BARRIERS TO USE OF HEALTHCARE DURING PREGNANCY IN NIGERIA

by

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STATEMENT BY AUTHOR

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DEDICATION

To my parents—thanks for letting me explore the world beyond our fencepost.

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ABSTRACT

INTRODUCTION: In sub-Saharan Africa, access to care during pregnancy and child birth is an abiding challenge for many women. Many women face socioeconomic, cultural, and physical barriers while attempting to access healthcare facilities during pregnancy. These barriers often result in women accessing healthcare facilities after life-threatening complications develop, ultimately leading to high rates of maternal mortality. In Nigeria, several locally endemic diseases, such as malaria and HIV, impinge on population health. Having access to care during pregnancy provides an opportunity for prompt diagnosis, treatment, and prevention of common endemic disease. This dissertation focused on access to care during pregnancy in Nigeria by using two indicators: malaria parasitemia and Cesarean-section (CS). Therefore, this dissertation had two overarching goals. The first was to estimate the prevalence of malaria parasitemia during pregnancy and to determine maternal risk factors associated with high malaria parasitemia in Enugu State, Nigeria. The second was to establish the incidence of CS and to determine the socioeconomic and medical risk factors that are associated with having a CS among women in Enugu State, as well as in Nigeria as a whole.

METHODS: Secondary analyses of two unique datasets —Healthy Beginnings Initiative (HBI) and the Nigerian Demographic and Health Survey (DHS) —were conducted. The HBI cohort study took place in Enugu State, Nigeria. The prevalence of peripheral malaria parasitemia in Enugu State was established within the context of HBI. Malaria parasitemia was scored according to the Malaria Plus System (0 to ++++). For this dissertation those in the 0 and + group were classified as low having parasitemia; while those in the ++ and +++ groups were classified as having high parasitemia. Person-

level maternal risk factors that were associated with high malaria parasitemia were estimated using crude and adjusted logistic regression modeling with malaria parasitemia as the main outcome. The incidence of CS in Enugu State was also estimated within context of the HBI cohort. Socioeconomic and medical risk factors associated with having a CS in Enugu State, Nigeria were estimated. To investigate the extent to which the findings from the HBI represent the rates of CS in Nigeria as a whole, the Nigerian DHS was utilized. The Nigerian DHS was a cross-sectional study that was conducted throughout Nigeria. The incidence of CS in all of Nigeria was estimated. Socioeconomic and medical risk factors associated with having a CS were also investigated. Crude and adjusted logistic regression models with CS as the main outcome are presented. Weights were applied to all analyses conducted with the DHS to make the data representative at the county level.

RESULTS: Over 99% of women in the HBI study tested positive for peripheral malaria parasitemia. For each additional person in the household, a 6% lower odds of high malaria parasitemia was found ($p<0.05$). Regarding CS, analyses of both datasets indicated that Nigeria has relatively low rates of CS compared to the World Health Organization's recommendations. In the HBI, 7.2% of women in Enugu State, Nigeria had a CS. Significantly lower odds of having a CS were observed among women who live in a rural setting compared to those who reside in an urban setting ($p<0.05$). Percentages of CS increased significantly as maternal age and/or education increased. HBI results demonstrated 53% higher odds of having a CS if participants had high malaria parasitemia compared to those with lower malaria parasitemia ($p<0.05$). Results of the DHS yielded even lower rates of CS with only 2.3% of women in Nigeria overall having had a CS during their last delivery. Consistent with analysis for Enugu State, in the DHS women living in rural areas had significantly lower odds of having a CS than those living in urban areas ($p<0.05$). Likewise, religion was significantly associated with having had a CS; Muslim women had 54% lower odds of having a CS compared to Catholics ($p<0.05$). Women who had health insurance and women who received prenatal care from a skilled birth attendant had increased odds of having a CS compared to women who did not have insurance and received no prenatal care (adjusted OR [aOR] 1.78: 95%CI 1.18-2.67, $p<0.05$; aOR 3.00: 1.51-5.96, $p<0.05$).

DISCUSSION: Based on the high prevalence of malaria among women in the HBI study, education on best practices to prevent malaria during pregnancy, and resources in support of these practices are urgently needed. Likewise, low rates of CS in both Enugu State and across Nigeria indicate that Nigerian women may not have adequate access to obstetric care during delivery. Results from this dissertation also indicate that Nigerian women face barriers in obtaining adequate perinatal healthcare, ultimately perpetuating the cycle of high maternal mortality and gross health deficiencies that are common to Nigerian women.

CHAPTER 1

INTRODUCTION

The Problem

In 2014, an estimated 134,028,000 live births occurred globally; the equivalent of 255 births per minute [1]. Many of these births occur in developing countries, where adequate prenatal care and healthcare facilities are either not accessible or nonexistent [2]. Obtaining prenatal care is essential to the prevention, detection, and treatment of health-related complications during pregnancy [3]. However, a number of individual and health system characteristics contribute to the delay in women seeking healthcare services during pregnancy [2, 4]. A disproportionate number of maternal and fetal deaths occur in sub-Saharan Africa [5, 6].

As one of the fastest growing populations in the world, Nigeria is a key location for studying woman's access to healthcare during pregnancy. This rapid population growth is at least partially attributable to Nigeria's relatively high crude birth rate, at 38.03 births per 1,000 women [1]. In 2013, Nigeria had the second largest proportion of global maternal deaths at 14% (n=40,000) [7]. Nigeria is also an important location for examining the burden of infectious disease during pregnancy as two key contributors to maternal mortality are human immunodeficiency virus (HIV) and malaria are common in Nigeria [8]. Therefore, accessing health care during pregnancy is essential to decreasing maternal mortality.

