors in which pregnancies occur in malaria-endemic areas.    In this research we examine the effect of HIV infection and malaria parasitemia on pregnancy outcomes, the relationship between HIV infection and risk, frequency, and severity of maternal parasitemia, and the effect of G6PD deficiency on parasitemia risk and pregnancy outcomes.

Between 2005 and 2006, we followed pregnant women attending two antenatal care clinics in southern Malawi from the second trimester of gestation until delivery.  HIV was associated with increased risk of LBW (adjusted prevalence ratio, $PR_{adj}$=3.08, 95% confidence interval, CI, = 1.40, 6.79).  Placental parasitemia was associated with an elevated risk of LBW ($PR_{adj}$=1.79, 95% CI = 0.83, 3.84), as was having $\geq$3 episodes of peripheral parasitemia during follow-up ($PR_{adj}$=2.68, 95% CI = 1.06, 6.79).

HIV was not associated with increased risk of parasitemia among primigravidae over follow-up (RR=1.0, 95% CI: 0.4, 2.8). Among multigravidae, the risk of parasitemia over follow-up among HIV-infected women was 2.2 times (95% CI: 1.5, 3.2) that of HIV-uninfected women.  Further, the odds of having $\geq$3 episodes of parasitemia among HIV-infected multigravidae were 4.8 (95% CI: 2.1, 10.9) times that of HIV-uninfected multigravidae.

Among both primigravidae and multigravidae, placental parasite density among HIV-infected women was on average 3.6 (95% CI: 1.8, 7.2) times as high as among HIV-uninfected women.

G6PD A- primigravidae were as likely to have placental parasitemia as G6PD-normal primigravidae (PR=1.0, 95% confidence interval (CI): 0.7, 1.3). Among multigravid G6PD A- carriers, the prevalence of placental parasitemia was 0.9 (95% CI: 0.8, 1.0) times that of G6PD-normal women. Further, their placental parasite density was on average 0.22 times that of G6PD-normal multigravidae.  Among primigravidae, G6PD A- carriers had 1.7 (95% CI: 1.0, 2.9) times the average risk of maternal anemia over follow-up when compared to G6PD-normal women. Across gravidities, G6PD deficiency was associated with an increased risk of low birth weight (PR=2.5, 95% CI: 1.2, 5.2).   Understanding the contribution of these different coexistent factors should aid in the design of effective interventions to improve maternal and infant health in malaria-endemic areas.

For my father, Christopher Timothy Nkhoma

## ACKNOWLEDGEMENTS

I wish to acknowledge my advisor, Steven R. Meshnick, and my committee: Charles Poole, Rick Fairhurst, Sonia Napravnik and Michael Hudgens for their support and guidance throughout the process. I have benefited tremendously from their knowledge and expertise during all aspects of this work, from the study design, analysis, to writing the final papers.

Words cannot express my gratitude for the love, support and encouragement I have received from my family (including my late father Christopher T. Nkhoma, and my mother Winnie Nkhoma, my sister Dalitso and my brother Robinson) and my friends both at UNC and elsewhere, who have supported me during my studies.

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# LIST OF ABBREVIATIONS

AIDS            acquired immunodeficiency syndrome

ANC             antenatal care

AP              attributable proportion (for interaction)

CI              confidence interval

CSA             chondroitin suphate A

G6PD            glucose-6-phosphate dehydrogenase

g/dL            grams per deciliter

GEE             generalized estimating equations

Hb              hemoglobin

HIV             human immunodeficience virus

IC              interaction contrast

IgG             gamma immunoglobulin

IPT             intermittent presumptive therapy

IQR             interquartile range

IUGR            intrauterine growth retardation

LBW             low birth weight

MAR             missing at random

MCAR            missing completely at random

OR              odds ratio

RERI            relative excess risk due to interaction

RR          risk ratio

S           synergy index

SD          standard deviation

SP          sulfodoxine-pyrimethamine

VCT         voluntary testing and counseling

WBC         white blood cell

# CHAPTER ONE - SPECIFIC AIMS

In malaria endemic areas, pregnant women are at high risk of morbidity from malaria. Malaria during pregnancy can affect the health of the mother through maternal anemia and the health of the infant by contributing to low birth weight [1]. HIV infection has also been associated with low birth weight [2]. Furthermore, being infected with HIV can increase the risk of malaria[3]. In many malaria endemic areas, however, the prevalence of glucose-6 phosphate dehydrogenase (G6PD) deficiency, an enzymopathy hypothesized to protect against clinical malaria, is relatively high[4]. Therefore, in areas such as Eastern Africa, where the prevalence of both HIV and G6PD deficiency is high, there can be a push and pull of factors influencing not only malaria risk, but also the risk of adverse pregnancy outcomes.

The objective of this study is to determine the effects of malaria, HIV infection and glucose-6-phosphate dehydrogenase (G6PD) deficiency on maternal anemia and low birth weight in a cohort of women delivering at the Mpemba and Madziabango clinics in Blantyre District, Malawi. Between 2005 and 2006, data were collected on HIV seropositivity, placental parasitemia, hemoglobin levels, and birth weight. Additionally, blood samples collected from the women were genotyped for carriage of the A- allele, corresponding to the mutation that confers G6PD deficiency among sub-Saharan African populations.

Malaria is a tremendous public health challenge in Malawi, with over 2 million reported cases each year out of a population of 12 million people[5]. The entire population of Malawi is at risk of malaria, with 97% living in malaria endemic areas and 3% living in areas subject to malaria epidemics. Each year in Malawi, there are approximately 500,000

pregnancies, all at risk of malaria [5]. Given this widespread risk and the deleterious effects of malaria during pregnancy, intermittent presumptive therapy (IPT) in the form of sulfadoxine-pyrimethamine (SP) is provided to pregnant women through antenatal care clinics. However, recent evidence suggests that SP treatment failure due to parasite resistance is increasing rapidly in Malawi as in other parts of Eastern Africa [6].

As SP treatment failure becomes more frequent, other antimalarial drugs will need to be considered for IPT. Uptake of certain classes of antimalarial drugs can elicit acute hemolysis in G6PD deficient individuals [7]. Given this adverse interaction, it is very important to understand the distribution of this enzyme deficiency and its contribution to malaria risk. HIV also represents another major contribution to malaria risk in sub-Saharan African countries. Malawi, again like many countries in Eastern Africa, has not been immune to the HIV/AIDS epidemic with over 12% of women visiting antenatal clinics infected [8]. Understanding HIV infection and G6PD deficiency as elements of the disease context of malaria will enable public health policymakers to more comprehensively address adverse pregnancy outcomes in Malawi.

**Specific Aim 1.** What is the effect of malaria and HIV infection during pregnancy on maternal anemia and birth weight?

- **Objectives:**
    1. To determine the separate effects of HIV infection and malaria on maternal anemia and low birth weight.
    2. To assess interaction between the effects HIV infection and malaria on

maternal anemia and low birth weight.

**Specific Aim 2.**  What is the effect of HIV infection on parasitemia risk at enrollment, during follow-up and at delivery?

- **Objectives:**

    1. To describe the pattern of parasitemia risk over the second and third trimester.

    2. To estimate the effect of HIV on the risk of parasitemia at enrollment, over the follow-up period and at delivery.

    3. To determine the relationship between HIV infection and frequency and severity of parasitemia at delivery.

**Specific Aim 3.**  What is the relationship between carriage of the G6PD A- allele and parasitemia and anemia among pregnant women?

- **Objectives:**

    1. To estimate the prevalence of carriers of the G6PD A- allele among a cohort of pregnant women in Southern Malawi.

    2. To determine if the risk of peripheral parasitemia during pregnancy and placental parasitemia at delivery among G6PD deficient women is different from the risk among G6PD-normal pregnant women.

    3. To determine if the risk of low birth weight of infants born to G6PD deficient women is different from that of infants born to G6PD-normal pregnant women.

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# CHAPTER TWO - BACKGROUND AND SIGNIFICANCE

Each year, 20 – 50 million women become pregnant in malaria-endemic areas [1-3]. In these women, exposure to and subsequent infection with malarial parasites, especially *Plasmodium falciparum*, can have remarkable implications during the course of pregnancy and may affect birth outcomes, with an estimated 75,000 – 200,000 infant deaths per year associated with malaria infection during pregnancy [3].

In Malawi, like in many countries in Southern Africa, the national prevalence of HIV infection among antenatal care clinics is high, estimated at 12% [4]. HIV infection among pregnant women is associated with adverse pregnancy outcomes including low birth weight and infant mortality [5]. HIV infection has also been found to increase the risk of malaria among pregnant women [6].

Glucose-6-phosphate dehydrogenase deficiency is thought to protect against clinical malaria among people living in endemic areas, by impeding the parasites ability to establish infection. Some studies have confirmed this relationship among children and non-pregnant adults, while others have failed to find an association [7-9]. Only one cross-sectional study in the literature has examined this relationship among pregnant women [10].

The primary goals of the proposed study are to evaluate the relationship between malaria and HIV infection on pregnancy outcomes and to determine the effect of G6PD deficiency on maternal anemia and malaria.

**Malaria and pregnancy**

The unusual relationship between pregnancy and malaria infection has been described for more than 60 years [3]. While the immune status of women to malaria generally mirrors that of men, pregnant women, especially primigravidae, are usually at much increased risk of malaria infection than both males and their non-pregnant female counterparts [11, 12]. At first, this was thought to be caused through the immuno-suppression triggered by pregnancy-associated hormones and proteins. This immuno-suppression would then increase the mother's susceptibility to various infections, including malaria. However, it was noted that while mean gamma-immunoglobulin (IgG) concentrations decrease with the progression of the pregnancy, with the lowest titers in the third trimester of pregnancy, the risk of maternal malaria infection, however, is highest in the first trimester, and lowest in the third trimester [11].

The more likely hypothesis, however, is that "pregnancy establishes within an otherwise effectively immune host an extremely vascular organ that shields parasite[s] from destruction by extra-uterine immune effector mechanisms" [11]. This hypothesis was originally posited in 1984, but there was no supporting evidence until recently. A number of recent studies have demonstrated that pregnant women are infected by particular variants of *P. falciparum* that have special affinity for the chondroitin sulphate A (CSA) receptor expressed on the surface of placental cells[13, 14]. These variants do not infect non-pregnant hosts and differ in their ability to evade immune response. Therefore, even if a woman was previously immune, the introduction of this novel variant renders her highly

susceptible to infection, especially during the first pregnancy. With successive pregnancies, the woman gradually develops immunity to this new parasite population [11, 15].

The condition described above is known as placental malaria (PM) and has adverse consequences for the mother and the fetus. During placental malaria, the parasites are sequestered in the placenta, but are not present in the rest of the body. Thus, blood smears, the usual mode of malaria diagnosis, will often give false negative results [16]. In contrast, among non-pregnant adults, a negative blood smear is more likely to indicate the lack of infection [12]. Among pregnant women, histological examination of the placenta is the most sensitive method of malaria detection [17-19].

**Malarial anemia and pregnancy**

Placental malaria has been associated with spontaneous abortion, stillbirth, low birth weight (LBW), intrauterine growth retardation (IUGR), prematurity, and severe maternal anemia. Severe maternal anemia, defined as hemoglobin <7g/dL, is a major effect of maternal malaria in endemic regions [11, 12, 16]. A number of cross-sectional surveys have found that the prevalence of severe anemia was greater in women infected with malaria when compared to those without malaria [2].

In the systematic review by Guyatt & Snow, the prevalence of severe anemia was higher in primigravidae when compared to multigravidae [2]. The median prevalence of severe anemia among primigravidae for 18 studies was 11.3% (interquartile range (IQR): 7.0-19.5%) with a prevalence of 8.2% (IQR: 4.5-10.3%) among all gravidities [17]. In a study, conducted in Ethiopia, placental parasitemia was associated with anemia among both

pregnant women living in stable transmission areas (relative risk (RR) = 2.0, p<.001) and women in unstable transmission areas (RR=4.4, p<0.001) [20]. Another study conducted in Tanzania, found that anemia among pregnant women was associated with the rainy season (high malaria transmission period, odds ratio (OR) = 6.02, p<0.001)[21]. Yet another cross-sectional survey, conducted in Mali reported increased risk of anemia during the rainy season (OR = 1.93, 95%CI 1.10, 3.39, in rural areas, OR = 3.55, 95% CI: 1.46, 8.62) [22].

In a prospective intervention study conducted in Malawi, women were followed during the course of pregnancy and monitored for malaria status. At delivery, blood from the placenta was examined for the presence of parasites. Placental examination was associated with an increase in the number of cases of maternal malaria of 12%. In associations of maternal malaria with LBW, the authors reported increased risk with both maternal malaria (minus placental malaria, OR=1.84, 95%CI: 1.26, 2.68) and placental malaria (OR=2.57, 95%CI 1.77, 3.72). Similarly, for associations with anemia, there was increased risk of anemia among women with maternal malaria (minus placenta malaria, OR=1.85, 95% CI: 1.45, 2.36) and placental malaria (2.0, 95%CI: 1.5, 2.7) [19].

Severe maternal anemia has various consequences for the mother and the fetus. Anemia resulting from placental parasitization is often asymptomatic and so may be overlooked until it becomes very severe [16]. Severe anemia may result in maternal heart failure and decrease the ability of mothers to endure blood loss associated with delivery. This increases the mother's risk for blood transfusion and maternal death [23].In the fetus, maternal anemia is associated with LBW. This is thought to be mediated through the reduced oxygen transport which contributes to IUGR. Furthermore, the chronically inflamed state of the placenta is thought to hasten labor, thus resulting in prematurity. All of these three

conditions are associated with infant mortality, especially in developing countries [2, 23].

**HIV and pregnancy**

HIV infection in pregnancy has been associated with spontaneous abortion, stillbirth, infant mortality, growth retardation, low birth weight and preterm delivery [5]. A systematic review/meta-analysis of studies published before 1997 from different countries on HIV infection and adverse birth outcomes reported that HIV infection results in a four-fold increase in infant mortality (Peto OR = 3.69; 95% CI: 3.03, 4.49; 9 studies). Additionally, HIV infection was found to increase the risk of low birth weight (Peto OR = 2.09; 95%CI: 1.86, 2.35; 17 studies). The standard mean difference in birth weight was reported as -0.342 (95%CI: -0.421, -0.262; 7 studies). In a study conducted in Kigali, Rwanda, HIV-positive women were 1.8 (95%CI: 1.1-2.9) times as likely to deliver low birth weight babies when compared to HIV-negative women [24]. The relationship between HIV infection and maternal anemia is unclear. In the study conducted in Rwanda, no relationship was observed between HIV infection and anemia (defined as Hb <10g/dL) [24]. However, in a study conducted in Kenya, HIV-positive pregnant women were found to have 1.7 (95%CI: 1.3 - 2.0) times the risk of anemia (defined as Hb < 11g/gL) [25]. Another study conducted in Kenya reported that being HIV seropositive increased the odds of anemia (defined as <8g/dL OR=2.0, 95%CI: 1.2-3.3) among primigravidae [26].

**HIV, malaria, and pregnancy**

According to recent evidence, not only does HIV infection increase the risk of malaria, the infection may also interact with malaria to cause adverse pregnancy outcomes [6, 27]. Among pregnant women, HIV infection increases the risk of peripheral and placental malaria, high parasite density, fever and severe anemia [27]. Generally, among pregnant women living in malaria endemic areas, the risk of malaria decreases with increasing gravidity. However, HIV, even without development to AIDS, seems to interfere with the development of immunity that is normally correlated with increasing gravidity [27]. For example, in a study conducted in Kenya, the relative risk for parasitemia in the third trimester was similar among primigravidae, secundagravidae, and multigravidae (RR=2.40, 95% CI 1.64-3.53; RR=2.54, 95% CI 1.32-4.86; 2.66, 95% CI 1.10-6.43). Similar results were also observed for symptomatic clinical malaria across all three categories of gravidity [28]. Thus HIV infection disrupts the typical relationship between gravidity and malaria risk.

The increased risk in malaria coupled with the effects of HIV infection may interact to result in adverse pregnancy outcomes more pronounced than those that would be expected with HIV infection or malaria alone. In a study conducted in Malawi, the odds of neonatal mortality among infants with low birth weight born to mothers with placental malaria was 2.0 (95% CI: 0.9 – 4.6). Among pregnant women with placental malaria and HIV infection, the odds of neonatal mortality among low birth weight infants was 8.98 (95% CI: 3.1-25.9) [29]. Another study conducted in Tanzania reported that among pregnant women infected with HIV, any parasitemia at the first visit was associated with an increased risk of low birth weight (defined as <2500 g, $RR_{adjusted}$ = 2.66, p=0.01) [30]. In a study conducted in Kenya,

the odds of low birth weight among primigravidae with malaria were 2.3 (95% CI 1.3-4.2)

times that of aparasitemic primigravidae. However, among primigravidae infected with both

HIV and malaria, the odds of low birth weight were 3.5 (1.7-7.1) times that of primigravidae

with neither infection [26]. Similar results were reported for maternal anemia. Multigravidae

infected with both HIV and malaria had 3.2(95% CI: 1.7 – 6.2) times the odds of anemia

(defined as Hb<8g/dL) as multigravidae with HIV only. In the same way, multigravidae with

dual infection had 2.8 (95% CI:1.3-6.0) times the odds of maternal anemia as multigravidae

with malaria only. This relationship was observed among primigravidae as well with the

reference as parasitemic primigravidae [26].


**G6PD deficiency**


Glucose-6-phosphate deficiency is the most prevalent enzyme deficiency with over

300 million people affected worldwide [31]. The enzyme catalyzes the first rate-

determining step in the pentose phosphate pathway that forms part of glycolysis. In the

erythrocyte, the conversion of glucose-6-phosphate into 6-phosphogluconolactone, catalyzed

by G6PD is a very important source for the production of NADPH (hydrogenated

nicotinamide). This compound acts by reducing glutathione and stabilizing catalase, two

compounds that have crucial anti-oxidant properties in the red blood cell. Thus, G6PD

activity helps the erythrocyte to withstand oxidative stress [32].

The gene encoding for G6PD is found on the long arm of the X chromosome (Xq28),

and consists of 13 axons with a length of 18kb [33]. The G6PD locus is thought to be one of

the most polymorphic loci among humans with almost 400 allelic variants reported [32]. The

variants are subdivided into five classes based on level of deficiency, with Class 1

characterized by severe deficiency resulting in chronic non-spherocytic anemia ranging to

Class 5 with greater than 150% of normal activity [33]. The variants encountered in sub-

Saharan Africa fall in Class III (moderate deficiency, 10-60% of normal activity) and Class

IV (approaching normal to normal activity, (60-150% of normal activity). The main allelic

variants found in sub-Saharan Africa are G6PD B (wild type or normal variant), A (80%

activity), and the deficient variant A- (12% activity) [33].

The global prevalence of G6PD deficiency is most pronounced in Africa,

Mediterranean Europe, South-East Asia, Latin America, and among the black population and

the populations of Mediterranean origin in the United States [34]. In sub-Saharan Africa, the

prevalence of the G6PD A- type is estimated to range between 0 – 25%, with an average of

20% [33]. Some specific studies have reported prevalences of 22% (Gabon), 24% (Malawi),

and up to 28% (Nigeria) [9, 35, 36]. In Democratic Republic of Congo, the prevalence

estimate is 22% [37]. The high prevalence of G6PD deficiency in sub-Saharan Africa and

the global geographic distribution of G6PD deficiency can be super-posed onto that of

endemic malaria. This has led to the hypothesis that the enzyme deficiency may confer

protection against malaria [33].


**G6PD deficiency and malaria**


The hypothesis that G6PD deficiency may confer protection against malaria has been

tested through observational studies and also in vitro. In vitro studies indicate that the

biological mechanism behind a possible protective effect may take place in two ways. The

two main mechanisms though to be at play are 'abortive' infection and 'suicidal' infection. In 'abortive' infection, because of the red blood cell's inability to counter oxidative stress, there is an accumulation of various toxic substances, such as hemozoin from the malaria parasites. This accumulation then impairs parasite development and multiplication. Some in vitro studies also point to 'suicidal' infection. This can occur in two ways. One is through the formation of methemoglobin and release of ferriheme after exposure to oxidant stress. This then leads to the premature lysis of the erythrocyte. The second way is through peroxidation of lipids in the membrane of the cell. This is thought to lead to increased phagocytosis of infected cells with deficient variants of G6PD [33, 38].

Evidence concerning the role of G6PD deficiency on malaria has also come from observational studies. In a case-control study of over 2,000 children conducted in Kenya and the Gambia, G6PD deficiency, defined as carriage of the A- allele was found to be highly protective for mild malaria (odd ratio (OR) = 0.59, 95% confidence interval (CI): 0.36 – 0.94) and severe malaria (OR = 0.54, 95%CI: 0.34-0.84) [8]. A cross-sectional study conducted among 271 children in Gabon found that the prevalence of asymptomatic parasitemia among A- heterozygous females was lower than among wild type females (p = 0.03). No association was observed among male hemizygotes [39]. Another study in Gabon employing a longitudinal design among 300 children found a protective effect among females carrying A- allele for clinical malaria (p=0.026), but did not find a difference in parasitemia. Similar to the other study conducted in Gabon, no effect was observed among male hemizygotes [9].

For the purposes of this proposal, discussion has been limited to G6PD deficiency from the A- variants found in sub-Saharan Africa. However, there are studies from other

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regions of the world which have explored the G6PD deficiency and malaria hypothesis [8][33]. Although several studies have been conducted on children and some on adults, few studies have looked specifically at pregnant women. Even though the relationship between malarial and pregnancy has been recognized for some time, it is only recently that we have come to understand that the pregnant woman, especially when experiencing first pregnancy, represents a naïve host to a particular strain of *P. falciparum*. Furthermore, even with subsequent pregnancies, women in endemic areas are not constantly exposed to the strain as they are strains which infect the periphery circulation. In the literature, there is one cross-sectional study conducted among 529 pregnant women in Ghana [10].

In the Mockenhaupt et al study, carriage of the A- allele was found to be protective for parasitemia among all women (p=0.04). When stratified by gravidity, the association was observed only among multigravidae (p=0.01). When stratified by trimester, an association was observed only among women in their third trimester of pregnancy (p=0.007). Similar results were observed for anemia which was found to be more frequent among infected women compared to non-infected women (p<0.0001) and more frequent among primigravidae than multigravidae (p<0.0001) [10]. Although this study contributes much to the current literature, it is subject to several limitations. One, since a cross-sectional study was employed, risks of parasitemia and malaria were not directly estimable. Secondly, although the outcomes of parasitemia and anemia were very common in this study, the authors relied on logistic regression to obtain effect estimates. Another limitation was that exposure to G6PD deficiency was defined as carriage of the A- allele. However, it has been shown that heterozygous carriers of the allele can have normal activity since the alleles are additive for the phenotype [36]. Thus, without measuring G6PD activity, there is potential

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for misclassification error that is differential according to outcome. Another limitation is the lack of power for the study especially to detect associations with homozygous carriers of A-. In the present study, we propose to overcome some of these limitations through a longitudinal study with assessment of enzyme activity in the context of an ongoing study on malaria and pregnancy.

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# CHAPTER THREE - RESEARCH DESIGN AND METHODS

## Study design

There are two main questions upon which this study is focused. The first question concerns the effect of malaria and HIV infection on maternal anemia and birth weight. This question will be investigated using data from a cohort study conducted among pregnant women followed from the second trimester to delivery. The second question concerns the effect of G6PD deficiency on malaria, anemia and birth weight. This question will be explored through a case-cohort study in which the sample is drawn from the same cohort of pregnant women employed in the first question.

## Study population

The study population consisted of 1,496 pregnant women at <24 weeks of gestation attending the Mpemba and Madziabango Health Centres in Blantyre District, Malawi for antenatal care and delivery between March 2005 and February 2006. The average age of the women was 23.7 (standard deviation (sd) = 5.45) and about a quarter of the women were primigravid (see Table 1). The average gestational age at enrollment was 23 weeks (sd=3.6) and ranged between 14 and 28 weeks. Almost a third (31.8%) of the women had parasitemia at enrollment and 94% agreed to voluntary testing and counseling (VCT).

**Data collection**

Study nurses collected information on basic demographic characteristics, socio-economic data and malaria prevention activities from the women attending the Health Centers (see appendix for questionnaires). Women agreeing to VCT were HIV-tested on site, in addition to receiving pre- and post-test counseling. At each visit, a finger-prick blood sample was collected for each woman for thick blood film examination for malaria parasites and determination of hemoglobin level. For women delivering at the Health Centres, placental, cord and peripheral blood films were collected and examined for malaria parasites on site. Birth weight was also recorded. For women not delivering at the Health Centers, an attempt was made to identify the women and record birth weight within 24 hours of delivery. Dried blood spot samples were also prepared at enrollment and delivery. These samples were genotyped to determine carriage of the A- allele that confers G6PD deficiency in this population.

**Measurements**

**Parasitemia**

Malaria was assessed through microscopic examination of thick blood smear slides on site by trained laboratory technicians after collecting finger prick blood samples from the participating women. Malaria parasites were quantified against 200 white blood cells (WBC). For quality control purposes, a 10% random sample of slides were re-examined by the laboratory supervisor at Ntcheu District Hospital.

**HIV**

Among women agreeing to VCT, HIV infection was assessed using two rapid HIV-1 antibody tests: Determine and Unigold. There was 95% (95% confidence interval: 93.2 – 97.8%) agreement between the two tests in identifying HIV positive women.

**G6PD deficiency**

A 30% simple random sample was drawn from the cohort of women followed until delivery who had filter paper blood spots available. Additionally, all women with malaria at delivery for whom filter paper blood spots were collected were also identified. Carriage of the A- allele was assessed through genotyping of the blood spots according to a previously published method [1].

**Maternal anemia**

Hemoglobin levels were estimated from the finger prick blood samples using HemoCue on site at each visit.

**Birth weight**

For women delivering at the Health Centres, birth weight was assessed after birth using simple spring balances. Through Community Nurses and Traditional Birth Attendants, an attempt was made to identify women not delivering at the Health Centres in order to obtain the birth weight within 24 hours of delivery.

Table 1. Characteristics of the study population

| | | Parasitemia | |
| Variable | All (N=1,496) | Yes (N=476) | No (N=1020) |
|---|---|---|---|
| Maternal age (years), mean ± SD | 23.7 ± 5.5 | 21.5 ± 4.8 | 24.7 ± 5.4 |
| Education ≤ 8 years (%) | 86.3 | 88.2 | 85.4 |
| Married (%) | 91.9 | 90.1 | 92.7 |
| Unemployed (%) | 52.7 | 56.3 | 51.1 |
| Reported fever in previous week (%) | 11.9 | 12.6 | 11.6 |
| Previous antimalarials in this pregnancy (%) | 9.6 | 6.7 | 10.9 |
| Report always using bednet (%) | 21.5 | 15.1 | 24.5 |
| Report insecticide impregnation of bednet in past 6 months (%) | 19.9 | 13.9 | 22.7 |
| Gravidity (%) | | | |
| Primigravid | 24.7 | 45.4 | 15.5 |
| Secundagravid | 23.2 | 24.4 | 38.1 |
| Multigravid (≥3 pregnancies) | 52.1 | 30.2 | 46.4 |
| Gestational age (weeks), mean ± SD | 23.3 ± 3.6 | 22.8 ± 3.5 | 23.5 ± 3.6 |
| Weight (kg), mean ± SD | 54.7 ± 6.8 | 53.9 ± 6.4 | 55.1 ± 6.9 |
| HIV seropositive (%) | 14.1 | 16.9 | 12.8 |
| Hemoglobin (g/dL), mean ± SD | 11.0 ± 1.6 | 10.5 ± 1.6 | 11.25 ± 1.6 |
| Hemoglobin ≤ 10g/dL (%) | 23.22 | 34.5 | 17.7 |
| Hemoglobin ≤ 7g/dL (%) | 1.82 | 1.9 | 1.8 |

**Data analysis**

**Specific Aim 1.** What is the effect of malaria and HIV infection during pregnancy on maternal anemia and birth weight?

Exposure definitions

It is of interest to determine how malaria affects pregnancy outcomes while accounting for HIV infection, gravidity and other confounders (see Figure 1 for conceptual model). Malaria is defined in various ways, and each way has implications for the type of analysis required. Pregnancy outcomes may be affected by parasitemia at delivery, parasitemia throughout the pregnancy, number of episodes of parasitemia throughout the

pregnancy, parasite density at delivery, and parasite density of malaria episodes throughout the pregnancy. Parasitemia can be conceived of as a dichotomous variable where the subject either has or does not have parasitemia. Parasite density is a semi-continuous variable calculated from the number of parasites observed through microscopic examination of thick blood smears (see Table 2 for variable specifications).

Outcome definitions

In a manner similar to malaria, low birth weight and maternal anemia may also be dichotomized or treated as continuous variables (see Table 2 for variable specification).

Analysis plan

**Objective 1.** To determine the separate effects of HIV infection and malaria on maternal anemia and low birth weight

Binomial regression will be employed to estimate the relative risks for the separate (main) effects of HIV infection and parasitemia and parasite density at delivery on dichotomized low birth weight and moderate/severe anemia at delivery, adjusting for important covariates (see Figure 1 and Table 2). The effect of HIV infection and parasitemia and parasite density at delivery on continuous birth weight and hemoglobin levels at delivery will be assessed through linear models, adjusting for important covariates.

**Objective 2.** To assess interaction between the effects of HIV infection and malaria on maternal anemia and low birth weight.

Interaction on a multiplicative scale will be assessed by introducing an interaction term between HIV and parasitemia/parasite density in the above models. Additive interaction (for dichotomous outcomes) will be assessed through computation of relative excess risk due to interaction (RERI) [2].

**Specific Aim 2.** What is the effect of HIV infection on parasitemia risk at enrollment, during follow-up and at delivery?

**Objective 1.** To describe the pattern of parasitemia risk over the second and third trimester.

At each visit, the proportion of women with parasitemia according to gravidity and HIV status will be computed and presented in a graph. Graphical analysis will be employed to describe the pattern of parasitemia over the follow-up visits.

**Objective 2.** To estimate the effect of HIV on the risk of parasitemia and anemia at enrollment, over the follow-up period and at delivery.

Binomial regression will be employed to determine the effect of HIV infection on parasitemia at enrollment, anytime over the follow-up period and at delivery. Generalized estimating equations (GEE) will be used to determine the effect of HIV infection on parasitemia throughout the pregnancy, adjusting for demographic and socioeconomic variables, including education. GEE models are appropriate for clustered outcomes; however,

they are not robust to data not missing completely at random (MCAR). Hence, GEE models will be weighted with inverse-probability-of-missingness weights, using information obtained from the demographic and socioeconomic questionnaire to build the missingness models[3].

**Objective 3.** To determine the relationship between HIV infection and frequency and severity of parasitemia at delivery.

Polytomous logistic regression will be used to determine the effect of HIV infection on the frequency of parasitemia episodes. Since frequency is necessarily ordinal, proportional odds logistic regression will be most appropriate. However, if the proportional odds assumption is not met, generalized logistic regression with robust standard errors will be employed. The effect of HIV infection on parasite density will be assessed using zero-inflated negative binomial regression to account for excess zeros, since most women will not have parasitemia, and also to account overdispersion of parasite density.

**Specific Aim 3.** What is the relationship between carriage of the G6PD A- allele and parasitemia and anemia among pregnant women?

<u>Exposure definition</u>

Since the study population is a cohort of women and the gene for G6PD is on the X-chromosome, G6PD deficiency can be defined as a categorical variable with three values, homozygous carriage, heterozygous carriage, and non-carriage of the mutant A- allele.

Outcome definitions

Outcome definitions same as for specific aim 1.

Analysis Plan

A case- cohort design was employed to address the second specific aim. To estimate the effect of G6PD deficiency on birth weight and maternal anemia, analyses similar to those used for the first specific aim will be conducted among the 30% sub-cohort. In order to determine the effect of G6PD deficiency on parasitemia/parasite density, binomial regression will be employed modeling the probability of carriage of the A- allele among cases versus carriage among the subcohort. Binomial regression is appropriate especially for parasitemia since it is a common outcome in this population [4]. GEE will be used to determine the effect of G6PD deficiency on parasitemia and maternal anemia over follow-up, GEE will be used.
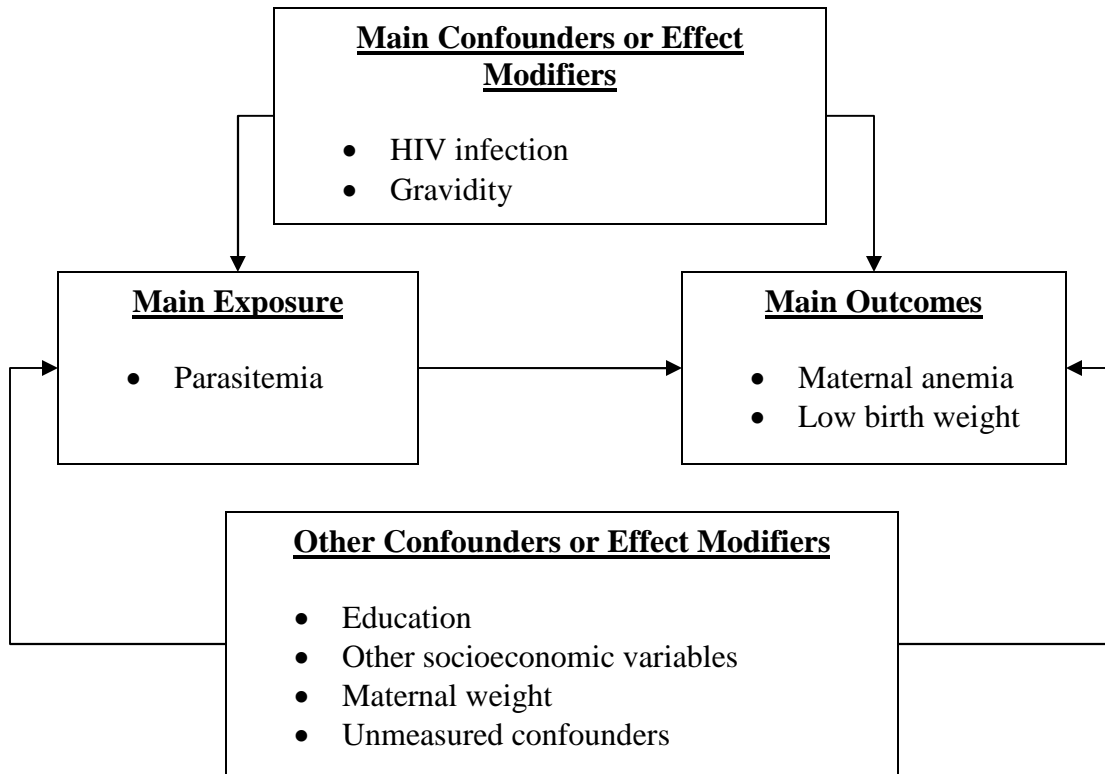
Figure 1. Causal model for specific aim 1.
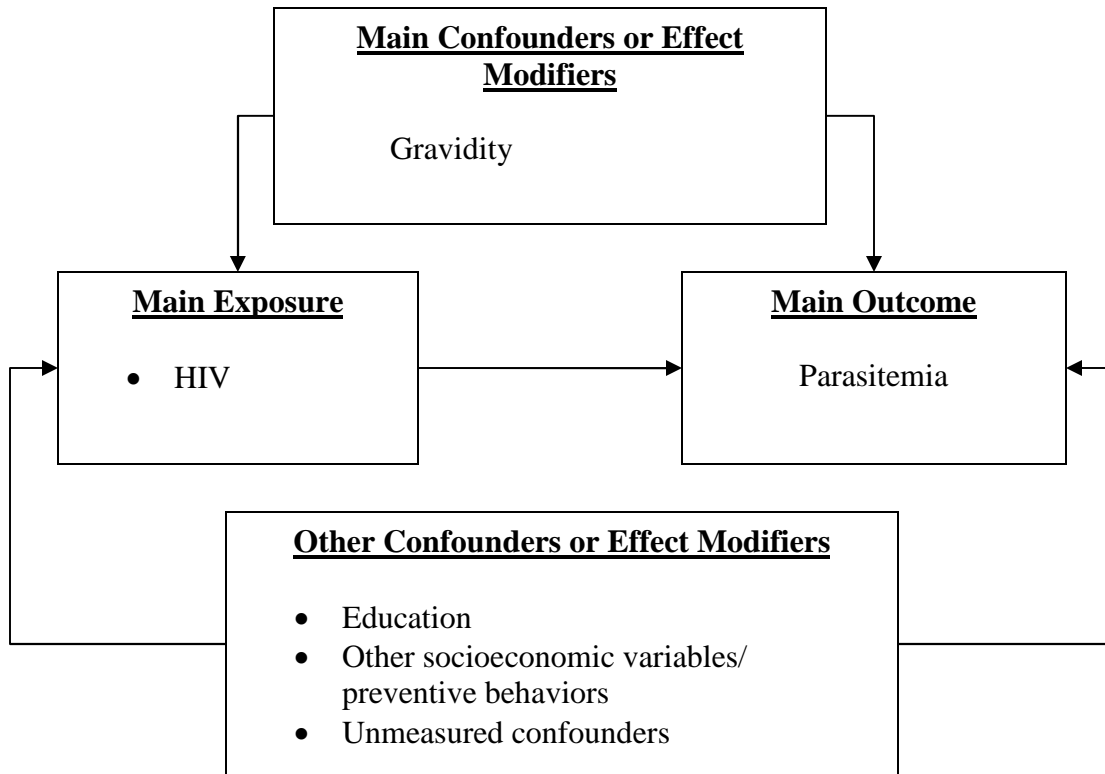
Figure 2. Causal model for specific aim 2.
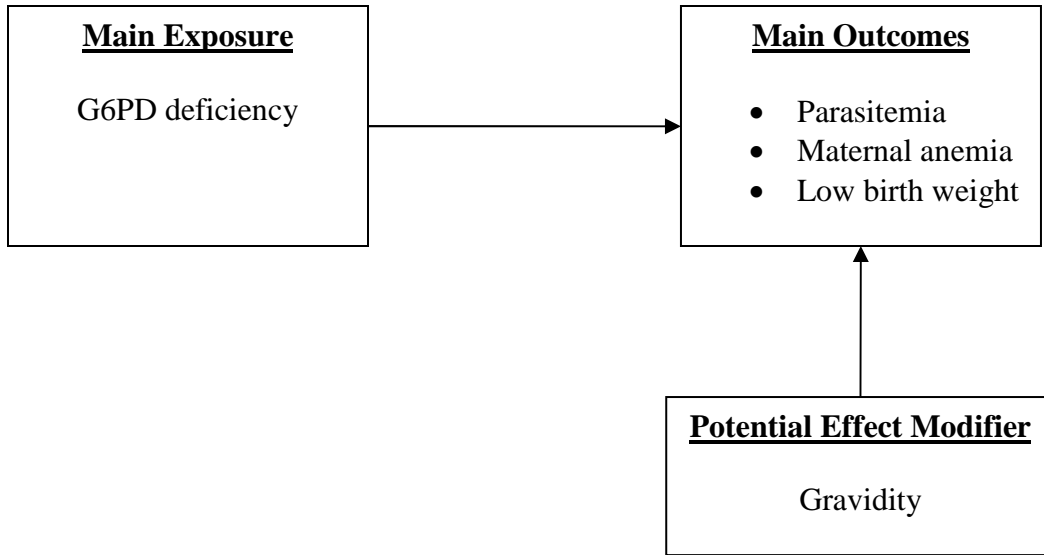
Figure 3. Causal model for specific aim 3.

Table 2. Variable specification

| Variable | Type | Description |
|---|---|---|
| **Exposures** | | |
| Parasitemia at delivery | Binary | 0 = No parasites detected, 1 = parasites detected |
| Parasitemia at visit | Binary | 0 = No parasites detected, 1 = parasites detected |
| Parasite density at delivery | Continuous | Parasite count per microliter, assuming 6000 WBC/μL |
| Parasite density at visit | Continuous | Parasite count per microliter, assuming 6000 WBC/μL |
| Visit | Categorical | 1=first visit, 2=second visit, etc |
| G6PD deficiency | Categorical | 0 = normal, 1 = heterozygous, 2 = homozygous |
| | | |
| **Outcomes** | | |
| Birth weight | Continuous | Measured in grams (g) |
| Low birth weight | Binary | 0 = birth weight ≥ 2500g, 1 = birth weight < 2500g |
| Hemoglobin at delivery | Continuous | Measured in grams per deciliter (g/dL) |
| Hemoglobin at visit | Continuous | Measured in grams per deciliter (g/dL) |
| Anemia at delivery | Binary | 0 = Hb ≥ 10g/dL, 1 = Hb < 10g/dL |
| Anemia at visit | Binary | 1 = Hb ≥ 10g/dL, 1 = Hb < 10g/dL |
| Severe anemia at delivery | Binary | 0 = Hb ≥ 7g/dL, 1 = Hb < 7g/dL |
| Severe anemia at visit | Binary | 1 = Hb ≥ 7g/dL, 1 = Hb < 7g/dL |
| | | |
| **Covariates** | | |
| HIV | Binary | Determined by either Determine or Unigold Test |
| Gravidity | Continuous | Number of pregnancies carried |
| Primigravid | Binary | 0 = second or later pregnancy, 1 = first pregnancy |
| Gravidity Category | Ordinal | 1 = Primigravid, 2 = Secundagravid, 3 = >2 pregnancies |
| Education | Ordinal | 1 = none, 2 = primary, 3 = secondary or higher |
| Marital Status | Binary | 0 = Currently married, 1= Currently not married |
| Mother's occupation | Categorical | 1 = none, 2 = vendor, 3 = works for a wage |
| Husband's occupation | Categorical | 1 = none, 2 = vendor, 3 = works for a wage |
| Maternal weight | Continuous | Measured in kilograms (kg) |
| IPT received | Ordinal | 1 = one dose, 2 = 2 doses, 3 = 3 or more doses |
| | | |

**Strengths and limitations of methods**

The aim of the proposed study is to estimate the effects of malaria, HIV and G6PD

deficiency on pregnancy outcomes in a cohort of pregnant women in southern Malawi.  This

is an important and timely question, particularly in the development of new anti-malarial drugs. The population of women sampled is appropriate for this study given the underlying prevalence of HIV, G6PD deficiency and malaria. Furthermore, since Malawi has over 95% ANC coverage, by sampling from women attending ANC clinics, the cohort approximates a population-based sample [5]. This enhances the generalizability of the obtained results. Furthermore, collecting information on various socioeconomic variables lends strength to the study by providing a more complete picture of the context in which the disease occurs and allowing for a more thorough examination of factors that can confound or modify the effects of interest. The longitudinal study design helps in assessing risk over the pregnancy. This is especially important as many studies in the literature have been limited to effects at delivery or the third trimester.