Only an estimated 35% of Nigerian women deliver at a healthcare facility [9] as compared to 47% in Kenya[10] and 40% in Cameroon[11]. This is problematic because obstetric complications occur most often around the time of delivery. Increasing access to emergency obstetric care, such as Cesarean sections (CS), decreases maternal and infant morbidity and mortality [12, 13]. These deaths would be largely preventable with improved quality and increased coverage of healthcare [8]. However, previous research has demonstrated that women encounter numerous barriers while attempting to access healthcare during pregnancy, e.g. disease, distance to healthcare facility, lack of transportation, and cost of procedures [2, 4]. Therefore, this dissertation had two overarching goals. The first goal was to estimate the malaria burden of pregnant women and to explore maternal risk factors associated with high malaria parasitemia in Enugu State, Nigeria; the second goal was to estimate the incidence of CS in Nigeria and determine what socioeconomic and medical risk factors are associated with having a CS among women in Enugu State, as well as in Nigeria overall. This will all be discussed within the framework of Thaddeus and Maine's Three Delay Model [2] and the United Nations' Millennium Development Goals [14] in the introduction.

Goals and objectives

This dissertation will focus on common complications of pregnancy in Nigeria, by using data from the Healthy Beginning Initiative Cohort in Enugu State, Nigeria, as well as data from the Demographic and Health Survey. The Specific Aims for this dissertation were to:

1. Estimate the population-based malaria burden during pregnancy in Enugu State, located in southeastern Nigeria;
2. Explore person-level maternal risk factors associated with high malaria parasitemia;

3. Estimate the incidence of CS among pregnant women in Enugu State, Nigeria using data from the Healthy Beginnings Initiative;
4. Determine if socioeconomic or medical risk factors are associated with having a CS within Enugu State, Nigeria;
5. Estimate the incidence of CS among pregnant women throughout Nigeria using the Demographic and Health Study dataset; and to
6. Determine if socioeconomic or medical risk factors are associated with having a CS in Nigeria.

Role of the author in the present research

Each Aim for this dissertation required the author to conduct a secondary data analysis. For Specific Aims 1 – 4, data from the Healthy Beginnings Initiative cohort was used, which were collected in Enugu State, Nigeria. The purpose of the Healthy Beginnings Initiative was to determine if providing onsite laboratory testing of common diseases to pregnant women and their spouses increased testing uptake. The author developed a statistical analysis plan based on guidance provided by the principal investigator of the overall study. The author also wrote two manuscripts using this data: one based on Specific Aims 1 and 2, and another based on Specific Aims 3 and 4. The parent study was approved by the Institutional Review Board of the University of Nevada, Reno, and the Nigerian National Health Research Ethics Committee. This secondary analysis was considered exempt from human subjects review by the Mel and Enid Zuckerman College of Public Health Research Office.

A third manuscript explored Specific Aims 5 and 6. For this manuscript a secondary analysis of the Demographic and Health Surveys (DHS) conducted in Nigeria was performed. The DHS data are publically available to researchers. The author developed the statistical analysis plan based on the recommendations of the authors of the DHS, and was responsible for all data analysis and interpretation. This secondary analysis was also considered exempt from human subjects review by the Mel and Enid Zuckerman College of Public Health Research Office.

Background

It is well-documented that increasing women's access to care during pregnancy has long-lasting positive implications for her health and the health of her unborn fetus, reducing morbidity and mortality during pregnancy. However, pregnant women in developing countries often delay seeking care because of the large number of obstacles they must overcome to obtain treatment at a healthcare facility [2, 4]. These barriers include lack of knowledge of the importance of seeking care during pregnancy, poverty, gender inequalities in household decision-making, cultural barriers, and geographical and transportation barriers [2, 4]. Overcoming these barriers is essential to decrease maternal and perinatal mortality in developing countries. Improving access to care decreases the negative effects of common comorbid conditions associated with pregnancy, including malaria, HIV, and anemia. These diseases require medical or behavioral interventions to prevent future negative consequences for mothers and their neonates. Decreasing the burden of malaria during pregnancy is essential, as malarial infection during pregnancy is associated with an increase in maternal and perinatal mortality [15-18]. Therefore, determining the prevalence of malaria among pregnant women in holoendemic areas is crucial to evaluating if women are obtaining adequate prenatal care (Aims 1 and 2).

An understanding of the common barriers associated with accessing healthcare during pregnancy is also important. Prompt access to life saving obstetric services, such as CS, are needed to decrease maternal mortality in many developing countries. However, many women face ongoing barriers that restrict them from accessing healthcare during delivery. By determining the factors that are associated with uptake of CS, we can better understand how to best allocate resources for future programing that targets improving healthcare utilization among pregnant women (Aims 3-6).

In this dissertation I will first discuss maternal mortality worldwide and in sub-Saharan Africa (SSA). Next, I will provide an overview of the United Nations Millennium Development Goals, which helped to provide a context for the Aims of this dissertation. Additionally, I will discuss the Three Delay Model, a framework to organize factors affecting women's access to care in developing countries. Based on the Millennium Development Goals and the Three Delay Model, this dissertation took a two-pronged approach to discussing maternal mortality by investigating the disease-related, and socioeconomic and cultural factors commonly associated with access to healthcare and maternal mortality. Next, CS and its role in reducing maternal mortality in SSA will be presented. Finally, the focus will turn to Nigeria, which contributes 14% of the global burden of maternal deaths, as addressing factors that lead to maternal mortality in Nigeria is essential to reducing global maternal mortality rates [7]. This dissertation includes 3 unique studies and 6 Aims, which will be discussed in Chapters 2 – 4.