Despite these strengths, there are a number of limitations to be recognized. One of the main limitations concerns the ability to minimize loss to follow-up. In this population of women, delivery frequently occurs at the home, thus making it difficult to collect birth weight and other information at delivery. This may reduce the generalizability of the study and introduce bias, particularly if women who are lost to follow-up are different from women who remain in the study until delivery. To address this limitation, the probability of missingness for the outcome will be modeled in order to understand the factors contributing to loss-to-follow up and adjust analyses appropriately.

Another limitation, particularly for the third aim is that given the sample size, there will most likely be insufficient statistical power to detect effects among homozygous carriers. However, the number of homozygous carriers is usually small in most populations and would thus have a minimal impact on population attributable fraction of protection by G6PD

deficiency.  The study site and population also present some limitations.  The population is ethnically diverse, and by extension, is genetically heterogeneous.  This admixture may present unequal distributions of other genetic 'responses' to endemic malaria (e.g. HbS, α/β-Thalassemia, etc.) that may be differential by outcome.  However, since these other characteristics have not been shown to be related to G6PD deficiency, they would not confound the results but may contribute to within- subjects error in the statistical models.

A final consideration involves the study population and is a limitation inherent to most studies on pregnancy.  Since women usually have their first ANC clinic visit during the second trimester, women with very early pregnancy loss may be missed.  There is some indication that HIV, G6PD deficiency and malaria may independently contribute to pregnancy loss [6, 7].  Hence this may reduce generalizability since the women who do enroll are women who had sufficiently healthy pregnancies to reach the second and third trimester.

Despite these limitations, the proposed study has a number of strong points and will add significantly to the literature on HIV, G6PD deficiency and malaria during pregnancy.  One, the study will be conducted in a population of women living in a malaria endemic area among which there is a high frequency of both HIV infection and G6PD deficiency.  Two, because the study employs a prospective design, it will allow for the direct estimation of risk and will allow for the observation of temporality between the outcome and important confounding or modifying factors, an advantage not afforded by cross-sectional studies conducted at delivery.  Additionally the inclusion of socioeconomic variables will enhance understanding of the relationship between HIV, malaria and G6PD deficiency on pregnancy outcomes in terms in proximal and distal causal mechanisms.

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# CHAPTER FOUR - THE EFFECT OF HIV INFECTION AND *PLASMODIUM FALCIPARUM* PARASITEMIA ON PREGNANCY OUTCOMES IN SOUTHERN MALAWI

## Abstract

**Background.** *Plasmodium falciparum* parasitemia and HIV are important independent risk factors for low birth weight and maternal anemia. The two infections may interact to increase risk of adverse pregnancy outcomes.

**Methods.** Between 2005 and 2006, we followed pregnant women enrolled between 14-28 weeks gestation attending two antenatal care clinics in southern Malawi until delivery.

**Results.** Among 831 pregnant women followed until delivery, the prevalence proportions of HIV and placental parasitemia were both 13%. HIV was associated with increased risk of LBW (adjusted prevalence ratio, $PR_{adj}=3.08$, 95% confidence interval, CI, = 1.40, 6.79) and a 138g reduction in birth weight. Placental parasitemia was associated with an elevated risk of LBW ($PR_{adj}=1.79$, 95% CI = 0.83, 3.84), as was having $\geq 3$ episodes of peripheral parasitemia during follow-up ($PR_{adj}=2.68$, 95% CI = 1.06, 6.79). Among multigravidae, dual infection resulted in 9.59 (95% CI: 2.51, 36.6) times the risk of LBW when compared to multigravidae with neither infection. HIV infection was associated with increased risk of anemia (adjusted odds ratio, $OR_{adj} = 2.12$, 95% CI: 1.09, 4.14), as was placental parasitemia ($OR_{adj} = 3.60$, 95% CI = 1.95, 6.64). There was no interaction in the effect of HIV and placental parasitemia on anemia risk. **Conclusions.** HIV infection and parasitemia are important independent risk factors for adverse pregnancy outcomes. Among multigravidae, HIV infection and placental

parasitemia may interact to produce an impact greater than the sum of their independent effects. These results underscore the need for malaria prevention strategies that appropriately target pregnant women across gravidities.

**Introduction**

Malaria is a tremendous public health challenge in Malawi, with almost 3 million reported cases, resulting in 7,000 deaths annually [1]. Each year in Malawi, there are approximately 500,000 pregnancies all at risk for malaria [2]. For these women, infection with *Plasmodium falciparum* parasites may result in adverse pregnancy outcomes including malaria-associated infant deaths [3, 4]. These adverse outcomes are mediated through placental malaria, a condition associated with intrauterine growth retardation, prematurity, and severe maternal anemia [5, 6]. These conditions can lead to low birth weight (LBW), one of the most important risk factors for neonatal mortality and developmental impairment [7, 8].

HIV prevalence in Malawi is also high, with an estimated 12% of antenatal care clinic attendees affected [9]. HIV infection has also been found to be associated with adverse pregnancy outcomes, such as infant mortality and LBW [10, 11]. The increased risk of malaria coupled with HIV infection may interact to cause adverse pregnancy outcomes more pronounced than would be expected with HIV infection or malaria alone [12]. Studies conducted in Malawi, Tanzania, and Kenya have reported increased risk of neonatal mortality, maternal anemia and LBW in the presence of dual infection by malaria parasites and HIV, versus malaria parasitemia or HIV alone [13-15]. However, previous studies have

relied on a cross-sectional design, determining malaria parasitemia status at single points during pregnancy.  In this study, we evaluate the effects of HIV and malaria parasitemia during pregnancy on maternal anemia and LBW among a cohort of pregnant women in Malawi.

**Methods**

*Study population and data collection procedures*

The study was conducted at the Mpemba and Madziabango Health Centres in a rural area of Blantyre District, Malawi. Between March 2005 and February 2006, healthy pregnant women in their second trimester attending the antenatal care clinics at the study sites were invited to participate .  At enrollment, study nurses collected information on basic demographic characteristics, socio-economic data and malaria prevention activities from participating women.  Women agreeing to voluntary counseling and testing (VCT) were tested for HIV on site, in addition to receiving pre- and post-test counseling.  HIV-infected women and their infants were given nevirapine according to national guidelines. Additionally, women found to be infected with HIV were referred to an antiretroviral treatment program.  Women attending the antenatal care clinics were administered sulfadoxine-pyrimethamine (SP) for intermittent presumptive therapy in pregnancy (IPTp) and treated for clinical malaria also according to national guidelines.

Women attended visits according to the standard antenatal care guidelines with visits scheduled after enrollment occurring at approximately 26, 32 and 36-38 weeks of gestation. At each visit, a finger prick blood sample was collected for thick blood film examination for malaria parasites and determination of hemoglobin level.  For women delivering at the

Health Centres, placental, cord and peripheral blood films were collected and examined for malaria parasites on site. Placental biopsies were not collected. Birth weight, measured with simple spring balances, was also recorded. For women not delivering at the Health Centers, an attempt was made to identify the women and record birth weight within 24 hours of delivery.

*Laboratory procedures*

Peripheral malaria parasitemia was assessed through microscopic examination of thick blood smear slides on site by trained laboratory technicians. Malaria parasites were quantified against 200 white blood cells (WBCs). Placental parasitemia was also assessed through thick blood smear. For quality control, a 10% random sample of slides were re-examined by the laboratory supervisor at Ntcheu District Hospital. HIV infection was assessed using two rapid HIV-1 antibody tests: Determine® and Unigold™. There was 95% (95% confidence interval: 93.2 – 97.8%) agreement between the two tests in identifying HIV positive women. CD4+ cell counts were not evaluated. Hemoglobin levels were estimated from the finger prick blood samples using HemoCue on site at each visit and after partum.

*Definitions*

Parasitemia was defined as the presence of parasites in thick blood smears. Parasite density per μL was computed assuming 6,000 WBC/μL of blood. Among women with placental parasitemia, mild parasitemia was defined as <20,000 parasites/μL, and severe parasitemia as ≥ 20,000 parasites/μL. Peripheral parasitemia over follow-up was defined as the number of episodes of parasitemia over the follow-up visits. Since we could not

distinguish between recrudescence and reinfection, measurements of parasitemia were assumed to be independent across visits. Malaria parasitemia at delivery was defined as peripheral and/or placental parasitemia upon delivery. Fever was rarely observed, and was thus not included in the definition of parasitemia. Anemia at delivery was defined as hemoglobin < 11g/dL. HIV infection was defined as a positive result on two rapid tests: Determine® and Unigold™. Discordant results were excluded from analyses. Neonates were considered as having LBW if they weighed less than 2500g. For the sociodemographic characteristics, unsafe water sources were defined as unprotected wells, lakes, rivers or ponds. Low housing quality was defined as housing with grass roofs and mud or grass walls with open unscreened windows.

*Statistical analysis*

Bivariate and multivariate analyses for dichotomous outcomes were conducted using binomial regression. When the binomial regression model was unstable, logistic regression was used. Continuous birth weight and hemoglobin were analyzed using general linear models.

In order to assess confounding for dichotomous outcomes, bivariate analyses with potential confounding variables were performed for both the outcomes and the main exposures. In the case of binary variables, if the resultant relative risks were less than or equal to 0.7 or greater than or equal to 1.3, they were considered as potential confounders. The number of potential confounders included in final models was further narrowed using a change-in-estimate approach with a 10% cut-off. Covariate inclusion for continuous outcomes models mirrored that of the binary outcomes models. Interaction effects were assessed through the inclusion

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of interaction terms in the models and using the spreadsheet by Andersson and colleagues to determine the relative excess prevalence due to interaction (REPI) [16]. All analyses were performed using SAS v9.1 (Cary, North Carolina).

*Ethical considerations*

Informed consent was obtained from all participating women in Chichewa. The study was reviewed and approved by the institutional review boards at the University of North Carolina at Chapel Hill and the University of Malawi College of Medicine.

**Results**

The study enrolled 1,496 women, of whom 831 completed follow-up until delivery with 590 delivering at the health centers. Descriptive characteristics of the study population are reported in Table 1. On average, participating women attended three follow-up visits between enrollment and delivery. The average age of the women was 23.4 years (standard deviation (SD) = 5.4). The average gestational age at enrollment was 23 weeks (SD=3.6) and ranged between 14 and 28 weeks. The prevalence of HIV was 13%. There were no significant differences in the main exposures and sociodemographic characteristics between women who were lost to follow-up and women who completed follow-up. There was, however, a difference in maternal weight, with women not lost to follow-up being on average 0.81 kg heavier than women lost to follow-up. There were no significant differences in the main variables  among women delivering at the health centers versus women delivering

elsewhere, except for peripheral parasitemia, with women delivering elsewhere slightly more likely to have peripheral parasitemia than women delivering at the health centers (PR = 1.11, 95% CI: 1.06, 1.17).

### *Low birth weight*

The incidence of LBW was 9%, and mean birth weight was 3055g (SD=478).  In bivariate analyses, HIV infection, placental parasite density ≥20,000/µL, and having ≥3 episodes of peripheral parasitemia over follow-up were strongly associated with LBW (Table 2).  Placental parasitemia was marginally associated with LBW in both bivariate and multivariate analyses. Further, only HIV infection and having ≥3 episodes of peripheral parasitemia over follow-up were strongly associated with LBW after adjusting for potential confounders (Table 2).  Additionally, the risk of LBW among primigravidae was nearly two times the risk among multigravidae, after adjusting for maternal weight, unsafe water source, gravidity and HIV status (Table 2).

Multivariate analyses of mean birth weight revealed a 138g reduction in birth weight by HIV infection (Table 2).  Placental parasitemia at delivery did not result in a notable reduction in birth weight; however, the mean difference for experiencing peripheral parasitemia ≥3 times over follow up was large (-163, 95% CI: -351 to 26). Decreasing number of SP IPTp doses received was associated with decreasing birth weight (p <0.01, Table 2). Being primigravid was associated with a 208g decrease in mean birth weight (Table 2).

*Maternal anemia*

HIV infection and placental and peripheral parasitemia were associated with an increased risk of maternal anemia and reductions in mean hemoglobin, with placental parasitemia having the strongest effect (Table 3). Maternal anemia risk varied with the degree of parasite density with a parasite density ≥20,000 parasites/µL resulting in the highest increased risk and the greatest reduction in mean hemoglobin. Maternal anemia risk was also associated with number of parasitemia episodes. Women who experienced 2 or more parasitemia episodes had an increased anemia risk and significant reductions in mean hemoglobin. Compared to women who received ≥3 doses of SP IPTp, receiving only 1 dose was associated with an elevated unadjusted risk of maternal anemia, but there were no significant differences in anemia risk according to doses of SP received after multivariate adjustment (Table 3).

*Interaction between effects of HIV and parasitemia on pregnancy outcomes*

No significant interaction effects between HIV and peripheral or placental parasitemia were found during multivariate analyses for both LBW and maternal anemia when all gravidities were analyzed together. The overall relative excess prevalence due to interaction (REPI) for LBW was 6.72 (95% CI: -3.65, 17.08). The REPI indicates the amount of observed prevalence that is beyond the sum of the independent effects of HIV and placental parasitemia. However, after stratifying by gravidity, the odds of LBW among multigravidae with both HIV infection and placental parasitemia were 9.6 (95% CI: 2.5, 36.6) times as high as the odds among women with neither infection (Table 4).

Even after stratifying by gravidity, there was no evidence of interaction for the joint effects of HIV infection and placental parasitemia on maternal anemia risk (Table 4). However, the reduction in mean hemoglobin was significantly higher among dually infected multigravidae than in multigravidae with neither infection. Among primigravidae, women with dual infection had the highest absolute reduction in hemoglobin levels, but the estimated difference was imprecise (Table 4).

**Discussion**

In this cohort of pregnant women HIV infection was associated with LBW and maternal anemia. Both peripheral and placental parasitemia at delivery were associated with maternal anemia and LBW, with a stronger effect observed on maternal anemia. Additionally, having ≥3 episodes of parasitemia over follow-up was associated with increased LBW risk. Among multigravidae, there was some evidence of superadditive interaction between HIV infection and placental parasitemia at delivery in their joint effects on LBW. No interaction effects were observed for maternal anemia.

*Low birth weight*

HIV infection has previously been associated with an increased risk of LBW [10, 17]. HIV-infected mothers in Kenya gave birth to infants weighing on average 99g less than infants born to HIV-uninfected mothers, but this reduction did not translate into a significant increase in the risk of LBW [13]. In this study, we found a 138g reduction in mean birth weight and a significant increase in the risk of LBW by HIV infection after adjusting for

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gravidity and placental parasitemia. Consistent with previous studies, placental parasitemia was found to be associated with elevated risk of LBW[18]. Peripheral parasitemia at delivery, however, was not significantly associated with LBW.

A high parasite density (>20,000 parasites per μL) was associated with a substantial reduction in mean birth weight. These high parasitemias were rarely accompanied by fever. In Zimbabwe, the risk of LBW among pregnant women with symptomatic malaria was 10 (95%CI: 6.50, 15.65) times that of women without malaria [17]. Taken together, these studies suggest that risk of LBW may be influenced by the severity of the malaria infection as indicated by degree of parasitemia and/or resulting morbidity.

Our data suggest that the number of episodes of parasitemia is a key predictor of risk of LBW. Women who had ≥3 episodes of peripheral parasitemia over follow-up had a significantly increased risk (PR 2.68; CI 1.06-6.89) of LBW. We are only aware of one other published study which examines the relationship between frequency of parasitemia and birth outcomes. In the Democratic Republic of Congo, Landis and colleagues reported that women having ≥3 infections were at increased risk of intrauterine growth retardation [19]. Recently, in a similar population in Malawi, a significantly increased risk of LBW and maternal anemia was found in women experiencing ≥2 parasitemia episodes during follow up (Kalilani L, Mofolo I, Chaponda M, Rogerson SJ, Meshnick, SR; The effect of timing and frequency of *Plasmodium falciparum* infection during pregnancy; submitted). This study also reports an attenuated increase in risk due to one episode; however, number of episodes includes parasitemia at delivery, whereas in the current study number of episodes excludes parasitemia at delivery. These results suggest that parasitemia throughout the gestation

period, and not only at delivery should be considered when assessing the effect of malaria on LBW risk.

Similar to previous studies, we did not detect any interaction between HIV infection and placental parasitemia in the effect on LBW overall [13, 20]. In the study conducted in Kenya, among HIV infected primigravidae, parasitemic women had 2.5 (95% CI: 1.0, 5.9) times the risk of LBW compared to aparasitemic HIV-infected women [13]. In contrast, in our study, in stratified analyses, among primigravid women, neither infection increased the risk of LBW in the presence of the other. However, among multigravid women with placental parasitemia, HIV-infected women had almost 10 times the risk of LBW when compared to HIV-uninfected parasitemic women. Although the confidence limit ratio of this estimate was 15 indicating imprecision, the magnitude of the estimate is strongly indicative of superadditive interaction. Furthermore, at the specified alpha-level of 0.10, the interaction term among multigravidae reaches statistical significance, further adding to the evidence of interaction.


*Maternal anemia*

HIV and peripheral and placental parasitemia were strong independent risk factors for maternal anemia at delivery and corroborating previous literature. In the study by Van Eijk and colleagues, malaria and HIV were associated with a slightly elevated risk of anemia among all gravidities [21]. In the study conducted in Malawi by Rogerson and colleagues, both placental and peripheral parasitemia were associated with anemia (peripheral parasitemia OR=1.85, 95% CI: 1.45, 2.36; placental parasitemia OR = 2.0, 95% CI: 1.5, 2.7), where placental parasitemia was determined through histological examination [22]. In a

similar population, 2 or more episodes of parasitemia have been associated with a doubling

of maternal anemia risk (Kalilani L, Mofolo I, Chaponda M, Rogerson SJ, Meshnick, SR;

The effect of timing and frequency of *Plasmodium falciparum* infection during pregnancy;

submitted). In the study conducted in Kenya by Ayisi and colleagues, however, among

primigravidae, only HIV infection was independently associated with anemia (defined as Hb

< 8g/dL).  We saw few women with such profound anemia. Among Kenyan multigravidae,

no independent effects of HIV and parasitemia were detected; however, co-infection was

strongly associated with increased risk of anemia [13].  These differences in results may be

due to the differing definitions of maternal anemia.


In contrast to the study in Kenya, we did not detect any interaction between HIV and

peripheral or placental parasitemia in their effect on maternal anemia at delivery among both

primigravidae and multigravidae [13].  Although the risk of anemia was highest among

dually infected women, the estimate for the joint effect was not greater than the additive

effects of HIV infection and placental parasitemia only. Furthermore, primigravidae

exhibited a significant reduction in hemoglobin levels only among women with placental

parasitemia in the absence of HIV infection.  Among multigravidae, we found no indication

of interaction between HIV infection and placental parasitemia in their effect on mean

hemoglobin levels. These differences in the influence of both HIV and malaria on maternal

anemia risk may be due to differing distributions of HIV infection and gravidity differing

definitions of anemia, and the multi-factorial nature of the etiology of anemia.

*Limitations*

The interpretation of these results is subject to some limitations. One, placental parasitemia was determined through placental blood film instead of histological examination, a more sensitive method [22-24]. However, this would have resulted in misclassification that would have biased the estimates towards the null. Two, we did not have information on CD4+ counts or other clinical measures of the stage of HIV infection. Given the association of HIV infection with age, this may have influenced the effects of HIV differentially according to gravidity. Three, birth weight was available for approximately 70% of the women. While there were no significant differences in HIV status, malaria preventive behaviors and demographic variables between women delivering at the health centres and those delivering elsewhere, women delivering at the health centres were more likely to have peripheral parasitemia. This may limit the generalizability of the results to women delivering at the health centres versus women in the catchment area of the health centres. A fourth limitation concerns the multiple factors contributing to maternal anemia. In this study, we did not assess nutritional status and the presence of other parasitic infections associated with anemia risk. However, we were able to adjust for variables linked to anemia, such as socioeconomic factors and maternal weight.