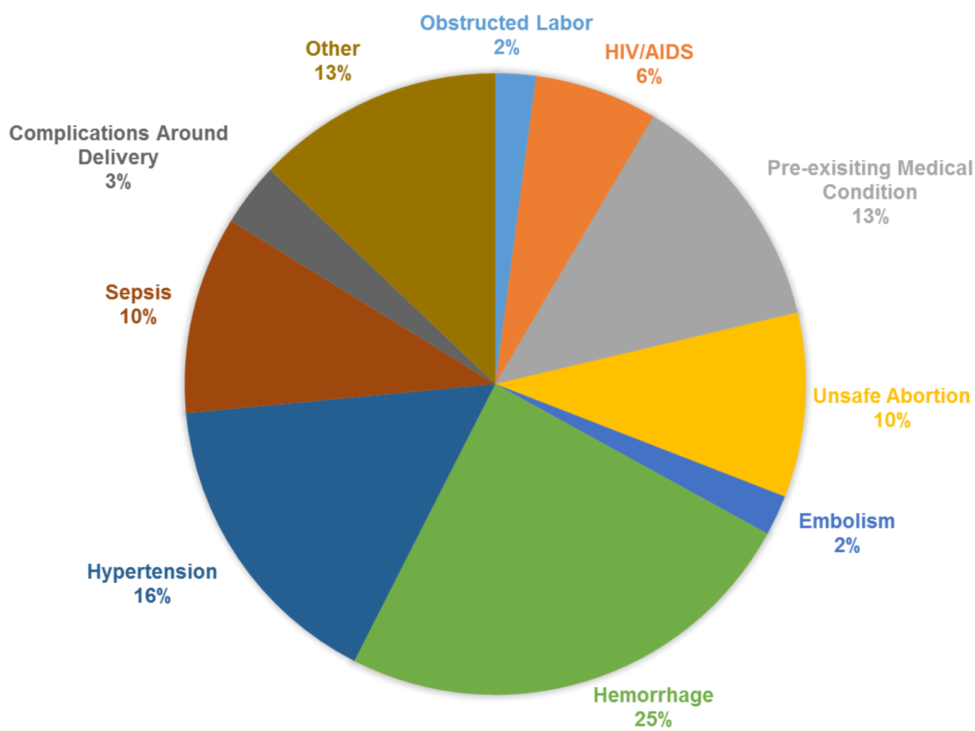
Worldwide maternal mortality: Developed vs. developing countries

In 2013, 289,000 women died of mostly preventable pregnancy complications [5]. This equates to approximately 800 women per day who die from complications during pregnancy or directly after childbirth [5]. Much of this burden is borne by women in developing countries, where over 99% of maternal deaths took place in 2013. In that year, the maternal mortality ratio in developing countries was 230 per 100,000 live births compared to 16 per 100,000 live births in developed countries [5]. Furthermore, according to the World Health Organization (WHO): “A woman’s lifetime risk of maternal death – the probability that a 15 year old woman will eventually die from a maternal cause – is 1 in 3700 in developed countries, versus 1 in 160 in developing countries” [5]. Worldwide, 75% of maternal deaths can be attributed to 5 factors: severe bleeding (most common after childbirth), infections (most common after childbirth), pre-eclampsia or eclampsia during pregnancy, complications associated with delivery, and unsafe abortion [19]; the remaining 25% of maternal deaths are attributed to diseases such as acquired immunodeficiency syndrome (AIDS) or malaria during pregnancy [5].

Maternal mortality in sub-Saharan Africa

Sub-Saharan Africa has historically carried a disproportionate burden of adverse health related outcomes for women and children [14]. The adult lifetime risk of maternal death is 1 in every 31 women in SSA [20]. According to a recent meta-analyses, maternal deaths in SSA can be attributed to: obstructed labor [2.1% (95% CI: 0.7–5.2)], HIV/AIDS related complications [6.4% (4.6–8.8%)], pre-existing medical conditions [12.8% (7.0–22.3%)], unsafe abortion [9.6% (5.1–17.2%)], embolism [2.1% (0.8–4.5%)], hemorrhage [24.5% (16.9–34.1%)], hypertension [16.0% (11.7–21%)], sepsis [10.3% (5.5–18.2%)], complications of delivery [3.3% (1.5–6.7)] and other causes (13%) (See Figure 1) [19]. Therefore, the majority of maternal deaths in SSA can be attributed to lack of access to adequate care during pregnancy and birth. However, addressing a woman's access to proper healthcare during pregnancy in SSA and many other parts of the world is often not straightforward. In order to address health disparities such as this, the United Nations created the Millennium Development Goals.

Figure 1. Reasons for Maternal Mortality in sub-Saharan Africa



Adapted from Say, L., et al. Global causes of maternal death: A WHO systematic analysis, 2014.

United Nations Millennium Development Goals

The Millennium Development Goals include 8 target goals with the aims of decreasing poverty, hunger, illiteracy, gender inequality, and diseases; while also attempting to increase environmental sustainability and access to healthcare, and were established by the United Nations in 2000 [14]. These goals were created in an attempt to focus efforts toward achieving common global objectives, including: 1) eradicating extreme poverty and hunger; 2) achieving universal primary education; 3) promoting gender equality and empowering women; 4) reducing child mortality; 5) improving maternal health; 6) combating diseases such as HIV/AIDS and malaria; 7) ensuring environmental sustainability; and 8) growing a global partnership for development [14].

Millennium Development Goals 5—improving maternal health—and 6—combating diseases—are particularly relevant to the present research. Goal 5 has two sub-goals: 1) to reduce the worldwide maternal mortality ratio by 75%, from 380 maternal deaths per 100,000 in 1990, to 95 maternal deaths per 100,000 in 2015; and 2) to achieve universal access to reproductive healthcare by 2015 [21]. Millennium Development Goal 6, has three sub-goals: 1) to halt and begin reversing the spread of HIV by 2015; 2) to provide universal access to treatment for HIV/AIDS by 2010; and 3) to halt and begin to reverse the incidence of malaria and other major diseases by 2015 [21]. Although progress has been made toward the Millennium Development Goals, they have been difficult to achieve, in part because women in developing countries are disproportionately burdened by barriers to reaching these goals, including lack of education, equality, access to adequate care, and poverty [22].

In order to meet Millennium Development Goals 5 and 6, vast improvements in facilities, infrastructure, and training of skilled obstetricians needed to be made within SSA. However, in 2013, SSA continued to have the largest maternal mortality ratio at 510 deaths for every 100,000 live births in women between the ages of 15–49 [7]. Although this is a vast improvement to 1990 estimates of 990 deaths for every 100,000 live births in women aged 15–49 years, it is still more than double any other area in the world [7]. It is also more than the initial world estimate of 330 maternal deaths for every 100,000 live births [7]. In order to achieve Millennium Development Goal 5, an average annual decrease of 5.5% in maternal mortality ratio was needed in SSA [14]. However, between 1990–2013, the average annual decrease in the maternal mortality ratio in SSA was only 2.9%; approximately half of the target goal [7]. Furthermore in 2013, SSA still accounted for 62% of the global burden of maternal deaths [7]. Accessing healthcare during pregnancy is essential to achieving Millennium Development Goals 5 and 6. However, women must overcome multiple barriers in order to gain access to care in SSA. Understanding when women seek—or do not seek—treatment during pregnancy is essential to understanding why it has been difficult to improve maternal health (Goal 5) and combat diseases (Goal 6) in SSA.

Three Delay Model

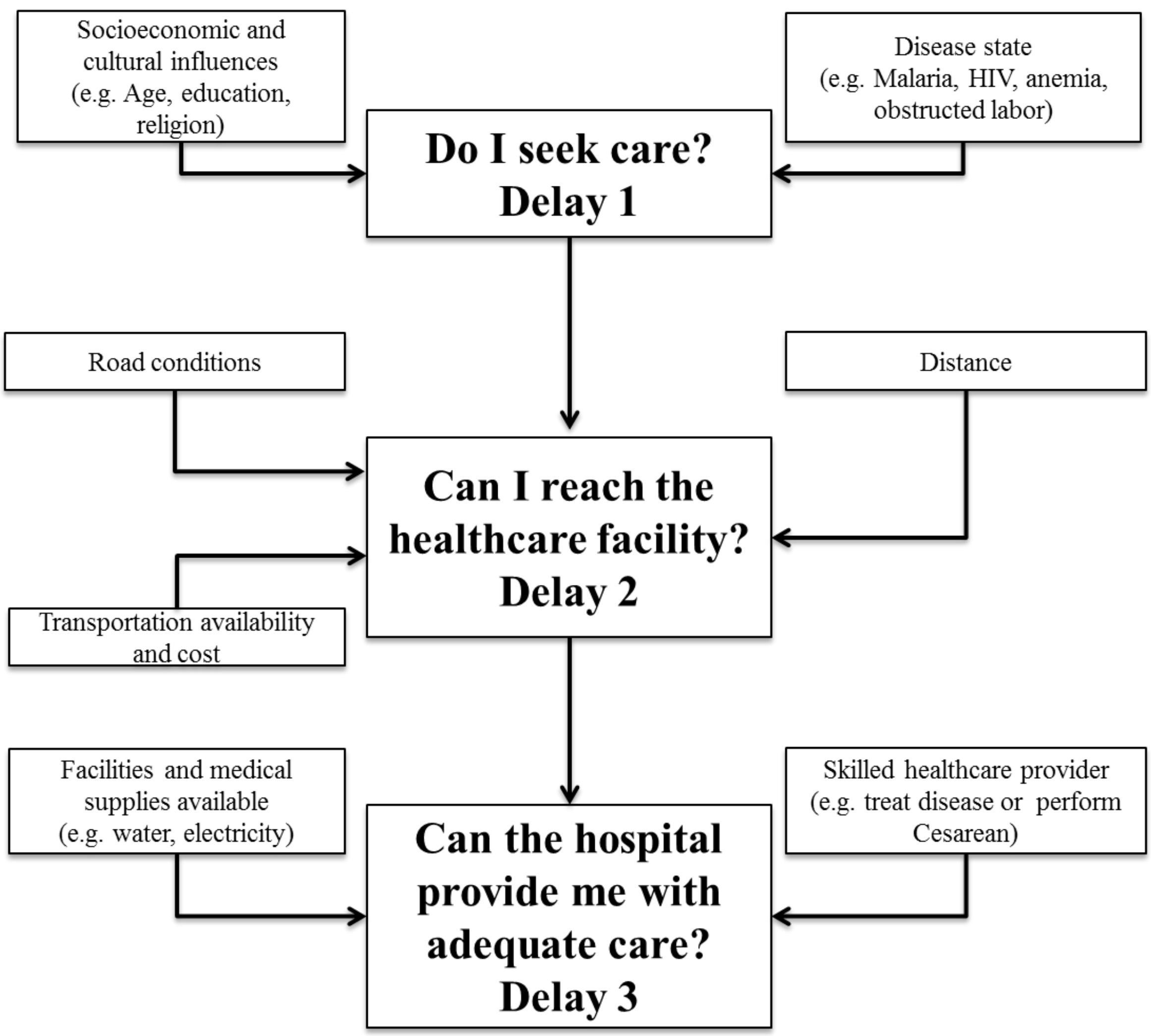
The Three Delay Model is a conceptual framework that provides organization for the factors affecting why women delay accessing care in developing countries. A number of individual and health system characteristics obstruct access to healthcare and contribute to the delay in women seeking healthcare services during pregnancy [2]. In order to provide a framework for the obstacles women face while obtaining adequate healthcare during pregnancy, Thaddeus and Maine developed the widely accepted Three Delay Model [2]. This framework includes three kinds of delays: 1) delays in decisions to seek care, 2) delays in arrival at a healthcare facility, and 3) delays in receiving adequate treatment for obstetric complications (see Figure 2).