*Conclusions*

This study confirms the deleterious effects of HIV infection and parasitemia during pregnancy. We report an increase in LBW risk due to multiple parasitemia episodes. Further, we found evidence of interaction between HIV infection and placental parasitemia in increasing risk of LBW among multigravidae. Contrasting previous results, we found no

evidence of interaction between HIV infection and placental parasitemia on maternal anemia risk. These results underscore the need for continued research to understand the role of HIV in contributing to adverse pregnancy outcomes in malaria-endemic areas. Furthermore, the differential effects according to gravidity highlight the importance of targeted malaria prevention programs to ensure that the benefits of protective measures, whether through IPTp or insecticide-treated nets, accrue to all pregnant women.

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# Tables

Table 1. Characteristics of the study population

| | All women | | Primigravida | | Multigravida | |
|---|---|---|---|---|---|---|
| | N* | % | N | % | N | % |
| *Overall* | *831* | *100%* | *235* | *28.4* | *592* | *71.6* |
| Age | | | | | | |
| 15 - 19 | 227 | 27.3 | 186 | 79.2 | 40 | 6.8 |
| 20 - 24 | 286 | 34.4 | 43 | 18.3 | 241 | 40.7 |
| ≥ 25 | 318 | 38.3 | 6 | 2.5 | 311 | 52.5 |
| Education ≤8 years | 686 | 82.7 | 174 | 74.0 | 508 | 86.0 |
| Married | 757 | 91.3 | 184 | 78.6 | 569 | 96.3 |
| Unemployed | 433 | 52.1 | 144 | 61.3 | 287 | 48.5 |
| Unsafe water source | 197 | 23.7 | 48 | 20.4 | 148 | 25.0 |
| Low housing quality | 199 | 24.0 | 59 | 25.2 | 138 | 23.4 |
| Report always using bednet | 203 | 24.4 | 40 | 17.0 | 163 | 27.5 |
| Report insecticide impregnation of bednet in past 6 months | 182 | 21.9 | 32 | 13.7 | 150 | 25.3 |
| Weight (kg), mean ± SD | 828 | 55.1 ± 6.9 | 235 | 54.4 ± 5.8 | 589 | 55.4 ± 7.3 |
| HIV seropositive | 102 | 13.5 | 14 | 6.5 | 88 | 16.5 |
| Placental parasitemia | 111 | 13.4 | 50 | 21.3 | 60 | 10.1 |
| Peripheral parasitemia | 112 | 13.5 | 49 | 20.9 | 62 | 10.5 |
| No. times parasitemic over pregnancy | | | | | | |
| 0 | 444 | 53.4 | 69 | 29.4 | 372 | 62.8 |
| 1 | 237 | 28.5 | 77 | 32.8 | 159 | 26.9 |
| 2 | 84 | 10.1 | 46 | 19.6 | 38 | 6.4 |
| ≥3 | 66 | 7.9 | 43 | 18.3 | 23 | 3.9 |
| SP doses received | | | | | | |
| 0 | 34 | 4.1 | 8 | 3.4 | 26 | 4.4 |
| 1 | 156 | 18.8 | 35 | 14.9 | 121 | 20.4 |
| 2 | 329 | 39.6 | 89 | 37.9 | 239 | 40.4 |
| ≥3 | 312 | 37.6 | 103 | 43.8 | 206 | 34.8 |
| Hemoglobin (g/dL), mean ± SD | 732 | 12.3 ± 1.7 | 196 | 12.3 ± 1.8 | 532 | 12.3 ± 1.7 |
| Hemoglobin <11g/dL | 65 | 8.9 | 17 | 8.7 | 48 | 9.0 |
| Low birth weight (<2500g) | 49 | 8.7 | 20 | 12.7 | 28 | 7.0 |
| Birth weight (grams), mean ± SD | 585 | 3055 ± 478 | 168 | 2902 ± 447 | 415 | 3118 ± 476 |

SD = standard deviation, g/dL= grams per deciliter, kg=kilograms
*Sums may not add up to 831 because of missing values

Table 2.  Predictors of risk of low birth weight and mean birth weight

| Variable | <2500g (N=49) n (%) | ≥ 2500g (N=517) n (%) | Crude prevalence ratio (95% CI) | Adjusted prevalence ratio* (95% CI) | Adjusted mean birth weight (g) difference** (95% CI) |
|---|---|---|---|---|---|
| HIV† | 12 (26.7) | 54 (11.5) | 2.32 (1.34 - 4.00) | 3.08 (1.40 - 6.79) | -138 (-250 to -26) |
| Placental parasitemia at delivery | 12 (24.5) | 75 (14.7) | 1.67 (0.98 - 2.85) | 1.79 (0.83 - 3.84) | -36 (-137 to 66) |
| Peripheral parasitemia at delivery | 12 (24.5) | 77 (15.0) | 1.63 (0.96 - 2.77) | 0.58 (0.27 - 1.25) | -59 (-42 to 159) |
| No. of times parasitemic over follow-up | | | | | |
| 0 | 23 (46.9) | 276 (53.4) | Reference | Reference | Reference |
| 1 | 13 (26.5) | 151 (29.2) | 1.03 (0.51 - 2.10) | 0.90 (0.41 - 1.98) | 6 (-110 to 122) |
| 2 | 2 (4.1) | 53 (10.3) | 0.45 (0.10 - 1.98) | 0.34 (0.07 - 1.59) | 35 (-136 to 205) |
| 3+ | 11 (22.5) | 37 (7.2) | 3.57 (1.61 - 7.91) | 2.68 (1.06 - 6.79) | -163 (-351 to 26) |
| Parasite density at delivery (parasites/µL) | | | | | |
| 0 | 37 (75.5) | 437 (85.4) | Reference | Reference | Reference |
| 1 - 20,000 | 9 (18.4) | 69 (13.5) | 1.54 (0.71 - 3.33) | 1.53 (0.66 - 3.56) | -53 (-183 to 78) |
| ≥ 20,000 | 3 (6.1) | 6 (1.2) | 5.91 (1.42 - 24.6) | 3.51 (0.76 - 6.28) | -220 (-727 to 287) |
| SP doses received | | | | | |
| 0 | 4 (8.2) | 21 (4.1) | 2.60 (0.78 - 8.62) | 2.00 (0.55 - 7.22) | -242 (-498 to 14) |
| 1 | 10 (20.4) | 93 (18.0) | 1.47 (0.63 - 3.43) | 0.95 (0.36 - 2.54) | -135 (-281 to 10) |
| 2 | 21 (42.9) | 212 (41.0) | 1.35 (0.67 - 2.73) | 1.27 (0.60 - 2.67) | -46 (-162 to 70) |
| 3+ | 14 (28.6) | 191 (36.9) | Reference | Reference | Reference |
| Primigravid | 20 (41.7) | 140 (27.1) | 1.54 (1.07 - 2.21) | 1.98 (1.01 - 3.88) | -208 (-290 to -126) |

CI = confidence interval, g = grams.

*All parasitemia outcomes and SP doses entered in separate models. All models adjusted for maternal weight, water source, gravidity and HIV status.

**All models adjusted for maternal weight, housing quality, gravidity and HIV status.

† n= 45 and 496, respectively

Table 3. Predictors of risk of maternal anemia and mean hemoglobin

| Variable | Hb < 11g/dl (N=65) n (%) | Hb ≥ 11g/dl (N=667) n (%) | Crude prevalence ratio (95%CI) | Adjusted prevalence ratio (95% CI) | Adjusted mean hemoglobin (g/dl) difference (95% CI) |
|---|---|---|---|---|---|
| HIV† | 17 (27.4) | 70 (11.6) | 2.36 (1.49 - 3.74) | 2.12 (1.09 - 4.14) | -0.74 (-1.13 to -0.36) |
| Placental parasitemia at delivery | 21 (32.3) | 84 (12.6) | 2.57 (1.71 - 3.85) | 3.60 (1.95 - 6.64) | -1.07 (-1.44 to -0.70) |
| Peripheral parasitemia at delivery* | 19 (29.2) | 90 (13.5) | 2.17 (1.42 - 3.31) | 2.84 (1.53 - 5.28) | -1.08 (-1.45 to -0.72) |
| Parasites density at delivery (parasites/μL) | | | | | |
| 0 | 44 (67.7) | 583 (87.4) | Reference | Reference | Reference |
| 0 - 20,000 | 17 (26.2) | 75 (11.2) | 3.00 (1.63 - 5.52) | 3.30 (1.73 - 6.28) | -1.04 (-1.43 to -0.65) |
| ≥ 20,000 | 4 (6.2) | 9 (1.4) | 5.89 (1.74 - 19.9) | 5.97 (1.58 - 22.52) | -1.26 (-2.21 to -0.32) |
| No. of times parasitemic over follow-up* | | | | | |
| 0 | 30 (46.2) | 366 (54.9) | Reference | Reference | Reference |
| 1 | 16 (24.6) | 187 (28.0) | 1.04 (0.56 - 1.90) | 1.07 (0.54 - 2.13) | -0.30 (-0.72 to 0.11) |
| 2 | 10 (15.4) | 66 (9.9) | 1.85 (0.86 - 3.96) | 2.27 (1.00 - 5.16) | -0.74 (-1.34 to -0.12) |
| 3+ | 9 (13.9) | 48 (7.2) | 2.29 (1.02 - 5.11) | 2.34 (0.96 - 5.72) | -0.96 (-1.66 to -0.26) |
| SP doses received* | | | | | |
| 0 | 4 (6.2) | 29 (4.4) | 1.74 (0.56 - 5.45) | 1.44 (0.45 - 4.63) | -0.04 (-0.89 to 0.81) |
| 1 | 19 (29.2) | 118 (17.7) | 2.03 (1.04 - 3.95) | 1.50 (0.73 - 3.07) | -0.40 (-0.91 to 0.10) |
| 2 | 22 (33.9) | 268 (40.2) | 1.03 (0.55 - 1.94) | 0.99 (0.52 - 1.90) | -0.12 (-0.52, 0.29) |
| 3+ | 20 (30.8) | 252 (37.8) | Reference | Reference | Reference |
| Primigravid | 17 (26.2) | 179 (27.0) | 0.97 (0.63 - 1.49) | 0.88 (0.47 - 1.67) | 0.04 (-0.25 to 0.34) |

CI = confidence interval, g/dl = grams per deciliter, Hb=hemoglobin

*All parasitemia outcomes and SP doses entered in separate models. All models adjusted for maternal weight, water source, gravidity and HIV status.

**All models adjusted for maternal weight, water source, gravidity and HIV status.

† n = 62 and 602, respectively

Table 4. HIV and placental parasitemia interaction effects

| Factor | Adjusted prevalence ratio (95% CI)** | | Adjusted mean differences (95% CI)** | |
|---|---|---|---|---|
| | Primigravida | Multigravida | Primigravida | Multigravida |
| | Low birth weight (<2500g) | | Birth weight (g) | |
| Neither infection | Reference | Reference | Reference | Reference |
| HIV only | 2.52 (0.44 - 14.47) | 2.02 (0.62 - 6.57) | -220 (-614 to 174) | -99 (-305 to 106) |
| Parasitemia only* | 1.65 (0.51 - 5.35) | 0.59 (0.08 - 4.66) | -162 (-377 to 53) | 127 (-95 to 349) |
| Dual infection | 6.37 (0.46 - 88.5) | 9.59 (2.51 - 36.6) | -174 (-843 to 495) | -216 (-559 to 126) |
| Interaction p-value | 0.8 | 0.1 | 0.4 | 0.2 |
| | Anemia (Hb<11g/dl) | | Hemoglobin (g/dl) | |
| Neither infection | Reference | Reference | Reference | Reference |
| HIV only | 5.42 (1.79 - 16.40) | 3.73 (1.47 - 9.47) | -0.14 (-1.80 to 1.52) | -0.89 (-1.51 to -0.28) |
| Parasitemia only | 2.56 (0.27 - 24.01) | 2.87 (1.25 - 6.60) | -1.02 (-1.86 to -0.17) | -1.16 (-1.92 to -0.40) |
| Dual infection | 9.03 (0.68 - 120.09) | 5.21 (1.53 - 17.7) | -1.72 (-4.16 to 0.72) | -1.68 (-2.82 to -0.54) |
| Interaction p-value | 0.8 | 0.4 | 0.6 | 0.5 |

CI = confidence interval, g/dl = grams per deciliter.

*Placental parasitemia

**All models adjusted for maternal weight, water source, gravidity and HIV status.

**CHAPTER FIVE - CHARACTERIZING THE EFFECT OF HIV ON MATERNAL**
***PLASMODIUM FALCIPARUM* PARASITEMIA RISK, FREQUENCY**
**AND SEVERITY DURING PREGNANCY**

**Abstract**

**Objective.** To examine the relationship between HIV infection and the risk, frequency and severity of malaria parasitemia among pregnant women.

**Methods**.  Between 2005 and 2006, we followed pregnant women attending two antenatal care clinics in southern Malawi from the second trimester of gestation until delivery. Information was collected on HIV infection and socio-demographic and malaria preventive behaviors.  Malaria parasitemia was assessed at each follow-up visit.

**Results.** A total of 1,370 pregnant women were included in the analyses.  The prevalence of HIV infection was 14% and 32% of the women were parasitemic at enrollment. The prevalence of parasitemia at enrollment among HIV-infected primigravidae was 0.85 (95% CI: 0.34, 2.12) times that of HIV-uninfected primigravidae. and the risk ratio was 1.0 (95% confidence interval (CI): 0.4, 2.8) over follow-up.  Among multigravidae, the prevalence of parasitemia among HIV-infected women was 2.3 (95% CI: 1.6, 3.3) times that of HIV-uninfected women at enrollment, and the risk ratio was 2.2 (95% CI: 1.5, 3.2)during follow-up. HIV infection was not associated with frequency of parasitemia among primigravidae (Odds ratio (OR) = 1.49, 95% CI: 0.64, 3.51 for ≥3 episodes) . However, HIV-infected multigravidae were 4.8 (95% CI: 2.1, 10.9) times as likely to have ≥3 episodes of parasitemia compared to HIV-uninfected multigravidae.  Among both primigravidae and multigravidae,

placental parasite density among HIV-infected women was on average 3.6 (95% CI: 1.8, 7.2) times as high as among HIV-uninfected women. **Conclusions.** HIV-infection resulted in a constant elevated risk of parasitemia over follow-up among multigravidae but not among primigravidae. HIV infection was also associated with increasing number of parasitemia episodes among multigravidae, but not among primigravidae. However, HIV infection was associated with higher peripheral and placental parasite densities across gravidities. These results underscore the need to target both primigravidae and multigravidae in malaria prevention.

**Introduction**

Each year, more than 20 million women become pregnant in malaria endemic areas [1, 2]. For these women, exposure to and subsequent infection with *Plasmodium falciparum* parasites may result in adverse pregnancy outcomes leading to an estimated 75,000 – 200,000 malaria-associated infant deaths per year [3]. These adverse outcomes are thought to be mediated through placental malaria, a condition associated with spontaneous abortion, stillbirth, intrauterine growth retardation, prematurity, and severe maternal anemia [4]. The latter three conditions can lead to delivery of an infant with low birth weight, one of the most important risk factors for neonatal mortality [5].

Placental malaria occurs when pregnant women are infected by particular variants of *P. falciparum* that have special affinity for receptors expressed on the surface of placental cells. These variants do not infect non-pregnant hosts and differ from other variants in their ability to evade host immune response. For this reason, primigravidae are generally at

highest risk of infection.  With successive pregnancies, women gradually develop immunity to these new parasite populations, and the risk of placental malaria decreases [6].

However, this gravidity-dependent immunity seems to be disrupted by HIV infection, with HIV-infected multigravidae being at increased risk of placental parasitemia [7][8-10]. However, the relationship between HIV and parasitemia among primigravidae remains unclear.  The purpose of the current study is to evaluate the effect of HIV infection on malaria parasitemia during pregnancy in a cohort of pregnant women in Malawi followed from the second trimester until delivery.  In order to appropriately characterize this relationship, we examine parasitemia in different ways: as parasitemia risk over the follow-up period, number of parasitemia episodes, and peripheral and placental parasite density at delivery.

## Methods

The study population comprised women in their second trimester of pregnancy attending the Mpemba and Madziabango Health Centres in Blantyre District, southern Malawi for antenatal care and delivery between March 2005 and February 2006.  These two health centres are located in rural areas outside of Blantyre, the largest city in Malawi. Women consenting to participate in the study were administered a questionnaire by the study nurses at enrollment to collect information on basic demographic characteristics, socio-economic factors and malaria prevention behaviors.  Enrolled women who also consented to receiving voluntary testing and counseling (VCT) were counseled and tested for HIV using two rapid HIV-1 antibody tests: Determine (Inverness Medical Innovations, Inc., Waltham,

MA) and Unigold (Trinity Biotech, Bray, Ireland). HIV-infected women were given

nevirapine according to national guidelines and referred to an antiretroviral treatment

program.

Participating women were encouraged to attend visits scheduled according to

standard antenatal care guidelines. At each visit, a finger prick blood sample was collected

for thick smear examination for malaria parasites and determination of hemoglobin level.

Peripheral malaria parasitemia was assessed through microscopic examination of thick blood

smear slides on site by trained laboratory technicians. Malaria parasites were quantified

against 200 white blood cells (WBCs). Women were administered sulfadoxine-

pyrimethamine (SP) for intermittent presumptive therapy in pregnancy (IPTp) and treated for

clinical malaria according to national guidelines. At delivery, placental, cord and peripheral

blood samples were collected and examined for malaria parasites through thick smear. For

quality control purposes, a 10% random sample of slides were re-examined by the laboratory

supervisor at Ntcheu District Hospital. Placental histology was not performed.

*Exposure*

HIV infection was defined as a positive result on the two rapid tests employed. There

was 95% (95% confidence interval (CI): 93.2 – 97.8%) agreement between the two tests in

identifying HIV infected women. Discordant results were excluded from analyses.

*Outcome*

Parasitemia was defined as the presence of parasites in thick blood smears. Parasite

density was computed assuming 6,000 WBC/μL of blood. Frequency of peripheral

parasitemia over follow-up was defined according to the number of episodes of parasitemia over the follow-up visits. Since we could not distinguish between recrudescence and re-infection, measurements of parasitemia were assumed to be independent across visits. Fever was rarely observed and was thus not included in the definition of parasitemia. Parasitemia was analyzed in four ways: a) as parasitemia risk (any or none) at enrollment, delivery, and any time over the follow-up period, b) as a longitudinal outcome averaging parasitemia risk across follow-up visits, c) as the number of episodes of parasitemia over follow-up, d) as peripheral and placental parasite density at delivery.

### *Statistical analysis*

Binomial regression was employed with the log link to estimate prevalence and risk ratios for parasitemia at enrollment, delivery and anytime during follow-up. Parasitemia risk over visits was analyzed using weighted generalized estimating equations (WGEE) in order to account for the possibility that data were missing at random (MAR). In these models, each individual response is weighted by the inverse probability of a missing response given the other responses, i.e., the probability of a missing measurement given the other measurements for a given subject [11]. We used the following model of covariates to estimate the weight:

$$Logit(\theta_{hi}) = \alpha + \sum_{k=1}^{7} \beta_k X_{hik}$$

where

$$\theta_{hi} = Pr\{missing\ response\ at\ visit\ h\ |\ non\text{-}missing\ response\ at\ visit\ h\text{-}1\}$$

In the above model, h indexes visits, i indexes subjects and h indexes covariates. The set of covariates used in the dropout model included continuous maternal weight in kg, maternal age at enrollment, and indicators for access to an unsafe water source, less than 8 years of

education, primigravida, husbands' occupation, and low housing quality. The polytomous outcome, number of parasitemia episodes, was analyzed using generalized logistic regression with robust standard errors. Parasite density at delivery was analyzed using zero-inflated negative binomial regression.

Covariate inclusion in regression modeling was decided using a causal diagram. Figure 1 presents a causal diagram of the relationship examined in the current study. Malaria preventive behaviors are endogenous to socioeconomic/demographic factors and are thus coupled together. In this data, age and gravidity were extremely correlated to the point of exchangeability. Hence, the path going through gravidity was chosen for analysis, since this is the main confounder of interest.

Interactions on ratio and difference scales were assessed through the inclusion of product terms in the models, and calculation of the relative excess risk or prevalence due to interaction (RERI or REPI) [12, 13].Weighted generalized estimating equations was conducted using the macro developed by Molenbergh & Verbeke (2005). All analyses except one were conducted using SAS v9.1 for Windows (SAS Inc., Cary, NC, USA). Zero-inflation negative binomial regression was conducted using StataSE v10 (StataCorp., College Station, Texas, USA).

*Ethical considerations*

Informed consent was obtained from all participating women in Chichewa. The study was reviewed and approved by the institutional review boards at the University of North Carolina at Chapel Hill and the College of Medicine at the University of Malawi.

**Results**

The overall study population comprised 1,496 pregnant women. Among these women, 111 did not agree to receive VCT and 15 women had discordant HIV rapid test results, yielding 1,370 women for inclusion in the analyses, of whom 61% were followed until delivery. The characteristics of women included in the study are presented in Table 1. Among primigravidae, there were stark differences between HIV-infected and HIV-uninfected women in educational attainment and malaria prevention behaviors (Table 1, Figure 2). A much higher proportion of HIV-infected primigravidae reported both using a bed net and treating the bed net with insecticide at regular intervals compared to HIV-uninfected primigravidae. Furthermore, HIV-infected primigravidae had higher educational attainment and a much greater proportion reported having received the first dose of SP IPTp prior to enrollment in the study. A much higher proportion of HIV-infected primigravidae were also reported receiving antimalarials for treatment during pregnancy prior to enrollment. Similar differences were not observed among multigravidae.

Over the follow-up period, 41% of the women experienced at least one episode of parasitemia. Among these women, 35% experienced more than one episode over follow-up. Figure 2 presents the proportion of women parasitemic at each visit according to HIV infection status and gravidity. Among primigravidae, at the enrollment visit, the proportion of women parasitemic was 18% lower among HIV-infected women, than HIV-uninfected women. However, for subsequent visits, higher proportions of parasitemia were generally observed among HIV-infected primigravidae. Among multigravidae, however, the proportion of women parasitemic was similar to that observed among primigravidae for HIV-

infected women, and consistently higher among HIV-infected women versus HIV-uninfected women across all visits.

There was significant negative interaction between HIV infection and being primigravid at enrollment and anytime over follow-up, but not for parasitemia at delivery. At enrollment, there was antagonistic interaction between HIV and being primigravid on a ratio scale ($\chi^2$=4.79, p = 0.03) and a difference scale ($\chi^2$=5.70, p = 0.02). The REPI was -0.851 (95% CI: -1.351, -0.350). Similarly, for parasitemia anytime over the follow-up visit, there was antagonistc interaction on both the ratio scale ($\chi^2$=7.04, p = 0.01) and a difference scale ($\chi^2$=10.42, p = 0.001). The RERI was -1.032 (95% CI: -1.884, -0.179). However, there was no evidence of interaction at delivery on neither the ratio ($\chi^2$=0.67, p = 0.41) nor the difference scale ($\chi^2$=0.06, p = 0.81). The REPI for placental parasitemia at delivery was 0.039 (95% CI: -3.654, 3.731).

The results of multivariate analyses are presented in Table 2. After adjusting for various factors, HIV-infected primigravidae were slightly less likely to have parasitemia at enrollment; however, the result is imprecise with a confidence limit ratio (CLR) of 6.2. The maximum likelihood estimate (MLE) for parasitemia anytime over the follow-up period indicates no difference between HIV-infected and HIV-uninfected women and has a CLR of 7.4. At delivery, however, the MLE indicates that the risk of placental parasitemia was 66% higher among HIV-infected primigravidae when compared with HIV-uninfected primigravidae, and the estimate is more precise (CLR = 3.9). Due to zero cell counts at some visits, the results from the wGEE analysis for primigravidae were unreliable and are thus not reported.

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The risk ratio estimates for parasitemia over follow-up are much more consistent and precise for multigravidae.    The risk ratio estimates for parasitemia at enrollment, anytime during follow-up and at delivery are all indicative of an approximate doubling of risk due to HIV infection.  This is further confirmed by the results from the wGEE analysis. After adjusting for possible differential attrition (missingness at random), the estimate for the effect of HIV on parasitemia over the follow-up period is over 20% higher compared to the risk of having parasitemia anytime during follow-up (see Table 2).

Low educational attainment among primigravidae was associated with parasitemia at enrollment (PR=2.25, 95% CI: 1.29, 3.91) and anytime over follow-up (RR= 1.80, 95% CI: 1.01, 3.30). This was not the case among multigravidae, where  risk ratios were 1.76 (95% CI: 0.40, 2.15) and 1.10 (95% CI: 0.64, 1.90), respectively.  Reported use of a bednet was inversely associated with parasitemia at enrollment among primigravidae (PR=0.64, 95% CI: 0.34, 1.21) and multigravidae (PR=0.67, 95%CI: 0.47, 0.97). For developing parasitemia anytime over follow-up, the effect estimate was greater among primigravidae (RR=0.47, 95% CI: 0.23, 0.94), than among multigravidae (RR=0.89, 95% CI: 0.61, 1.30).

Among multigravidae, HIV infection was associated with increasing frequency of parasitemia episodes (Table 3).  A similar relationship was not observed among primigravidae. However, among primigravidae, low educational attainment was associated with increasing frequency of parasitemia episodes, and an inverse association with bed net usage was also observed. Receiving 2 doses of SP IPTp was also observed to be inversely associated with increasing frequency of parasitemia episodes, particularly among primigravidae.

Differences with respect to gravidity were not observed, however, for parasite density at delivery. Across gravidities, HIV was associated with higher parasite density, with a greater effect estimate observed for placental parasite density (Table 4). Independent of HIV infection, being primigravid was associated with nearly 300% higher placental parasite densities, but 100% higher peripheral parasite densities. The predicted change in parasites/µL for placental parasitemia was similar for HIV infection and being primigravid. While both reporting regular use of bed net and treating the bed net with insecticide at regular intervals were associated with lower peripheral parasite densities, only reporting insecticide treatment of bed nets was associated with lower placental parasite densities.

**Discussion**

In the current study, we report the effect of HIV infection on malaria parasitemia in a cohort of pregnant women observed from the second trimester until delivery. Whereas among multigravidae we observed a constant effect of HIV infection on parasitemia risk over the course of follow-up, primigravidae exhibited a gradual strengthening of the effect of HIV infection across visits. Additionally, parasitemia risk across follow-up visits among HIV-infected multigravidae was most similar to the risk among HIV-uninfected primigravidae. Further, HIV infection was observed to be associated with increasing frequency of parasitemia episodes among multigravidae, but not among primigravidae. At delivery, however, the effect of HIV infection was associated with increased peripheral and placental parasite density across gravidities.

### *Parasitemia at enrollment*

One of the more unexpected results was the lower prevalence of parasitemia among HIV-infected primigravidae relative to HIV-uninfected primigravidae at enrollment. In light of previous literature, we would have expected to see either a similar or a higher prevalence [7, 8, 10, 14]. The observed direction of this association, however, seems congruous with the stark differences in educational attainment and malaria preventive behaviors observed between HIV-infected and HIV-uninfected primigravidae. For example, for most of the women, the enrollment visit was the first ANC visit and hence the first visit in which women would have received SP IPTp. Yet, HIV-infected primigravidae were five times as likely to report already having received an SP IPTp dose before enrollment compared with HIV-uninfected. Furthermore, HIV-infected primigravidae were three times as likely to report using and treating bed nets with insecticide at regular intervals compared with HIV-uninfected primigravidae. These differences are consistent with the observed higher educational attainment among HIV-infected primigravidae, and the finding of a strong elevated risk of parasitemia at enrollment associated with low educational attainment.

After adjusting for the factors, the value of the effect estimate was attenuated, but did not change direction, suggesting other potential factors. Another possible explanation is that HIV-infected primigravidae may have been more likely to experience parasitemia earlier than the second trimester when they enrolled in the study. HIV-infected primigravidae were more than 4 times as likely to report having received antimalarial treatment during the pregnancy prior to enrollment. A similar relationship was not observed among primigravidae. The finding of higher previous antimalarial use among HIV-infected women during pregnancy before initiation of SP ITPp has been observed in another study [7]. This

may indicate high parasitemia risk among HIV-infected women during early gestation.  In their study also conducted in southern Malawi, Verhoeff and colleagues report elevated prevalence of parasitemia in HIV-positive women at less than 16 weeks of gestation[7]. Recent malaria treatment prior to enrollment among HIV-infected women may also explain the lower prevalence of parasitemia at enrollment.

### *Parasitemia risk over follow-up*

In this study we examine the risk of parasitemia over the follow-up period in two ways. The first way is experiencing parasitemia anytime over the follow-up period regardless of frequency; and secondly, as the average risk of experiencing parasitemia over the follow – up visits accounting for frequency.  We report the results of the latter analysis only for multigravidae due to sparseness of data for primigravidae.  Among primigravidae, there was no evidence of elevated risk of parasitemia anytime over the follow-up period due to HIV infection.   In contrast, among multigravidae, HIV infection doubled the risk of parasitemia anytime over the follow-up period.  This effect was further strengthened when estimating the average risk of parasitemia over follow-up.  Nonetheless, there were no large differences among the effect estimates for HIV infection among multigravidae at enrollment, anytime during follow-up and averaged over follow-up visits, suggesting a constant effect over the follow-up period.  In contrast, the pattern of parasitemia risk observed across visits among primigravidae and the changing direction of effect estimates from enrollment until delivery suggested that the effect of HIV infection on parasitemia risk may vary over the course of pregnancy.  This may be due to the differential effects of SP IPTp between HIV-infected and HIV –uninfected women [15].

*Frequency of parasitemia episodes*

The finding that HIV infection is associated with increasing number of parasitemia episodes corroborates previous literature.  In a study conducted in Malawian non-pregnant adults by Patnaik and colleagues, HIV infection increased the hazard of one episode 1.8 times (95% CI: 1.2, 2.7), and two episodes 2.5 times (95% CI: 1.5, 4.2) [16].  In the present study, we report similar and more precise estimates for one and two episodes among multigravidae. Further, we found a very strong association between HIV infection and having three or more episodes of parasitemia among multigravidae. We did not observe a similar relationship among primigravidae.  Consistent with other findings in the current study, low educational attainment was a strong risk factor for two or more episodes of parasitemia among primigravidae.

*Parasitemia risk at delivery*

The elevated risk of placental parasitemia observed among primigravidae and multigravidae support finding from previous studies.  In a meta-analysis of the effect of HIV infection on malaria parasitemia, ter Kuile and colleagues summarizing data from four studies, three of which were conducted in Malawi,  reported a summary estimate of 1.27 (95% CI: 1.06, 1.51) for the effect of HIV infection on placental parasitemia among primigravidae and 2.39  (95%  CI: 1.87, 3.07) among multigravidae[10].  The latter estimate is very similar in magnitude of effect to that reported in the current study, though the former differs most likely due to sparseness of data. This study confirms the hypothesis that the effect of HIV infection on placental parasitemia is more marked among multigravidae than among primigravidae.

*Parasite density at delivery*

A difference in effect according to gravidity was not observed, however, for parasite density. Across gravidities, HIV infection was associated with higher parasite density. The result that HIV infection is associated with higher parasite density also corroborates previous research [8, 17, 18]. Interestingly, report of bed net use was associated with lower peripheral parasite densities, but not placental parasite densities. While, reporting treatment of bed nets with insecticide was associated with both lower peripheral and placental parasite densities.

Departing from previous studies, we employed zero-inflated negative binomial regression (ZINB) to examine the effect of HIV infection and other factors on parasite density. The advantage of ZINB regression is that it takes into account the semi-continuous nature (excess zeros) of parasite density and allows for overdispersion in non-zero values of parasite density. This is preferable to comparing geometric mean parasite density using student's t-test and analysis of variance or performing analyses on log-transformed parasite density for two reasons. One, because of the high degree of skew in parasite density, log-transformation may not result in a normal distribution of transformed values precluding use of tests with a normality assumption. Additionally, the optimal Box-Cox transformation that would result in a normal distribution may vary from population to population, given factors such as seasonality and transmission intensity that tend to differ across populations. Two, back-transformation of transformed values may not result in sensible results. Whereas, ZINB regression appropriately takes into account the distribution of parasite density and allows for the estimation of a predicted mean change in parasite density which has utility in determining the impact of the risk/protective factors of interest on parasite burden.

*Limitations*

There are a number of considerations in the interpretation of the results from this study. One of the main limitations is loss to follow-up. Almost 40% of women included in the analyses were lost to follow-up over the course of the study. This may limit generalizability of the results, although no substantial differences were observed between the cohort at enrollment and women followed until delivery. Additionally, in the longitudinal analysis, we accounted for loss to follow-up by weighing our analysis model by the inverse probability of being missing given a set of covariates that could potentially influence missingness. Secondly, placental parasitemia was determined through thick smear instead of placenta histology, a more sensitive method. By the same token, because we were unable to distinguish if measurements of parasitemia at different visits were the result of recrudescence or re-infection, some women labeled as having multiple parasitemia episodes may have had chronic infection.

An additional limitation is that we did not obtain information on CD4+ count which has been associated with parasitemia in previous studies (Laufer, 2006; Patnaik, 2005; Whitworth, 2000) [16-18]. The differences observed among primigravidae and multigravidae in the effect of HIV infection on parasitemia may be reflective of more advanced HIV disease in multigravidae. Further, since HIV infection may also be associated with early pregnancy loss, there may have been some selection bias in that HIV-infected women enrolled in the study were women with sufficiently healthy pregnancies to reach the second trimester. This may be associated with a complex of other factors such as socioeconomic

status and may be differential according to gravity, since primigravidae are generally at much higher risk of adverse pregnancy outcomes than multigravidae.

These limitations, however, are counterbalanced by the strengths of this study. Firstly, the current study employed a longitudinal study design and analytical procedures enabling examination of parasitemia risk from the second trimester until delivery. Secondly, although there was substantial loss to follow-up, there were no significant differences between women at enrollment and women followed until delivery. Furthermore, potential residual differences were accounted for in the analyses. By the same token, the richness of socio-demographic information recorded from the women that allowed for a comprehensive missingness model, also allowed for the examination of the role of other individual factors in determining parasitemia risk over the follow-up period.

*Conclusions*

With the reduced effectivenesss of SP IPTp among HIV-infected women, it is important that antenatal care services promote the full repertoire of malaria prevention methods. Our findings speak to the effectiveness of the use and regular treatment of bed nets with insecticide in reducing parasitemia risk and placental parasite density. The study underscores the necessity of targeting both primigravidae and multigravidae for malaria prevention in areas with a high prevalence of HIV infection. Further, although we report a constant elevated risk of parasitemia among multigravidae, our results suggest that the effect of HIV infection on parasitemia risk may vary over time among primigravidae. Further studies are needed to understand the effect of HIV infection on parasitemia risk over the course of pregnancy.

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## Tables

Table 1. Study population characteristics

| | Primigravida | | Multigravida | |
|---|---|---|---|---|
| | **HIV-infected** | **HIV-uninfected** | **HIV-infected** | **HIV-uninfected** |
| **Variable** | **(N=23)** | **(N=316)** | **(N=162)** | **(N=865)** |
| Maternal age(years), mean ± SD | 19.2 ± 2.7 | 18.3 ± 1.9 | 26.5 ± 4.2 | 25.3 ± 5.3 |
| Education ≤8 years (%) | 43.5 | 80.7 | 89.4 | 89.9 |
| Married (%) | 65.2 | 81.6 | 94.4 | 96.1 |
| Unemployed (%) | 52.2 | 61.1 | 46.3 | 51.1 |
| Report always using bednet (%) | 34.8 | 13.0 | 24.1 | 25.9 |
| Report insecticide impregnation of bednet in past 6 months (%) | 30.4 | 11.5 | 22.8 | 22.3 |
| Weight (kg), mean ± SD | 56.9 ± 7.8 | 53.6 ± 5.7 | 54.7 ± 6.8 | 55.1 ± 6.9 |
| Parasitemia at enrollment (%) | 43.5 | 58.9 | 37.0 | 20.7 |
| Placental parasitemia (%) | 28.6 | 22.3 | 19.3 | 8.3 |
| Peripheral parasitemia at delivery (%) | 28.6 | 21.3 | 20.5 | 8.3 |
| No. times parasitemic over pregnancy (%) | | | | |
| 0 | 47.8 | 27.9 | 50.0 | 67.7 |
| 1 | 26.1 | 37.0 | 30.9 | 26.1 |
| 2 | 4.4 | 20.9 | 8.6 | 5.6 |
| 3+ | 21.7 | 14.2 | 10.5 | 1.2 |
| SP doses received (%) | | | | |
| 0 | 8.7 | 5.1 | 8.6 | 5.9 |
| 1 | 26.1 | 19.6 | 32.7 | 26.9 |
| 2 | 30.4 | 38.6 | 32.7 | 38.5 |
| 3+ | 34.8 | 36.7 | 25.9 | 28.7 |
| Anemia at enrollment (Hb ≤ 10g/dL) (%) | 31.8 | 43.1 | 35.8 | 15.0 |
| Anemia at delivery (Hb ≤ 10g/dL) (%) | 15.4 | 8.9 | 20.3 | 7.4 |
| Birth weight (grams), mean ± SD | 2767 ± 514 | 2931 ± 428 | 2968 ± 544 | 3140 ± 461 |

SD = standard deviation, g/dL= grams per deciliter, kg=kilograms

Table 2. Estimated effect of HIV on parasitemia risk at different time points and over follow-up.

| | Primigravidae | | | Multigravidae | | |
|---|---|---|---|---|---|---|
| | **Fraction Parasitemic (n/N)** | | **Prevalence Ratio (95% CI)** | **Fraction Parasitemic** | | **Prevalence Ratio (95% CI)** |
| **Time point** | **HIV+** | **HIV-** | | **HIV+** | **HIV-** | |
| Enrollment | 10/23 | 186/316 | 0.85 (0.34, 2.12) | 60/162 | 179/865 | 2.28 (1.58, 3.28) |
| Anytime during follow-up* | 8/20 | 243/291 | 1.04 (0.38, 2.81) | 48/140 | 148/760 | 2.16 (1.45, 3.21) |
| Delivery | 4/14 | 45/202 | 1.66 (0.72, 2.81) | 17/88 | 37/447 | 2.44 (1.28, 4.66) |
| Average risk over follow-up*† | ---- | ---- | ---- | varies | varies | 2.63 (1.08, 6.44) |

Models adjusted for education, maternal weight, bednet use, and number of SP doses received.

*Excludes enrollment visit, risk ratio

†Primigravidae wGEE results not reported due to sparseness of data across visits, average risk over follow-up, frequencies vary according to visit.

Table 3. Risk factors for number of parasitemia episodes over follow-up

| | One episode only PR (95%CI)* | | Two episodes RR (95%CI)* | | Three or more episodes RR (95%CI)* | |
|---|---|---|---|---|---|---|
| **Variable** | **Primigravida (n=123)** | **Multigravida (n=276)** | **Primigravida (n=67)** | **Multigravida (n=62)** | **Primigravida (n=50)** | **Multigravida (n=27)** |
| HIV infection | 0.76 (0.38, 1.54) | *1.31 (1.02, 1.67)* | 1.40 (0.68, 2.89) | *2.20 (1.53, 3.15)* | 1.49 (0.64, 3.51) | *4.82 (2.12, 10.96)* |
| Less than 8 years of education | 1.08 (0.78, 1.51) | 1.06 (0.77, 1.46) | *1.77 (0.99, 3.15)* | 0.86 (0.52, 1.43) | 1.98 (0.90, 4.37) | N/A |
| Report always using bednet | *0.64 (0.42, 0.99)* | 0.96 (0.77, 1.19) | 0.62 (0.32, 1.17) | 0.96 (0.18, 5.00) | 1.01 (0.55, 1.85) | 0.95 (0.41, 2.20) |
| SP doses received | | | | | | |
| 0-1 | 1.44 (0.96, 2.17) | 1.19 (0.93, 1.53) | 1.03 (0.57, 1.84) | 0.73 (0.45, 1.18) | 0.54 (0.19, 1.55) | N/A |
| 2 | 0.75 (0.55, 1.01) | 0.97 (0.78, 1.20) | *0.63 (0.42, 0.96)* | *0.68 (0.47, 1.00)* | *0.50 (0.29, 0.86)* | 0.48 (0.21, 1.10) |
| 3+ | Reference | Reference | Reference | Reference | Reference | Reference |

PR = Prevalence Ratio, RR = Risk Ratio, CI = Confidence Interval, Primigravidae (N = 339), Multigravidae (N=1122)

*Excluding enrollment visit

Table 4. Multivariate predictors of parasite density at delivery

| Variable | Peripheral | | Placental | |
|---|---|---|---|---|
| | Ratio of average densities (95%CI) | Predicted change in parasites/µL (SD) | Ratio of average densities (95%CI) | Predicted change in parasites/µL (SD) |
| HIV infection | 2.59 (1.25, 5.34) | 646 (108) | 3.56 (1.76, 7.21) | 1,700 (253) |
| Primigravid | 2.03 (1.11, 3.73) | 419 (131) | 3.97 (2.16, 7.30) | 1,700 (410) |
| Maternal weight, kilograms (continous) | 0.94 (0.89, 1.00) | -420 (74)** | 0.89 (0.85, 0.92) | -2,300 (310)** |
| Report always using bednet* | 0.42 (0.20, 0.87) | -130 (67) | 1.22 (0.57, 2.62) | ---- |
| Report insecticide impregnation in past 6 months | 0.35 (0.17, 0.73) | -150 (79) | 0.19 (0.04, 0.94) | -440 (260) |

CI = Confidence Interval, SD= Standard deviation, N=831

*All variables in model. Bednet model excludes insecticide impregnation variable.