In SSA, several factors affect a woman's decision to seek treatment during pregnancy. Delays in seeking treatment during pregnancy (Delay 1) may come from a variety of sources, including the financial costs of healthcare; the status of a woman within her family; culture; age; education; and a woman's overall health. Although healthcare-seeking behavior is typically influenced by illness [2], delaying care may increase the likelihood of medical complications and increase adverse events during pregnancy and delivery.

Delays in reaching an adequate healthcare facility (Delay 2) are caused by factors that affect a woman's ability to physically reach a healthcare facility [2]. In order to reach a healthcare facility, many women in SSA must either walk long distances—often in rough terrain—or access an alternative form of transportation. Women living in rural areas are often at an increased disadvantage as they must overcome limited transportation options, poor road conditions, and high costs associated with travel [2, 4, 23]. Delays in receiving adequate care at a healthcare facility (Delay 3) occur when factors affect a facility's ability to provide needed medical services. Healthcare facilities in developing countries often suffer from shortages in trained personal, supplies, and equipment.

The Three Delay Model acknowledges that no decision is made in isolation and that an overall connectedness between delays exists. The factors affecting seeking treatment during pregnancy and reaching an adequate healthcare facility—Delays 1 and 2—are so closely intertwined that it makes little sense to discuss them in isolation. Therefore, in the following sections this dissertation will focus on diseases that are common in pregnant women in SSA and the socioeconomic and cultural influences that affect a woman's decision to seek treatment.

Figure 2: The Three Delay Model: Why women delay accessing care in developing countries.



Note: This is a visual adaptation of the 3-delay model by Thaddeus and Maine (1994). Delays do not exist in isolation and are all interconnected.

Diseases influencing women's health during pregnancy in sub-Saharan Africa

As previously mentioned, healthcare seeking behavior is often influenced by illness [2]. Costs associated with healthcare utilization often deter women from accessing healthcare during pregnancy even when illness arises [2, 4, 24]. HIV/AIDS and malaria are common in SSA and both diseases have been associated with adverse pregnancy outcomes [15-18, 25-39]. HIV/AIDS may further decrease healthcare utilization because of stigma associated with the disease [40, 41]. Anemia is also an important risk factor for adverse pregnancy outcomes in SSA and is implicated in the relationship between malaria and adverse pregnancy outcomes [18, 30, 42-45]. The following sections will focus on how malaria, HIV/AIDS, and anemia affect pregnancy and neonatal outcomes in SSA.

Malaria

Malaria, a mosquito-borne disease, is caused by the parasite, *Plasmodium* [46]. Five species of the protozoan parasite of the genus *Plasmodium* infect humans and cause malaria: *P. falciparum, P. malariae, P. ovale, P. vivax,* and *P. knowlesi.* Of these, *P. falciparum* causes the most severe form of malaria and is responsible for the largest proportion of morbidity and mortality [47]. Malaria is transmitted when an infected female mosquito of the genus *Anopheles* bites a human [48].

Globally, malaria is accountable for approximately 1.24 million deaths per year [49]. It is also the leading cause of morbidity and mortality for children under five in SSA [50, 51]. More malaria-related deaths in both children and adults occur in western, eastern, and central Africa than any other part of the world [49]. Roughly 80% of all cases and 90% of all deaths from malaria occur in SSA [50]; pregnant women are among the most vulnerable to this disease.

Between 25 and 50 million women will become pregnant this year in malaria-endemic areas of SSA [52, 53]. Pregnant women are twice as likely to be bitten by the *Anopheles* mosquito than non-pregnant women [29, 54]. It is possible that this is because of an increase in respiration and blood flow to the skin during pregnancy [29] that entices the *Anopheles* mosquitos, which are attracted to moisture and heat, and consequently increasing the number of mosquito bites to pregnant women [29]. Malaria infections from *P. falciparum* show consistently high parasitemia levels throughout pregnancy, indicating an inability to mount a sufficient immune response to the malaria parasite during pregnancy [55-58]. Some cases of spontaneous recovery from malaria have been reported [58, 59]; however, these results have been inconsistent [60, 61]. Recent studies suggest that malaria, as either a primary or secondary infection, may contribute to almost 25% of maternal deaths in endemic areas [34, 62]. In addition to its effects on pregnant women, malaria infection during pregnancy is also linked to poor birth outcomes [25].

Maternal and fetal outcomes associated with malaria include maternal anemia [26], preterm delivery, eclampsia, postpartum hemorrhage, intrauterine growth restriction (IUGR), spontaneous abortion, puerperal fever, still-birth, and maternal and fetal deaths [27]. Malaria is estimated to contribute to preterm delivery, resulting in approximately 19% of low birth weight births [63]. In malaria endemic areas, up to 200,000 newborn deaths occur each year as a results of malaria during pregnancy [33]. Malaria is assumed to produce adverse fetal outcomes via systemic effects, such as maternal anemia [26, 64, 65], or local effects such as placental infection [65-68]. Maternal anemia decreases erythropoiesis [30, 69] and increases red blood cell apoptosis [30, 70], ultimately leading to a maternal hypoxic state. During this hypoxic state, impaired growth and vascularization occur within a pregnant woman, which in turn can lead to fetal hypoxia [71]. Decreased vascularization leads to a reduction in the exchange of important nutrients and gases across the placenta, including oxygen, which in turn, produces a fetal hypoxic state [66, 72, 73]. Intrauterine growth restriction may occur because of fetal hypoxia and decreased nutrient uptake [72]. Malaria infection is also thought to disrupt cytokine activity, resulting in an increase in placental infection; this is especially found during the first or second pregnancy, and can result in preterm infants with intrauterine growth restriction [67, 68, 74-80]. Even cases of malaria that are asymptomatic may pose a threat to an unborn fetus; therefore, prophylactic measures to prevent malaria during pregnancy are recommended and are essential to achieving Millennium Development Goals 5 and 6 [21, 81].