**Predicted change for maximum difference in maternal weight

**Figures**
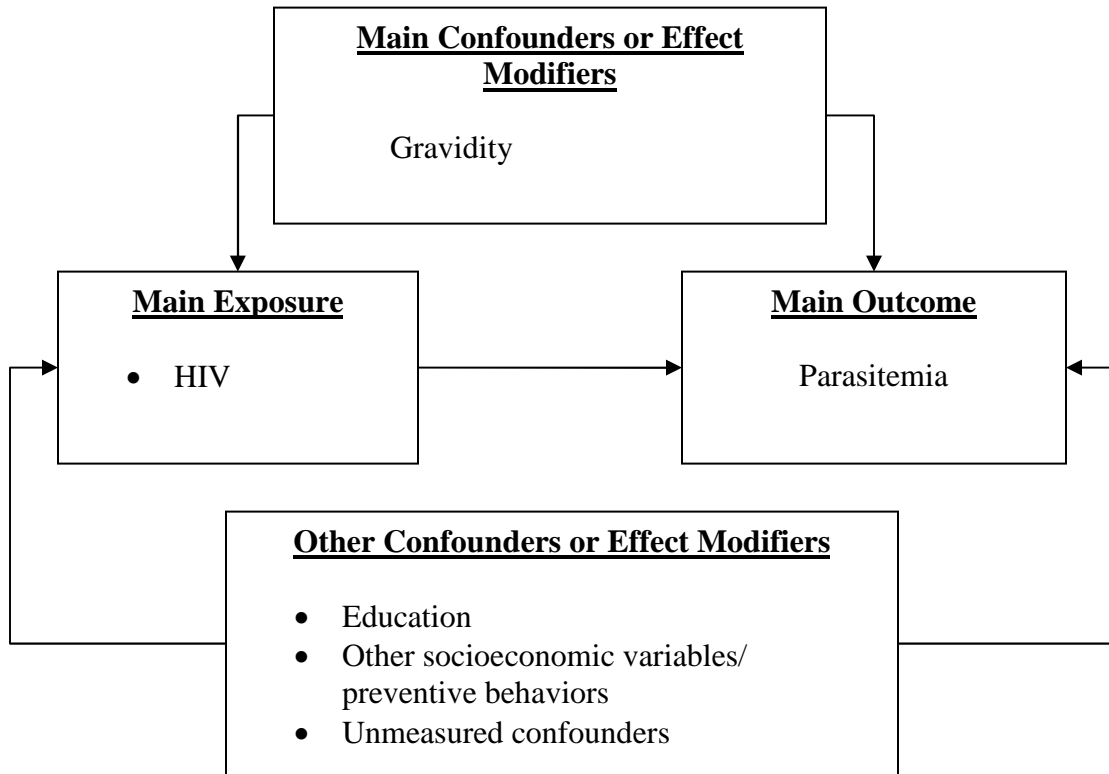


Figure 1. Causal diagram of HIV and parasitemia relationship in pregnancy
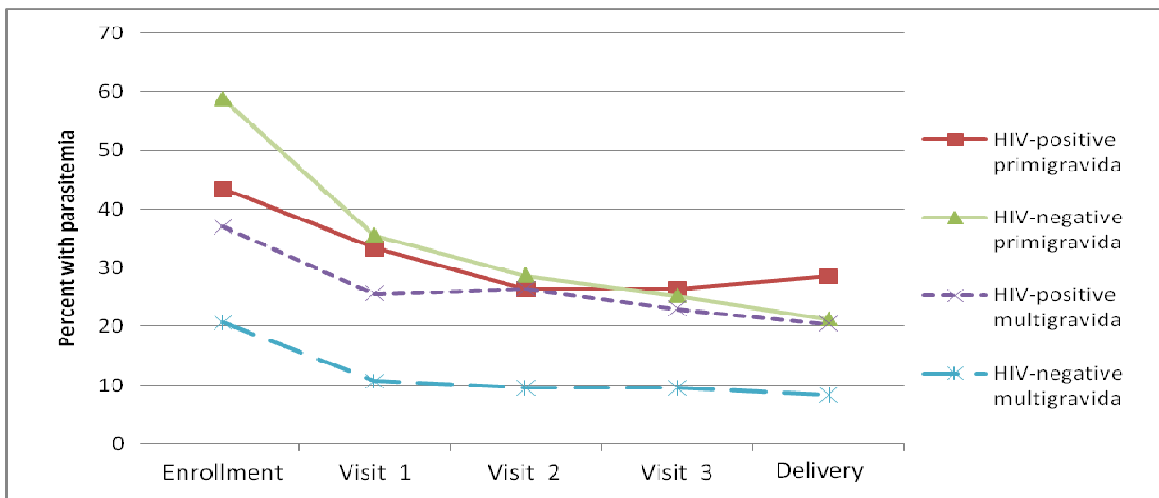


Figure 2. Parasitemia across visits among primigravidae and multigravidae (Note: Value for visit 2 carried over to visit 3 among HIV infected primigravidae due to sparseness of data).
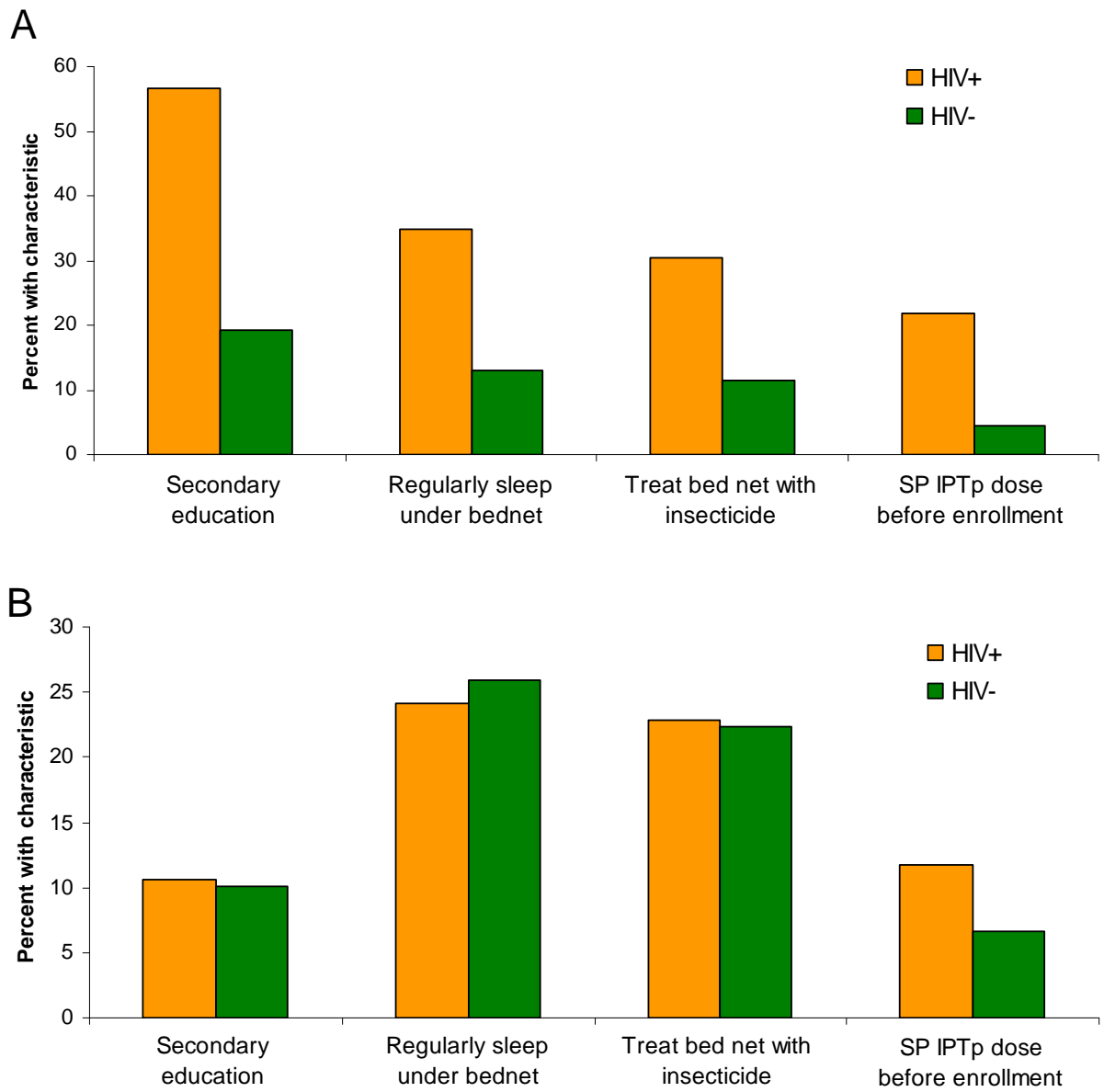
Figure 3. Frequency of selected factors among primigravidae (**A**) and multigravidae (**B**)

# CHAPTER SIX - THE EFFECT OF G6PD A- DEFICIENCY ON *PLASMODIUM FALCIPARUM* PARASITEMIA AND ANEMIA AMONG PREGNANT WOMEN IN SOUTHERN MALAWI.

## Abstract

**Objective**. The objective of this study was to determine the effect of carriage of the G6PD A- allele on maternal parasitemia, maternal anemia and low birth weight.

**Methods.** Between 2005 and 2006, we followed pregnant women attending two antenatal care clinics in southern Malawi from the second trimester of gestation until delivery. We conducted a case-cohort study by sampling all cases of placental parasitemia at delivery and a 35% random sample of all women followed until delivery comprised a sub-cohort. The G6PD status of the subcohort and all cases was assessed.

**Results.** There were 46 cases of placental parasitemia and 294 women were included in the subcohort. The prevalence estimates of placental parasitemia and G6PD A- allele carriage in the subcohort were 16% and 28% respectively. G6PD A- primigravidae were as likely to have placental parasitemia as G6PD-normal primigravidae (PR=1.0, 95% confidence interval (CI): 0.7, 1.3). Among multigravidae, G6PD A- carriers were 0.9 (95% CI: 0.8, 1.0) times as likely to have placental parasitemia as G6PD-normal women. Further, their placental parasite density was on average 0.22 times that of G6PD-normal multigravidae. Among primigravidae, G6PD A- carriers had 1.7 (95% CI: 1.0, 2.9) times the average risk of maternal anemia over the follow-up period when compared to G6PD-normal women. A similar relationship was not observed for multigravidae. Across gravidities, G6PD

deficiency was associated with an increased risk of low birth weight (PR=2.5, 95% CI: 1.2, 5.2). Among daughters, G6PD A- carriage by the mother was associated with a 286g reduction in mean birth weight (p = 0.02). A reduction in mean birth weight was not observed among sons.

**Conclusions.** The results of this study suggest a lack of a protective effect for G6PD A- carriage on parasitemia and anemia risk. However, among multigravidae, G6PD A- carriage may protect against high parasite density. We also found an increased risk of low birth weight due to maternal G6PD A- allele carriage. More studies will need to be conducted to understand the role of G6PD A- in adverse birth outcomes.

**Introduction**

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most prevalent enzyme deficiency with over 300 million people affected worldwide [1]. The enzyme catalyzes the first rate-determining step in the pentose phosphate pathway that forms part of glycolysis [2]. In the erythrocyte, the conversion of glucose-6-phosphate into 6-phosphogluconolactone, catalyzed by G6PD is very important for the production of reduced nicotinamide adenine nucleotide phosphate (NADPH), a compound crucial to the erythrocyte's ability to withstand oxidative stress [2].

The geographic distribution of G6PD deficiency and its spatial correlation with areas of historic and present endemic malaria have led to the hypothesis that the deficiency may confer protection against malaria. Various observational and *in vitro* studies have been

conducted to test this hypothesis [3-7]. Few studies, however, have been conducted among pregnant women.

In malaria-endemic areas, pregnant women, especially primigravidae, are generally at much increased risk of malaria infection than non-pregnant adults [8, 9]. This elevated risk is mediated through particular variants of *P. falciparum* that have special affinity for the chondroitin sulphate A (CSA) receptor expressed on the surface of placental cells leading to placental infection [10, 11]. Placental malaria has adverse consequences for the mother and the fetus, such as low birth weight, intrauterine growth retardation, prematurity, and severe maternal anemia [12, 13]. With increasing gravidity, there is increasing production of antibodies that help reduce adhesion of infected cells to the CSA receptor, leading to a lower risk of placental malaria among multigravidae [14].

In this study we employ a case-cohort design to assess the effect of G6PD deficiency on malaria parasitemia and maternal anemia risk among pregnant women followed from the second trimester until delivery.

**Methods**

*Study population.* This case-cohort study was drawn from a cohort of 1, 496 pregnant women in their second trimester attending the Mpemba and Madziabango antenatal care clinics located in a rural area outside of Blantyre, the largest city in Malawi. Women were enrolled between March 2005 and February 2006 in a cohort study examining the effect of HIV infection and malaria parasitemia on pregnancy outcomes. Women attended visits according to the standard antenatal care guidelines with visits scheduled after enrollment

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occurring at approximately 26, 32 and 36-38 weeks of gestation.  At each visit, a finger prick

blood sample was collected for thick blood film examination for malaria parasites and

determination of hemoglobin level.  For women delivering at the clinics, placental and

peripheral blood films were collected and examined for malaria parasites on site.  Placental

biopsies were not collected.  Women attending the antenatal care clinics were administered

sulfadoxine-pyrimethamine (SP) for intermittent presumptive therapy in pregnancy (IPTp)

and treated for clinical malaria also according to national guidelines.  The sub-cohort used

for the analyses comprised a 35% simple random sample without replacement of the 831

women who completed follow-up until delivery.

   *Case definitions.* Parasitemia was defined as the presence of parasites in thick blood

smears.  Malaria parasites were quantified against 200 white blood cells (WBCs) by trained

laboratory technicians.  For the purpose of the case-cohort analyses, a case was defined as a

woman presenting with placental parasitemia at delivery.  For sub-cohort analyses, case

definitions extended to peripheral parasitemia at delivery, enrollment, and the follow-up

visits.  Fever was rarely observed and was thus not included in the case definitions.  Anemia

was defined as hemoglobin $\leq 11$g/dL. Low birth weight (LBW) was defined as a birth weight

$< 2500$g.

   *Exposure definition.* G6PD deficiency was defined as heterozygous or homozygous

carriage of the A- allele. The presence of the G6PD A- allele was determined as previously

described [6]. G6PD normal status was defined as the absence of the G6PD A- allele.

Additionally, hemoglobin types and the presence of the 3.7-kb deletional determinant of

$\alpha(+)$-thalassemia was determined as described[15].

*Statistical analysis.* Binomial regression was employed to estimate the effect of carriage of the G6PD A- allele on parasitemia and anemia risk at delivery, enrollment, and any time over follow-up. For analyses involving parasitemia-related outcomes, both the case series and the subcohort were employed using inverse of sampling fraction weights. Binomial regression was also used to determine the effect of G6PD A- carriage on low birth weight. Analysis of variance was used to examine differences in mean birth weight. Generalized estimating equations were employed to determine the effect of G6PD A- allele carriage on parasitemia and anemia risk averaged over follow-up visits. Zero-inflated negative binomial regression was used to determine the effect of G6PD A- carriage on parasite density. Since G6PD deficiency is inherited at birth and the antimalarials used for IPTp and treatment are not known to induce hemolysis in G6PD-deficient individuals, there were no confounders of its relationship with parasitemia or anemia risk. However, gravidity was investigated as an effect modifier through inclusion of interaction terms and stratified analyses.

*Ethical considerations.* Informed consent was obtained from all participating women in Chichewa. The study was reviewed and approved by the institutional review boards at the University of North Carolina at Chapel Hill and the College of Medicine at the University of Malawi.

**Results**

Characteristics of the study population are presented in Table 1. There were no significant differences between the sub-cohort and the full cohort. A normal G6PD genotype

was observed for 72% of the sub-cohort and 80% of the cases. There were 5 homozygous carriers among the sub-cohort and none among the cases. The prevalence estimate for sickle cell trait was 6%. The prevalence estimates for heterozygous (-α/αα) and homozygous (-α/-α) α (+)-thalassemia trait were 45% and 10%, respectively. The relationship between there red blood cell polymorphisms and pregnancy outcomes will be explored in a subsequent paper. In comparison to the sub-cohort, there was a greater proportion of primigravidae among cases.

### *Plasmodium falciparum parasitemia*

According to the case-cohort analysis, both primigravid and multigravid G6PD A- carriers had a lower prevalence of placental parasitemia compared with G6PD normal women (PR= 0.77, 95% CI: 0.17, 3.42; PR=1.15, 95% CI: 0.50, 2.69, respectively). However, with confidence limit ratios (CLRs) of 20 and 5, respectively, these estimates were highly imprecise. In the weighted analyses, there was no evidence of a difference in prevalence of placental and peripheral parasitemia among G6PD A- carriers versus G6PD normal women (Table 2).

We also examined the effect of G6PD A- carriage on peripheral parasitemia at delivery, enrollment and over follow-up using the subcohort. A difference in prevalence was not observed among G6PD A- carriers and normal women at delivery and enrollment. Among primigravidae, G6PD A- carriers had a 28% higher prevalence of parasitemia when compared with G6PD normal primigravidae anytime over follow-up. However, a similar relationship was not observed with parasitemia averaged over the follow-up visits. The estimates among multigravidae were similar in magnitude, but exhibited higher variance

(Table 2).  For parasite density at enrollment, no significant effects were observed for both primigravidae and multigravidae. However, at delivery, multigravid G6PD A- carriers had on average much lower parasite densities than G6PD normal multigravidae (Table 3).

*Maternal anemia*

Maternal anemia prevalence was higher among cases than among the subcohort, but the difference was more pronounced at delivery than at enrollment (Table 1).  At delivery, the effect estimates for G6PD A- carriage were on the side of protection for both primigravidae and multigravidae. However, with CLRs of 67 and 11, the estimates were extremely imprecise to be reliable.  At enrollment, there was significant negative interaction between carriage of the G6PD A- allele and gravidity, with G6PD A- carriage being associated with two times the prevalence of parasitemia among primigravidae when compared with G6PD normal primigravidae. Among multigravidae, however, the effect estimate for anemia at enrollment was in the direction of protection.  This relationship persisted even after adjusting for parasitemia.  At enrollment, among primigravidae with parasitemia, G6PD A- carriage was associated with 1.31(95% CI: 0.66, 2.59) times the risk of anemia when compared to normal G6PD.  Among multigravidae with parasitemia, G6PD A- carriage was associated with 0.93 (95% CI: 0.22, 3.89) times the risk of anemia when compared with normal G6PD. Effects on opposite sides of the null for primigravidae and multigravidae were also observed when examining average anemia risk over the follow-up period although the interaction effect was not significant. There was no evidence of interaction between number of SP doses received for IPTp and G6PD A- carriage in their effect on anemia.

*Birth weight*

With regard to birth outcomes, across gravidities, G6PD A- carriers' offspring exhibited a higher prevalence of low birth weight compared to the offspring of G6PD normal women (Table 2). However, this translated into a significant difference in mean birth weight only among primigravidae. On average, G6PD A- primigravidae bore infants weighing 203g less than G6PD normal primigravidae (p = 0.05). Whereas, there was a 76g difference between G6PD A- and normal multigravidae (p=0.36, Figure 1). Further, among G6PD A- carriers, sons had a higher mean birth weight than daughters (+346g, p = 0.03). A similar difference was not observed among G6PD normal women (+36g, p=0.6). Daughters of G6PD A- carriers had a higher prevalence of low birth weight and significantly lower mean birth weight than daughters of G6PD normal women (Figure 2, Table 5). However, sons of G6PD A- carriers had a mean birth weight similar to sons of G6PD normal women, but a higher prevalence of low birth weight (Figure 2, Table 5). These differences differed according to gravidity. Among primigravidae, there was a reduction in mean birth weight for both sons and daughters born to G6PD A- deficient women. However, among multigravidae, sons born to G6PD A- deficient mothers had higher average birth weights (Table 5). There was no significant difference in the sex ratios according to G6PD status. The ratio of sons to daughters among G6PD A- carriers offspring was 1.2; and among G6PD-normal women, 0.95. Further, among G6PD normal women, the difference in proportions of daughters born to women with placental parasitemia versus sons was 4% (95% CI: -4%, 13%); for G6PD A- carriers, 0% (95% CI: -17%, 20%).

**Discussion**

In this study, we report the absence of a protective effect by G6PD A- carriage on the risk of malaria parasitemia and maternal anemia over pregnancy. At delivery, we report the absence of a protective effect of G6PD A- carriage on peripheral parasitemia among both primigravidae and multigravidae. However, we found a minimal protective effect among G6PD A- multigravidae. We also found a reduced peripheral and placental malaria parasite density among multigravid G6PD A- carriers. Additionally, we report an increased risk of low birth weight for neonates born to G6PD A- carriers. The difference in mean birth weight was found to be more pronounced for daughters than for sons.

*Plasmodium falciparum parasitemia*

To our knowledge this is the first study reporting the effect of G6PD A- carriage on malaria parasitemia at delivery. Using a case-cohort design, the resultant prevalence ratios suggested protection by G6PD A- carriage. However, the confidence limits crossed the null and the CLR's ranged between 4 and 5, indicating imprecise estimates. In contrast, for the sub-cohort analyses, the CLRs were much lower and the estimates closer to the null. For primigravidae, the maximum likelihood estimate was close to 1.0 indicating the lack of a relationship.

There is one other study that reports the effect of G6PD A- carriage among pregnant women. In the cross-sectional study conducted by Mockenhaupt and colleagues among pregnant women in Ghana, the authors report a prevalence odds ratio of 0.6 (95% CI: 0.4 – 0.9) for the effect of heterozygous carriage of G6PD A- allele on parasitemia among

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multigravidae [16]. However, given that parasitemia was a very common outcome in the population (over 60% of the women were infected with *P. falciparum*), the use of logistic regression would result in estimates strongly biased away from the null. Binomial regression is more appropriate when outcomes are common [17, 18]. Using binomial regression on the data from the study by Mockenhaupt and colleagues, the appropriate estimate of the prevalence ratio was found to be 0.81 (95% CI: 0.52, 1.26). This is similarly indicative of a lack of an association between G6PD A- carriage and parasitemia among mostly heterozygous multigravid G6PD A- carriers. Similar to the study conducted in Ghana, we also did not find a protective effect of G6PD A- carriage earlier in gestation.

Although we did not detect protection against parasitemia by G6PD A- allele carriage, in this study we found significantly lower parasite densities at delivery associated with G6PD A- carriage among multigravidae. Multigravid G6PD A- carriers had on average 78% lower placental and 65% lower peripheral parasite densities at delivery when compared to G6PD normal multigravidae. This finding suggests a possible interaction between G6PD deficiency and gravidity-dependent immunity to *P. falciparum* infection whereby the inhibition of parasite growth and enhanced phagocytosis of infected cells mediated by G6PD deficiency coupled with gravidity-dependent production of antibodies inhibiting adhesion of infected erythrocytes to CSA may result in an anti-parasitic effect stronger than would be expected by either factor acting alone [7, 11, 14, 19, 20]. *In vitro* studies are needed to understand the effect of G6PD deficiency on parasite adherence and infection of erythrocytes in the placental circulation.

*Maternal anemia*

In this study we report an increase in risk of maternal anemia at enrollment and averaging over the follow-up period associated with G6PD A- carriage among primigravidae. Because malaria parasitemia is most frequent early during the follow-up period and decreases as women approach delivery, due to the receipt of IPTp, the increased risk of parasitemia could be due to infection induced hemolysis among G6PD A- carriers. This would be more pronounced among primigravidae, who generally have a higher risk of *P. falciparum* parasitemia than multigravidae living in holoendemic areas. Among multigravidae, there is some evidence of a protective effect of G6PD A- carriage on anemia at enrollment and averaging over the follow-up period. The prevalence ratio reported in the current study for anemia risk over follow-up is similar to the prevalence ratio obtained from data from the Mockenhaupt study for infected multigravidae (PR=0.71, 95% CI: 0.39, 1.28, authors report OR=0.50, 95% CI: 0.3, 1.0) [16]. However, after accounting for infection, we did not find evidence of a protective effect of G6PD A- carriage on maternal anemia risk at delivery.

*Birth weight*

One of the most surprising findings was the adverse effect of G6PD A- carriage on birth weight. G6PD A- pregnant women bore infants with low birth weight more frequently than G6PD normal women. Furthermore, this difference in risk translated into a large difference in mean birth weight. This is consistent with a previous study in the Gambia in which G6PD A- carriage was associated with stillbirths, miscarriages and infertility [21]. Contrary to expectations, however, G6PD A- carriage of the mother seemed to have a bigger impact on reductions in mean birth weight among daughters compared with sons. It has been

hypothesized that a loss in fitness due to G6PD deficiency may be more pronounced for male hemizygous carriers and female homozygous carriers, compared to female heterozygotes [20]. Most of the G6PD A- carriers in this population of women were heterozygous carriers. We would expect 50% of their sons to be hemizygous carriers, and over 50% of daughters to be homo- or heterozygous carriers. Given that most female carriers would be heterozygous, we would expect to see a larger fitness cost among sons than among daughters. These results may also be observed if there was a higher reproductive cost among male hemizygotes and female homozygotes, so that female heterozygotes were more likely to survive until delivery. A study using mouse models found a higher lethality among female homozygous and male hemizygous G6PD deficient fetuses when compared to female heterozygous carriers [22]. The study also reported significantly lower fetal weight of female homozygous and male hemizygous carriers compared with female heterozygotes. However, female heterozygous fetuses also had significantly higher lethality and lower fetal weight compared to G6PD-normal fetuses. Additionally, another study conducted in Malawi among neonates found that given male gene frequencies for G6PD A- carriage, the gene frequency among females was higher than expected [23]. This would also suggest that female heterozygous carriers may have a survival advantage *in utero* compared to male hemizygotes. To further understand our finding in this population, information on neonates' G6PD status would be needed. Additionally, the range of phenotypic expression of G6PD A- carriage among heterozygous carriers in this population could further elucidate this relationship.

*Limitations*

The interpretation of the results of this study is subject to some limitations. One, placental parasitemia was determined through microscopic examination of placental blood film instead of histological examination, a more sensitive method [24-26]. This may have resulted in misclassification that would have biased the estimates towards the null. Two, unlike the study conducted in Kenya, we did not conduct genetic analyses to detect submicroscopic parasitemia. However, parasitemia detectable by microscopy is more likely to be clinically relevant. Thirdly, a clearer understanding of the effect of G6PD A- status on birth weight would have been aided by information on fathers' and neonates' G6PD A- allele carriage status.

These limitations are offset by the strengths of this study. Perhaps the main strength of the study is that we were able to determine the effects of G6PD A- carriage on placental parasitemia, maternal anemia at delivery and birth weight. To our knowledge, this is the first study to do so in the literature. Secondly, the longitudinal design enabled us to estimate the average effect of G6PD A- on malaria parasitemia and anemia over time. Thirdly, we employ appropriate analytic methods taking into account the frequency of the outcome in the study population and the special attributes of outcome measures, e.g. longitudinal parasitemia risk and semi-continuous parasite density.

*Conclusions*

To conclude, we found no clear evidence of protection against malaria parasitemia and anemia by G6PD A- status. Among multigravidae, there was some evidence of minimal protection against risk of parasitemia at delivery and high parasite density. However, in this

population of women, G6PD A- carriage was a risk factor for low birth weight, with daughters of G6PD A- experiencing the highest reduction in mean birth weight. More studies will be needed to understand the effect of G6PD A- carriage on birth outcomes in malaria-endemic areas.

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**Tables**

Table 1. Study Characteristics

| | Cases (N=46) | Sub-cohort (N= 294) | Full cohort (N=831) |
|---|---|---|---|
| Maternal age, mean±SD | 22.1 ± 5.0 | 23.2 ± 5.4 | 23.4 ± 5.4 |
| Gravidity, % | | | |
|    Primigravidae | 43.5 | 28.5 | 28.4 |
|    Multigravidae | 56.5 | 71.5 | 71.6 |
| Placental parasitemia, % | 100.0 | 15.6 | 13.4 |
| Peripheral parasitemia at delivery, % | 100.0 | 17.0 | 13.5 |
| Peripheral parasitemia at enrollment, % | 37.0 | 31.0 | 30.8 |
| Any parasitemia over follow-up, % | 65.2 | 40.4 | 41.9 |
| Number of parasitemia episodes over follow-up, % | | | |
|     0 | 0.0 | 53.7 | 53.4 |
|     1 | 34.8 | 25.2 | 28.5 |
|     ≥2 | 65.2 | 21.1 | 18.1 |
| Report always using bed net, % | 17.4 | 21.4 | 24.4 |
| Report insecticide treatment of bed net, % | 17.4 | 22.5 | 22.0 |
| SP doses received, % | | | |
|     0 | 6.5 | 6.5 | 4.1 |
|     1 | 26.1 | 16.7 | 18.9 |
|     2 | 32.6 | 37.4 | 39.6 |
|     ≥3 | 34.8 | 39.5 | 37.6 |
| HIV infection, % | 15.9 | 13.5 | 13.5 |
| Anemia at delivery (Hb ≤ 11g/dL), % | 26.7 | 8.8 | 8.9 |
| Anemia at enrollment (Hb ≤ 11g/dL), % | 32.6 | 20.3 | 23.2 |
| G6PD A- carrier, % | 19.6 | 27.6 | |
| HbS trait, % | 6.5 | 6.1 | |
| α-thalassemia, % | | | |
|    -α/αα | 15.2 | 9.9 | |
|    -α/-α | 41.3 | 44.5 | |
| Birth weight (mean±SD) | 2966 ± 428 | 3078 ± 503 | 3056 ± 478 |

Table 2.  Parasitemia and low birth weight according to G6PD status and gravidity

| | Total | | | Primigravidae | | | Multigravidae | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Prevalence* | | | Prevalence | | | Prevalence | | | Interaction P-value** |
| | G6PD A- % (n/N) | G6PD normal % (n/N) | PR (95% CI) | G6PD A- % (n/N) | G6PD normal % (n/N) | PR (95% CI) | G6PD A- % (n/N) | G6PD normal % (n/N) | PR (95%CI) | |
| Placental parasitemia | 13 (9/69) | 17 (37/215) | 0.74 (0.36, 1.49) | 11 (2/19) | 32 (18/56) | 0.87 (0.21, 3.61) | 14 (7/49) | 12 (19/158) | 1.21 ( 0.52, 2.81) | 0.08 |
| Peripheral parasitemia | 19 (13/69) | 21 (45/215) | 1.03 (0.64, 1.66) | 21 (4/19) | 34 (19/56) | 0.93 (0.43, 1.98) | 18 (9/49) | 16 (26/158) | 1.07 (0.59, 1.95) | 0.77 |
| Parasitemia at enrollment | 33 (24/73) | 27 (61/223) | 1.03 (0.87, 1.47) | 68 (15/22) | 58 (34/59) | 1.10 (0.85, 1.42) | 18 (9/50) | 17 (27/163) | 1.01 (0.65, 1.56) | 0.49 |
| Any parasitemia over follow-up | 47 (34/73) | 39 (86/223) | 1.28 (1.10, 1.56) | 77 (17/22) | 66 (39/59) | 1.16 (0.94, 1.42) | 34 (17/50) | 29 (47/163) | 1.29 (0.96, 1.73) | 0.54 |
| Parasitemia ≥ 3 | 4 (3/73) | 9 (21/223) | 0.69 (0.34, 1.43) | 9 (2/22) | 25 (15/59) | 0.55 (0.24, 1.27) | 2 (1/50) | 4 (6/163) | 0.81 (0.23, 2.91) | 0.62 |
| Average parasitemia risk over follow-up | 47 (34/73) | 39 (86/223) | 1.09 (0.74, 1.61) | 77 (17/22) | 66 (39/59) | 0.97 (0.59, 1.57) | 34 (17/50) | 29 (47/163) | 1.16 (0.69, 1.94) | 0.62 |
| Low birth weight | 21 (11/52) | 9 (13/152) | 2.47 (1.18, 5.18) | 30 (6/20) | 11 (4/38) | 3.70 (1.37, 9.98) | 13 (4/31) | 8 (9/113) | 2.12 (0.99, 4.56) | 0.48 |

PR= prevalence ratio, CI=confidence interval

* Proportion of case series with trait

**G6PD A- and gravidity interaction

Table 3. Parasite density according to visit and G6PD A- status

| | Total ARD (95% CI) | Primigravidae ARD (95% CI) | Multigravidae ARD (95%CI) |
|---|---|---|---|
| **Enrollment** | | | |
| Peripheral | 1.48 (0.81, 2.68) | 1.38 (0.76, 2.49) | 1.56 (0.41, 5.97) |
| **Delivery** | | | |
| Placental | 0.63 (0.23, 1.77) | 1.51 (0.29, 7.80) | 0.22 (0.08, 0.56) |
| Peripheral | 0.85 (0.33, 2.16) | 1.88 (0.41, 8.65) | 0.35 (0.14, 0.85) |

ARD = average ratio of densities, CI = confidence interval

Table 4. Anemia risk according to gravidity and G6PD A- carriage status

| | Prevalence | | Total | Prevalence | | Primigravidae | Prevalence | | Multigravidae | Interaction |
| | G6PD A-<br>% (n/N) | G6PD normal<br>% (n/N) | PR (95% CI) | G6PD A-<br>% (n/N) | G6PD normal<br>% (n/N) | PR (95% CI) | G6PD A-<br>% (n/N) | G6PD normal<br>% (n/N) | PR (95%CI) | P-value** |
|---|---|---|---|---|---|---|---|---|---|---|
| Anemia at delivery | 5 (4/77) | 10 (20/196) | 0.51 (0.18, 1.44) | 4 (1/24) | 12 (6/49) | 0.34 (0.04, 2.67) | 6 (3/51) | 10 (14/146) | 0.61 (0.18, 2.05) | 0.62 |
| Anemia at enrollment | 17 (13/78) | 22 (46/212) | 0.78 (0.44, 1.34) | 42 (11/26) | 29 (16/55) | 2.20 (1.27, 3.82) | 4 (2/50) | 19 (30/156) | 0.55 (0.26, 1.18) | 0.01 |
| Average parasitemia risk over follow-up | 30 (23/76) | 37 (76/207) | 0.78 (0.51, 1.21) | 54 (13/24) | 52 (28/54) | 1.70 (1.00, 2.89) | 20 (10/50) | 32 (48/152) | 0.76 (0.50, 1.16) | 0.32 |

Table 5. Effect of mother's G6PD A- carriage on birth weight according to gender of neonate

| | LBW | LBW | Birth weight  mean difference | (95% CI) | |
| Mothers' status | Prevalence | PR (95% CI) | Total | Primigravidae | Multigravidae |
|---|---|---|---|---|---|
| *Sons* | | | | | |
| G6PD A- | 19 (6/32) | 2.66 (0.88, 8.09) | 4 (-205, 197) | -149 (-497, 199) | 206 (-30, 443) |
| Normal | 7 (5/71) | reference | reference | reference | reference |
| *Daughters* | | | | | |
| G6PD A- | 25 (5/20) | 2.53 (0.93, 6.91) | -286 (-529, -42) | -319 (-703, 65) | -234 (-544, 76) |
| Normal | 10 (8/81) | reference | reference | reference | reference |

**Figures**
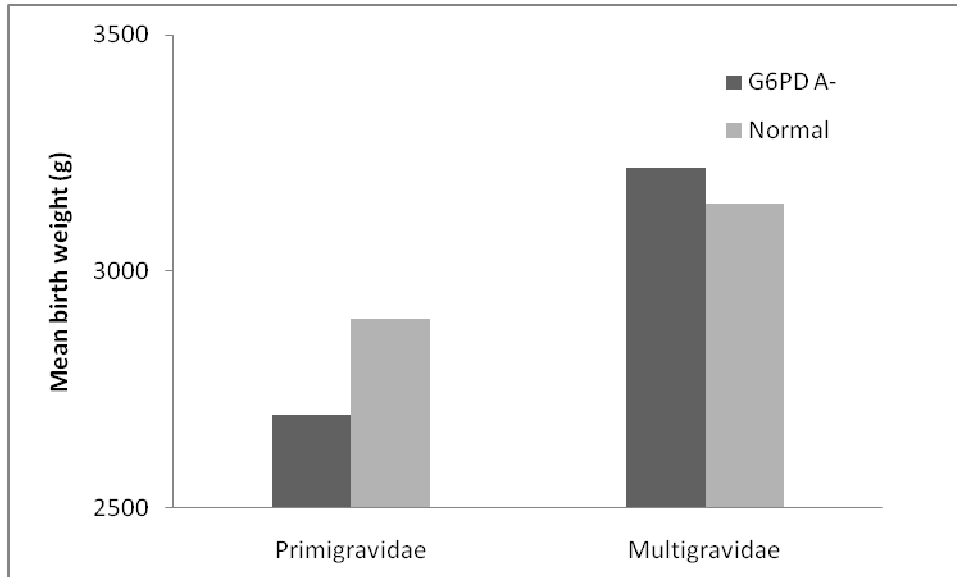


Figure 1. Mean birth weight according to gravidity and G6PD A- allele carriage status
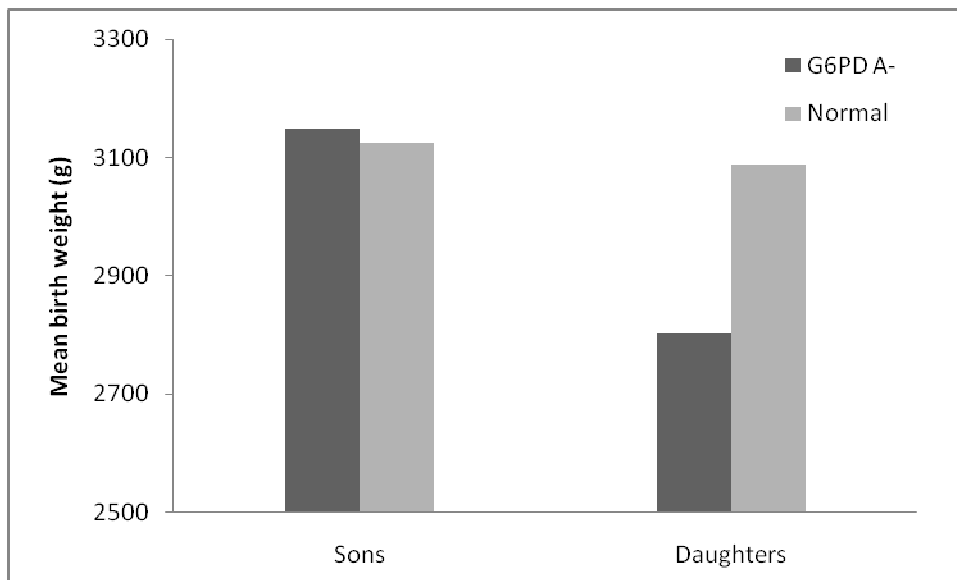


Figure 2. Mean birth weight according to gender of neonate and G6PD A- allele carriage status

# CHAPTER SEVEN - DISCUSSION

## Summary of findings

The purpose of this dissertation was to examine the role of HIV infection and G6PD deficiency in malarial parasitemia risk among pregnant women, and the effect of parasitemia on adverse pregnancy outcomes. The research had three main aims. First, we examined the separate and joint effects of HIV infection and parasitemia on low birth weight and maternal anemia. Second, we explored the impact of HIV infection on the risk, frequency and severity of malaria parasitemia. Third, we investigated the effect of G6PD A- allele carriage on parasitemia, maternal anemia and low birth weight.

### *HIV, malaria parasitemia, and pregnancy outcomes*

In the first part of this research, we found that HIV infection is associated with increased risk of LBW. Additionally, placental, but not peripheral parasitemia at delivery was also associated with increased risk of LBW. However, having three or more episodes of peripheral parasitemia over follow-up resulted in an increased risk of LBW. The results were similar for maternal anemia. Both HIV infection and malaria parasitemia at delivery were associated with increased risk of maternal anemia, and the increase in risk was reflected in differences in mean hemoglobin levels. Increasing number of episodes of parasitemia was also associated with decreasing hemoglobin levels.

Among all women, no interaction between HIV infection and parasitemia were observed in their effects on low birth weight and maternal anemia. However, among multigravidae, there was evidence of interaction between HIV infection and parasitemia in their effect on low birth weight. However, this association was not reflected in differences in mean birth weight. There was no evidence of interaction between HIV and parasitemia in their effect on maternal anemia. Although having dual infection resulted in the largest absolute difference in mean hemoglobin levels, this difference was not more than would be expected assuming the effects of HIV infection and parasitemia were independent.

The finding that both HIV infection and placental parasitemia are associated with increased risk of LBW corroborates previous literature [1-3]. Additionally, similar to previous literature, we found no evidence of interaction between HIV infection and placental parasitemia in their effect on LBW for all women. However, stratifying by gravidity, there was evidence of interaction among multigravidae. This is not consistent with an earlier study in which dual infection with HIV and malaria parasites resulted in a much increased risk of LBW relative to single infection only among primigravidae, but not multigravidae [4]. This difference could be due to dissimilar impacts of HIV infection on parasitemia according to gravidity, examined further in the second section of this research.

One of the more interesting findings of this research was that frequency of parasitemia episodes was associated with increased LBW risk. To our knowledge, there is only one other published study examining the effect of number of episodes of parasitemia on birth outcomes. In the study conducted by Landis and colleagues, having three or more episodes of parasitemia over gestation was associated with an increased risk of intrauterine growth retardation [5]. Since intrauterine growth retardation can result in low birth weight,

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these results suggest that frequency of parasitemia episodes has a deleterious impact on birth outcomes and should inform how parasitemia is defined in studies examining the effect of malaria on the risk of LBW.

The finding that both HIV infection and parasitemia at delivery are independently associated with increased risk of maternal anemia corroborates previous studies[6, 7]. In this study, however, we did not find evidence for interaction between HIV and parasitemia in their effect on maternal anemia. This result differs from an earlier study where the joint effects of HIV infection and placental parasitemia seemed to indicate synergistic interaction between the two infections in their relationship with maternal anemia [4]. However, the definition of anemia employed in that study involved a lower cut-off point (Hb<8g/dL). In the current study, we rarely observed such profound anemia. In addition to the different cut-off for anemia, another reason for the discrepancy may be the multi-factorial nature of anemia and its variation from population to population.