Prophylactic malaria measures during pregnancy include sleeping under insecticide-treated nets (ITN) and intermittent preventative treatment with the inclusion of sulphadoxine-pyrimethamine (IPTp-SP) as part of antenatal care [32]. Both of these interventions have been deemed largely successful at reducing malaria transmission rates during pregnancy, thereby decreasing both infant and maternal mortality [31, 82, 83]. This may be because a decrease in placental malaria infection is found in women who receive more than three doses of IPTp-SP and sleep under ITN [82].

Although IPTp-SP and ITNs have demonstrated great efficacy in randomized control trials, their real-world effectiveness has not been as high [84, 85]. Community-based assessments of clinics that are implementing IPTp-SP programs have found medication shortages in these facilities [86]. Furthermore, access to malaria preventative measures is not synonymous with utilization. Numerous studies in SSA have shown low utilization rates of ITN even when they are available in the house [85]. Discomfort associated with sleeping under the net, damaged nets, disruption of sleeping arrangements, forgetting, or an all-around dislike of sleeping under the nets, and perceived low mosquito density are all common reasons reported for not sleeping under an ITN [87]. Additionally, in SSA, a disproportionate number of women who do take IPTp-SP and properly use ITN are wealthier and more educated [83]. Therefore, even though mass distribution of malaria medications and ITN are essential to reducing malaria during pregnancy, the effectiveness of these programs remains debatable.

In addition to socioeconomic factors that influence the malaria burden, cultural beliefs often influence women's decisions to seek treatment for malaria during pregnancy. For example, in qualitative studies women have reported delaying antenatal care early in pregnancy because of fear that a community member would put a hex on them via witchcraft [88]. This link between modern medicine—including malaria control—and witchcraft is fairly well documented in qualitative literature [89-91]. Incorporating these beliefs into malaria control programs remains essential to reducing malaria during pregnancy.

In summary, a combination of socioeconomic and medical risk factors are related to perpetuate malaria, leading to high morbidity and mortality rates among pregnant women in malaria endemic areas. Therefore, by establishing the prevalence of malaria parasitemia among pregnant women in Nigeria and discussing the socioeconomic risk factors associated with malaria parasitemia, this dissertation will add to the overall assessment of programs aimed to reduce malaria during pregnancy in Enugu State, Nigeria.

Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome.

Human Immunodeficiency Virus (HIV) attacks the immune system and destroys T-cells, a type of white blood cell that fight off infections [92]. Therefore, HIV weakens a person's ability to defend against infections [93]. As the virus invades the body, it eventually makes the infected person immunodeficient. Acquired Immunodeficiency Syndrome (AIDS), the most advanced state of an HIV infection occurs when the infected individual's CD4 count—a protein on immune cells such as T-cells—falls below 200 cells/mm^3 [93]. HIV is primarily spread via unprotected sex, injection or transfusion of infected blood, needle sharing, and mother-to-child transmission [94].

In 2013, 35 million people worldwide were living with HIV, of which 2.1 million had become newly infected [93]. Sub-Saharan Africa accounted for 71%, or 25 million, of prevalent cases and 70% or 1.6 million of the global incident cases of the disease [93, 95]. In SSA, women have the highest proportion of new HIV cases, which makes children particularly vulnerable [20].

In 2008, the WHO estimated that 42,000 women died worldwide during pregnancy from AIDS; in SSA alone, that number was an estimated 18,000 [20]. In fact, AIDS was responsible for 9% of all maternal deaths in SSA in 2008 [20]. By 2013, the WHO reported a decline in the number of estimated maternal deaths from AIDS in SSA to 3.8% [7]. Therefore, in 2013, the AIDS-attributed maternal mortality ratio was 19 maternal deaths per 100,000 live births [7]. However, SSA contributed 91% of the estimated global number of AIDS-related maternal deaths in 2013 (n=7,500) [6, 7]. This high burden of HIV/AIDS in women within SSA also affects the region's children.

The prevalence of HIV in children in SSA is 6 – 762 times that of children living anywhere else in the world, with 1.6 million prevalent cases and 230,000 incident cases [95]. Mother-to-child transmission (MTCT) of HIV is responsible for 90% of all HIV infections in children [39]. Being HIV-positive (HIV+) during pregnancy is associated with many adverse fetal outcomes that include low birth weight [96-98], increased infant mortality [96-99], miscarriage [97, 98], small for gestation age [97, 98], and preterm birth [97, 98]. Mother-to-child transmission can occur during pregnancy, labor, delivery, or breastfeeding; in short, any time a child becomes exposed to infected maternal body fluids (e.g. blood or breast milk) [93].