### *Effect of HIV on malaria parasitemia*

In the second part of this research, we report a different effect of HIV on malaria parasitemia during follow-up according to gravidity. Among multigravidae, HIV infection was observed to increase the risk of parasitemia throughout the follow-up period. Further, the magnitude of this increase in risk was fairly constant at different points during follow-up and also averaging over follow-up. The prevalence and risk of parasitemia observed among HIV-infected multigravidae was similar to that observed among primigravidae. Among primigravidae, we did not observe a constant effect of HIV on parasitemia, with the magnitude of the effect estimates increasing with gestation. Among multigravidae, HIV

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infection was also associated with a higher frequency of parasitemia episodes. However, among both primigravidae and multigravidae, HIV infection was associated with higher parasite peripheral parasite density at delivery and higher placental parasite density.

We also reported protection by education and malaria preventive behaviors. At enrollment, HIV-infected primigravidae had a lower prevalence of parasitemia than HIV-uninfected primigravidae. This was mostly explained by higher educational attainment and higher uptake of malaria preventive behaviors by HIV-infected primigravidae when compared to their HIV-uninfected counterparts. Further, low educational attainment continued to be a risk factor for parasitemia over the follow-up period for primigravidae. At delivery, reporting nightly use of bed nets was associated with lower peripheral parasite densities. Reporting nightly use of bed nets and regular treatment of bed nets with insecticide was associated with both lower peripheral and placental parasite densities.

The finding that HIV infection increases the risk of malaria parasitemia among multigravidae to the levels observed among primigravidae corroborates previous literature [8-11]. Among primigravidae, we would have expected HIV infection to either increase the risk of parasitemiak or to have no effect. Our results suggest that the effect of HIV infection on malaria parasitemia among primigravidae may vary over gestation. However, in this population of women there were few HIV infected primigravidae, and the HIV-infected primigravidae differed from HIV-uninfected primigravidae in important ways, i.e. higher educational attainment and adoption of malaria preventive behaviors. More longitudinal studies will need to be conducted to elucidate the relationship between HIV infection and malaria parasitemia over gestation.

Few studies report of the effect of HIV infection on frequency of parasitemia episodes among pregnant women. However, the findings in the current study confirm the results of similar studies among non-pregnant adults [12]. In this study, we found that among multigravidae, HIV infection was strongly associated with having 3 or more episodes of parasitemia. We did not observe a similar effect among primigravidae. This result may explain the interaction of HIV infection and parasitemia in their effect on low birth weight reported in the first part of this research. The finding that HIV infection is associated with higher parasite densities is also in agreement with previous studies [9, 13, 14].

The results of this portion of the dissertation also highlight the effectiveness of malaria prevention. The reduced prevalence of parasitemia among HIV-infected primigravidae when compared to HIV-uninfected primigravidae was mostly explained by the much higher uptake of malaria preventive behaviors by the former. Further, reported use of bed nets and particularly regular impregnation of bed nets in insecticide was associated with lower parasite density at delivery.

### Effect of G6PD deficiency on parasitemia and pregnancy outcomes

In the third part of this research, we investigated the relationship between G6PD deficiency, defined as carriage of the G6PD A- allele, malaria parasitemia and pregnancy outcomes. We did not find evidence of protection against malaria parasitemia and maternal anemia by carriage of the G6PD A- allele. However, among multigravidae, carriage of the G6PD A- allele was associated with lower peripheral parasite density at delivery and lower placental parasite density. Additionally, among multigravidae, there was some suggestion of protection against maternal anemia by G6PD A- deficiency. Among primigravidae, G6PD A-

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deficiency was associated with an increased risk of maternal anemia at enrollment and averaging over follow-up.

Among both gravidities, however, we found that carriage of the G6PD A- allele was strongly associated with LBW.  Further, this translated into a large difference in mean birth weight among G6PD A- mothers compared to G6PD normal mothers.  While the risk of LBW was elevated for both sons and daughters of mothers carrying the G6PD A- allele, a large reduction in mean birth weight was only observed for daughters.

There is only one other study in the literature reporting the effect of carriage of G6PD A- deficiency on parasitemia and anemia risk among pregnant women [15].  After calculating prevalence ratios from the data presented in the study, the effect estimates of the effect of G6PD A- deficiency on malaria parasitemia and maternal anemia among multigravidae were similar to those reported in the current study.  The finding that G6PD A- deficiency among primigravide was associated with higher risk of maternal anemia may be due to infection induced hemolyis, since primigravidae are at higher risk of malaria parasitemia compared to multigravidae.

Although we did not find a strong protective effect of G6PD A- deficiency on parasitemia at delivery, among multigravidae, G6PD A- deficiency was strongly associated with lower parasite density. This may be due to the combination of gravidity-dependent immunity reducing adhesion of infected erythrocytes, and G6PD A- deficiency-mediated inhibition of parasite growth and enhanced phagocytosis of infected cells [16-20]. *In vitro* studies will need to be conducted to understand how malaria parasites interact with G6PD A- deficient erythrocytes in the placental circulation.

Perhaps the most novel finding of this research was the increased risk of LBW due to G6PD A- deficiency. A previous study has reported adverse obstetric outcomes, such as miscarriage and stillbirth associated with G6PD A- deficiency [21]. Further, G6PD deficiency has been hypothesized to reduce fitness of affected individuals. However, contrary to expectations, we found a larger reduction in mean birth weight among daughters of G6PD A- deficient mothers. This may signify that there is a greater reproductive cost among male hemizygous and female homozygous carriers, so that female heterozygous carriers are born alive more frequently, albeit with reduced fitness. This is in agreement with the results of a study conducted using mice models in which female homozygous and male hemizygous fetuses exhibited higher lethality and lower fetal weight than female heterozygous carriers and a survey conducted in southern Malawi among neonates in which the gene frequency for female heterozygous carries of the G6PD A- allele was higher than would be expected given the gene frequency among males [22, 23].

**Conclusions**

The results of this research underscore the importance of targeted interventions to improve pregnancy outcomes among women living in malaria-endemic areas. In this study we report adverse effects of frequency of parasitemia episodes on low birth weight. Further, we report increased risk and frequency of parasitemia due to HIV infection among multigravidae. Recent studies suggest that the current regimen of IPTp may not be effective in reducing malaria and improving pregnancy outcomes among HIV-infected women [24, 25]. The reduced effectiveness of prophylactic drugs should highlight the need for continued

attention to malaria prevention behaviors such as use of insecticide-treated bed nets. In the current research, we report the effectiveness of malaria preventive behaviors in reducing risk, frequency and severity of parasitemia. However, an integrated approach that involves both preventive behaviors and anti-malaria prophylaxis would be maximally beneficial in improving pregnancy outcomes. More research will need to be conducted to determine the most optimal dosing strategies for women of all gravidities, and to find alternative prophylactic regimens in the face of increasing resistance.

The search for alternative anti-malarial drugs must necessarily be informed by the frequency and effects of G6PD deficiency in malaria-endemic countries. In this study we report a high prevalence of G6PD deficiency among Malawian women. Further, we report the lack of a protective effect of G6PD deficiency of parasitemia, and maternal anemia risk. Additionally, we report increased risk of LBW due to G6PD A- deficiency. Future research will be needed to further understand the relationship between G6PD A- deficiency and birth outcomes.

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**APPENDIX A – Informed Consent**

1. <u>**CHIPHASO CHOLOLEDZA – AMAYI APAKATI**</u>

Tikukupemphani kuti mutenge nawo mbali mukafukufukuyu. Kutenga mbali kwanu ndi kwa ufulu. Simuyenera kuvomereza kupanga nawo kuti mulandire chisamaliro, ndikuti muudzabelekere pa chipatala chino. Mutasintha maganizo, mutha kusiya nthawi iliyonse popanda chovuta chilichonse

Matenda a malungo nthawi yomwe mai ali oyembekezera angathe kuononga umoyo wa mwana wake. Pachifukwa ichi, boma la Malawi limalangiza kuti mzimayi aliyense woyembekezera alandire makhwala otetezera malungo. Mogwirizana ndi sukulu ya University ya North Carolina, ife tikufuna kufufuza kuti tione ngati malangizowo akugwiradi ntchito. Kafukufukuyu adzathandiza kupeza malangizo oyenerera opewera matenda a malungo, mtsogolomu.

Ngati muvomereza kulowa kafukufukuyu anamwino adzatenga timagazi pang'ono, kuti akayeze ngati muli ndi malungo, chindoko, kapenanso kuchepa kwa magazi. Magazi onse adzatengedwa ndi zoyezera ndi zobayila zatsopano. Nthawi zina pangaoneke kabala (kachilonda) kakang'ono pamalo pomwe patengedwa magazi. Anamwino adzakuyezani sikelo ndikukufunsani mafunso angapo. Mutapezeka ndi malungo kapena chindoko, mudzapatsidwa mankhwala oti akuchiritseni. Mukadali oyembekezela chomwechi, mudzafunsidwa kukaunikidwa mwezi ndi mwezi kuti tione kuti mwana akukula bwanji. Mukadzabereka, tidzamuyezanso mwana wanu, ndi kutenga timagazi pang'ono kuchokela pa nchombo, ndi pa nsengwa ya mwana kukayezanso malungo. Ndondomeko yonse kuchokera pa ulendo woyambawu, ndi maulendo otsatira ndikubereka mwana wanu zidzaikidwa m'mabukhu achinsinsi, ndi kufufuzidwa bwino.

Kuonjezera apa tidzakufunsani ngati mungafune kuti mudziwe ngati muli ndi kachilombo ka HIV. Ngati mudzalore kuyezedwa, tidzakulongosolerani zonse zokhudzana ndi kuyeza kwa kachilombo ka HIV tisanatenge magaziwa. Tidzakudziwitsani zotsatira zake, ndipo tizakambirana nanu kutanthauza kwa zotsalirazi pamoyo wanu ndi banja lanu. Zonsezi zidzachitika mwachinsinsi, ndipo ndi inu nokha osati wina aliyense yemwe atazadziwe zotsatira za zomwe mwayezedwa. Muli ololedwa kukhala nawo mukafukufukuyi ngakhale mutakhala kuti simukufuna kuti tikuyezeni matenda a HIV. Tikadzakupezani ndi matenda a HIV, inu ndi mwana wanu akangobadwa kumene mudzapatsidwa

makhwala otchedwa nevirapine omwe amateteza theka la ana omwe anakatenga kachilombo ka HIV kuchokera kwa mai awo. Nthawi ino sitingathe kukupatsani mankhwala a HIV koma tizakuthandizani kuti mudzalandire mankhwalawa mwamsanga akazapezeka ku unduna wa zaumoyo ndi chiwerengelo cha anthu.

Tikutsimikiza kuti tidzasunga mapepala anu onse a zotsatira zonse zamukafukufukuyi mwa chimsisi ndipo palibe wina aliyense yemwe azathe kudziwa dzina lanu kuchokera pa mapepalawa. Mapepala anu onse tidzasunga pamalo obisika mu kabati ya chitsulo yomwe tidzizatsekamo ndi kiyi poti wina aliyense sazatha kupeza zotsatirazi.

**Ngati muli ndi mafunso apadera**

Ngati muli ndi mafunso pankhaniyi ngakhale zina zonse zokhuzana ndi kafukufukuyu, kapena chobvuta chili chonse chingabwere chifukwa chakafukufukuyi muyankhule ndi anthu awa pamaso ngakhale pa telefoni;

**Mr I. Mofolo  Telefoni  01671911 kapena 09957678**
**Mrs E. Chaluluka Telefoni 01671911 kapena 08308339**

Kodi mukuvomereza kulowa kafukufukuyu?

**Kuvomeredza**
Ine ndawerenga/ndamva zonse zimene zalembedwazi ndipo ndamvetsa tanthauzo la zinthu zimenezi. Choncho ndavomera kuti ndikhale mmodzi mwa anthu omwe atenge nawo mbali mu kafukufuku uyu.

*Chitsimikizo ............................................................... Tsiku_____/____/____*

**Chidindo cha chala:………………………………**

# NEW MALARIA IN PREGNANCY STUDY

SOP version 14 March 2005

## DESCRIPTION

A study of pregnant women to look at the effects of malaria, SES and HIV on birth weight and maternal haemoglobin.

## STUDY PARTICIPANTS

All pregnant women (14-26 weeks) at 1st antenatal visit in Mpemba and Madziabango.

## ENROLLMENT PROCEDURE

### 1st VISIT

1.  Identify pregnant women between 14 – 26 weeks of gestation at their first ANC visit
2.  Administer VCT including counseling and rapid tests
3.   Fill in VCT proforma
4.  Obtain informed consent
5.  Assign study number and write it on all proformas
6.  Place identity sticker on health passport
7.  Take relevant history; medical and obstetric
8.  Physical examination: BP, weight and height
9.  Lab tests: malaria, hemocue, syphilis, temperature
10. Administer SP (DOT), Folate and Iron
11. Fill in Enrollment proforma make a patient folder
12. Fill in SES proforma

### ALL SUBSEQUENT VISITS

1.  ANC Visit proforma
2.  Physical examination
3.  Administer SP if >4 weeks since 1$^{st}$ dose + Folate and Iron if needed
4.  VCT if didn't consent during the 1$^{st}$ visit
5.  Lab tests:  malaria and hemocue

### Other visits:

1.  Fill in a new ANC follow up proforma for each additional visit
2.  Do malaria tests and hemocues

DELIVERY

### During labour

1.  Administer nevirapine to HIV positive moms only

### At delivery

2.  Lab tests – malaria smears on mother and placenta;  Hemocue on mother
3.  Measure baby's birth weight and Ballard score
4.  Delivery information – mother proforma
5.  Delivery information – baby proforma

Administer nevirapine to babies of HIV-positive mothers and fill in nevirapine proforma

## *VCT Proforma*

## Agreed to VCT

Yes          No

## Pre-test counseling

Given by_____

Initials_____          Date_____

**Test results**

Determine  …….          Initials/Date _____
Unigold   ………          Initials/Date _____
Hema-Strip ……          Initials/Date _____

          1= positive
          2=negative
          3= indeterminate
          4= not done

## Post-test counseling

Given by_____

Initials_____          Date_____

## Enrollment  Proforma

IS THIS THE PATIENT'S FIRST VISIT? YES/NO Visit date __/__/____

If yes, proceed


Name _____ Age _____ Gravidity _____

Weeks pregnant _____ Determined by:   dates/ palpation

Residence location _____



Malaria history

Prior antimalarials during this pregnancy? Yes/No If yes, number of doses _____

Recent fever (in past week)?   Yes/No

Net user? (circle appropriate answer) 1 Never 2 Sometimes 3 Usually 4 Always

Net impregnated? 1 Never 2 > 6 mos. 3 within 6 mos. 4 Don't know



Physical exam/labs

Maternal height _____cm Maternal weight ____.___ kg

Mother's HemoCue _____g/dl

Syphilis rapid test result          positive/negative          Treatment given  yes/no

Temperature at clinic _____

Peripheral film 0 + ++ +++ ++++ Peripheral count _____



Procedures

Study number assigned?  Enter on all proformas

SP given at this visit? Yes/No Administered how? DOT Take home Unknown

Filter paper made and numbered?   Yes  No



**Obstetric or medical complications**

# SES Profoma

## 1.  INFORMATION ON PARTICIPANT

Age of mother (years) ....................................................................................I__I__I

What is the highest level of schooling you have had  (circle)       0=none

           1=standard 1-4

           2=standard 5-8

           3=form 1-2

           4=form 3-4

           5=university

## 2.  INFORMATION ON COMPOSITION OF HOUSEHOLD

Total number of persons in household (people who normally eat together).......................I__I__I

Total number of children in household  ...........................................................................I__I__I

What is mother's marital status? .......... 1 = Married with husband in the household

         2 = Single

         3 = Divorced or separated

         4 = Widowed

         9 = Not known

## 3.  INFORMATION ON FAMILY INCOME AND ECONOMIC STATUS

What do you do?            1 working for wages

              2 selling items

|                                                              |                      |
|--------------------------------------------------------------|----------------------|
|                                                              | 3 none               |
|                                                              | 9 not known          |

What does your husband do?                                       1 working for wages
                                                                 2 selling items
                                                                 3 none
                                                                 9 not known

Does your family earn enough to meet its needs for the year?
                                                                 1 Yes
                                                                 2. No

Does your family have a steady (regular) source of income over the year?
                                                                 1 Yes
                                                                 2. No

Does the household possess a

|          | Yes | No |
|----------|-----|----|
| Mattress |     |    |
| Radio    |     |    |
| Bicycle  |     |    |
| Car      |     |    |

## 4.   INFORMATION ON HOUSING

What is the building material of the walls of the main house?............. 1 = Poles and grass

2 = Poles and mud

   If other, please specify _____   3 = Mud

4 = Mud brick

5 = Fired brick

8 = Other

9 = Not known

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What is the building material of the roof of the main house? .............. 1 = Grass

                                                                                  2 = Iron sheets

       If other, please specify _____ 3 = Tiles

                                                                                  8 = Other

                                                                                  9 = Not known

What kind of windows does the main house have? ........................... 0 = None

                                                                        1 = Open windows

       If other, please specify _____ 2 = Screen or glass

                                                                                  8 = Other

                                                                                  9 = Not known

What is the source of drinking water? ............................................... 1 = Piped water

                                                                        2 = Borehole

       If other, please specify _____ 3 = Protected well

                                                                                   4 = Unprotected well

                                                                                  5 = Lake

                                                                                  6 = River, pond

                                                                                  8 = Other

                                                                                  9 = Not known

Sanitary facilities .............................................................................. 0 = None

                                                                        1 = Ventilated improved pit latrine

       If other, please specify _____ 2 = Regular pit latrine

                                                                                 8 = Other

                                                                                  9 = Not known

**(PLEASE FILL OUT A NEW SEPARATE SHEET FOR EACH VISIT)**

## ANC Visit Proforma

Visit date ___/___/_____

Name _____ Age _____ Gravidity _____

Visit number _____Weeks pregnant ____ Determined by dates estimated palpation

Antimalarials since last visit? Yes/No

Peripheral film 0 + ++ +++ ++++ Peripheral count _____

Recent fever (past 7 days)  Yes/No

Temperature at clinic _____

SP given at this visit? Yes/No Administered how? DOT Take home Unknown

Other medications given (such as quinine)?  Please give drug and dosage.

# Delivery information - Mother

**1.**                                                                                    **DELIVERY**

Date and time of delivery  _____

Place of delivery:                           ..............................1 = Home

                                                                          2 = TBA facility

If other, please specify: _____        3 = Health centre

                                                                          4 = District or CHAM

                                                                              hospital

                                                                          5 = Central hospital

                                                                          8 = Other

                                                                          9 = Not known

       Attendant during delivery ...........................................0 = None

                                                                          1 = TBA

                                                                          2 = Friend, relative or other

                                                                          3 = Nurse / midwife

                                                                          4 = Ward attendant

                                                                          5 = Clinical officer

                                                                          6 = Doctor

                                                                          9 = Not known

       Duration of delivery:.......................................................1 = Less than 12 hours

                                                                          2 =  12 -24 hours

                                                                          3 = More than 24 hours

       Number of children born: _____

       Presentation of child: ....................................................1 = Breach

                                                                          2 = Vertex

If other, please specify: _____        3 = Feet

                                                                          4 = Hands

                                                                          5 = Cord

                                                                          6 = Face

                                                                          8 = Other

                                                                          9 = Not known

Mode of delivery:.................................................................1 = Normal vaginal

2 = Normal vaginal with large perineal tear

3 = Elective caesarean section

4 = Emergency caesarean section

5 = Forcep

6 = Vacuum extraction

8 = Other                          If other, please specify: _____

9 = Not known


Were there any delivery complications? .................................0 = No

                                                                  1 = Yes


If yes, please describe:




Were you referred to a health centre or a hospital?..................        0 = No

   If yes, reason: _____        1 = Yes

**Physical exam/Lab results**

Fever in week before delivery   Yes/no

Fever at delivery  Yes/no  (Take temperature if in clinic)

Fever after delivery  Yes/No

Blood pressure _____

Hemocue  _____

Maternal peripheral parasitemia film 0 + ++ +++ ++++

                               count _____

Placental parasitemia film       0 + ++ +++ ++++    count _____

Filter paper taken?  Yes/no

# Delivery Information on Newborn

Date of birth ___/___/___         Date child seen ___/___/___

Age of child when seen  (Days) .................................................................._____

Was the child born alive?..............................................................................0 = No

1 = Yes

If *no*, type of stillbirth ...............................................................................1 =
Fresh

2 =

Macerated

Sex of the child .........................................................................................1 = Girl

2 = Boy

Child's size at birth:

      Weight (g) I__I__I__I__I

      Length (mm) I__I__I__I

      Chest circumference (mm) I__I__I__I

      Head circumference (mm) I__I__I__I

      Mid upper-arm circumference (mm) I__I__I__I

Ballard (0 – 4/5 points each):

Points:

| | |
|---|---|
| Skin | |
| Lanugo | |
| Plantar creases | |
| Breast | |
| Ear | |
| Genitals | |
| **Total:** | |

# Nevirapine proforma

Nevirapine tablet (200 mg) to mother

Given by _____

Initials _____     Time _____     Date _____

Nevirapine syrup (0.2 ml per kg) to baby

Given by _____

Initials _____     Time _____     Date _____