Treatment of HIV/AIDS in pregnant women decreases MTCT [93], and prevention of mother-to-child transmission (PMTCT) services are designed to prevent HIV transmission from an HIV-infected mother to her child [100]. When no preventative measures are taken, MTCT rates are highest during delivery at 15–45% [37, 93, 101]. When preventative measures are taken, MTCT rates can be reduced to less than 2% [101]. Prevention of mother-to-child transmission services include the short-term provision of antiretrovirals to prevent HIV transmission from mother to child, and lifelong antiretroviral treatment for HIV+ women [102]. A large increased effort has been made to administer antiretroviral treatment to HIV+ women during pregnancy; however, only 15% of young women in SSA aged 15-24 are aware of their HIV status [21]. Therefore, the majority of women of childbearing age do not receive PMTCT services when needed. The stigma associated with HIV (i.e., negative attitudes about persons living with HIV) complicates the use of PMTCT and remains an important barrier to women deciding to seek care [40, 41].

A community-based, cross-sectional survey of mothers in eastern SSA showed that stigma and lack of HIV knowledge were significantly associated with a decreased likelihood of maternal HIV testing [103]. Another study in rural SSA [104] also established that women who demonstrated stigma towards someone with HIV were less likely to seek care at a healthcare facility during delivery. The authors of that study reported—based on their qualitative data—that childbirth at a health facility was commonly viewed as most appropriate for women with pregnancy complications (e.g., HIV); therefore, women who deliver at health facilities may be labeled as HIV+ in the community [104]. This HIV stigma further delays women seeking treatment during delivery when complications do arise. The medical procedure that is the focus of Aims 3-6 (CS), can only safely be performed in the context of a healthcare clinic setting; therefore, a better understanding of the associations between stigma of HIV and accessing healthcare becomes essential.

Anemia

Hemoglobin is the red blood cell protein that carries oxygen in the blood [105]. The measurement of hemoglobin concentration in the blood is the most common strategy to assess the presence of anemia [106]. To measure hemoglobin concentration, a blood sample is placed into an automated machine where the red blood cells are broken down to get hemoglobin [106]. A chemical containing cyanide is exposed to the free hemoglobin, which then binds tightly with the hemoglobin molecule to form cyanomethemoglobin

[106]. Next, a light is shown through the solution and the amount of absorbed light is measured, determining the amount of hemoglobin [106]. The WHO classifies anemia in pregnant woman as hemoglobin below 11g/dL [107], and severe anemia is classified as a hemoglobin less than 7 g/dL [17].

Globally, 56 million pregnant women are affected with anemia [45]. In Africa, 19 million pregnant women are living with anemia, and it is estimated that 55.8% of all pregnant women in Africa suffer from anemia compared to 44.4% of non-pregnant women [45]. In SSA, anemia is seldom caused by one independent factor. Iron deficiency may be the primary cause of anemia; however, it often coexists with blood disorders and parasitic infections, such as malaria, hookworm and schistosomiasis [45, 108-112]. Because of the complex nature of anemia, few studies have assessed its etiology in SSA[113]. This gap in the literature is likely the result of inadequate diagnostic facilities in SSA and because parasitic infection and nutritional deficiencies often coexist in SSA[113]. To treat anemia and raise hemoglobin levels, correctly diagnosing the causal factors is essential [45]. As previously stated, this is often very difficult to accomplish in SSA because women usually live with overlapping conditions [43]. Therefore, treating any one of these conditions in isolation may not remedy anemia [43].

Pregnant women and children are two groups that are among the most susceptible to all types of anemia [45], and an increased risk of maternal and child mortality attributed to severe anemia has been well documented [42, 44]. It is commonly thought that other poor maternal and perinatal outcomes, such as congestive heart failure, fetal death, low birth weight, and preterm birth are linked to maternal anemia [17, 114]; however, these relationships are inconsistent. Some epidemiological studies have found a relationship between maternal anemia and poor birth outcomes [73, 115-117], while others have not [117-119]. This discrepancy in the literature is partly based on the complex nature of the different causes of anemia in developing countries where diseases such as malaria are often highly prevalent in women with anemia [45].

The effects of maternal hemoglobin levels on perinatal outcomes have not been well established in malaria endemic areas [65, 120]. Malaria infection complicates this relationship because little agreement exists on the mechanisms that mediate adverse fetal outcomes in neonates [53, 64]. Malaria may have systemic effects such as maternal anemia [26, 64, 65], or local effects such as placental infection [65-68] that may reduce fetal birth weight. Maternal anemia decreases erythropoiesis [30, 69] and increases red blood cell apoptosis [30, 70], which in turn may cause a fetal hypoxic state that then adversely affects fetal outcomes [71]. Research linking hemoglobin to adverse perinatal outcomes is contradictory, with results limited by small sample size and varying according to geography [53, 99, 121], and thus more research is needed to clarify this relationship.

Comorbid diseases influencing maternal mortality in sub-Saharan Africa.

A large number of pregnant women live in malaria endemic areas, putting women and their infants at risk for adverse outcomes. However, these diseases do not exist in isolation. A large proportion of women who are at risk for malaria live in areas with concomitant high HIV prevalence, and one consequence of the expanding HIV/AIDS epidemic is the increasing comorbidity of HIV and malaria [122]. This is of particular concern in SSA where malaria is endemic and 80% of the world’s HIV+ women reside, along with 90% of the world’s HIV+ children [123]. A woman’s susceptibility to malaria is higher if she is also co-infected with HIV [35]. Not only does an HIV+ woman have an increased susceptibility to malaria, but higher levels of malaria parasitemia are found in women with HIV [52, 124, 125]. Women infected with both HIV and malaria during pregnancy consistently show higher parasitemia densities and more severe anemia [52, 124]. By impairing the ability to develop immunity, HIV increases the burden of malaria during pregnancy, putting both the mother and the neonate at risk [52, 126].

The comorbidity of HIV and malaria are associated with adverse outcomes for both the mother and the neonate [96, 125]. Neonates born to women who had both HIV and malaria had significantly lower birth weights [125], and an increased risk of stillbirth or post-neonatal mortality [127, 128], preterm delivery [127], and maternal anemia [125], compared to those born to mothers who were negative for both diseases [127]. An increased risk of adverse perinatal outcomes is not found when comparing women who have the dual infection of HIV and malaria to women who have malaria alone [75]. However, the results relating these comorbid conditions to fetal outcomes remain understudied [125].

Because the malaria parasite affects red blood cells, pregnant women disproportionately suffer from anemia as a result of malaria infection [34]. In fact, it has been estimated that malaria is responsible for 25% of severe anemia during pregnancy in SSA [17]. HIV further complicates this relationship as the co-infection of HIV and malaria is often associated with even higher rates of severe anemia compared to those who have only one of these conditions [15]. Therefore, women who live in areas with high malaria transmission rates and high HIV rates also have an increased risk of anemia.

Treating these diseases during pregnancy is often not straight forward. Women often delay seeking treatment for common diseases associated with pregnancy because of socioeconomic and cultural beliefs. In order to have the greatest impression on most of the Millennium Development Goals, treatment and control measure need to be implemented that take into consideration the interconnectedness of disease and culture in SSA [43, 129]. To gain a deeper understanding of risk factors associated with adverse maternal and fetal outcomes, a holistic understanding—one that takes into consideration socio/demographic, as well as medical risk factors—of a woman's healthcare is required. Therefore, the next section will focus on the socioeconomic and cultural factors that influence maternal and perinatal health.

Socioeconomic and cultural risk factors associated with access to care during pregnancy in sub-Saharan Africa

Socioeconomic factors and culture influence decision-making and affect healthcare utilization in SSA (See Figure 2) [4]. Increasing healthcare utilization is essential to achieving Millennium Development Goal 5, because the use of healthcare services by women is a key determinate of her morbidity and mortality during pregnancy [2, 4]. Therefore, these influences are essential to understanding why women delay seeking treatment during pregnancy and will be discussed in the following sections.

Age

For many reasons, age is often used to represent experience utilizing healthcare services [4, 130]. Because of cultural norms in many countries in SSA, younger women are often less influential in their households than older women [131, 132]. This restricted autonomy tends to deter adequate utilization of healthcare services [131, 133]. Therefore, younger women—compared to their older counterparts—are less likely to receive more than minimal prenatal care and are less likely to have a skilled birth attendant during delivery-i.e. doctor, nurse, midwife [132]. In addition, age is also positively associated with socioeconomic status and pregnancy wantedness [130, 132]. As wantedness increases, more women attend prenatal care and have a doctor present at delivery in SSA [130]. Similarly, it is typically more acceptable for older women to deliver at a healthcare facility because of the increased risk of adverse maternal and fetal outcomes associated with mothers' age [130, 134, 135]. Therefore, older women are more likely to deliver at a healthcare facility than younger women, and having a skilled birth attendant present at delivery reduces maternal mortality [136].

Family composition

Family composition can also have a significant effect on a woman's ability to access healthcare. With multiple young children, women may have difficulty finding adequate child care in order to deliver at a healthcare facility[4]. In addition, because of a diffusion of power in family decision-making and resources, having extended family in the home may effect a woman's ability to make decisions about her own healthcare [4]. Being married may also affect a woman's access to healthcare. Single women are thought to have more autonomy, and therefore increase their utilization of healthcare services when they can financially afford services or have positive support from family[4]. However, some evidence suggests that single women may be less likely to deliver at a healthcare facility if they feel stigmatized because of being pregnant while unmarried [137], thus decreasing their chances of delivering with a skilled birth attendant, and increasing their risk of maternal and infant morbidity and mortality[138].

Education, employment and income

Education, employment status, and household income are typically highly correlated and are all positively associated with utilization of healthcare facilities [137, 139-141]. Women with more education and women who are employed often show more confidence in their household and decision-making skills [130, 137], which may increase women's rates of healthcare facility usage during pregnancy and using a skilled birth attendant during delivery[4]. Likewise, women with more education and women who are employed often have greater control over family resources and play a larger part in reproductive decision-making [23, 142-144]. Women with lower incomes often lack the financial ability to utilize healthcare services while pregnant, thereby increasing their rates of adverse maternal and fetal outcomes [144].

Distance of healthcare facility and skill level of provider

Although most African countries have healthcare facilities with perinatal services available, distance and poor road conditions often make healthcare facilities difficult to access [2]. Many pregnant women simply do not attempt accessing healthcare because transportation is not available and walking long distances is typically difficult and may even be impossible during labor [2]. Those that do attempt to walk to a healthcare facility for delivery, often deliver or die *en route* [2].

Even when facilities are in close proximity, rural healthcare clinics often lack the necessary skilled healthcare providers, thus, putting women at risk of increased morbidity and mortality during pregnancy [145]. The WHO estimates that two-thirds of the countries in SSA have skilled healthcare worker shortages, with 20% to 60% of all physicians trained in SSA working abroad [145]. According to the WHO, the lack of qualified healthcare workers in SSA necessitates a 140% increase in the healthcare workforce to make a positive change on health in the region [145]. Inequalities among healthcare workers' skill and quality of services are especially prominent in rural settings where twice as many rural healthcare workers lack appropriate medical training compared to urban healthcare workers [146].

Summary of the socioeconomic and cultural risk factors associated with access to care during pregnancy in sub-Saharan Africa

In summary, a variety of socioeconomic and cultural risk factors are associated with decreased access to care during pregnancy and labor in SSA. Women in SSA face a variety of obstacles while trying to obtain adequate healthcare during pregnancy. Gender inequalities and cultural acceptance of home deliveries are both common reasons women delay seeking treatment at a healthcare facility [2, 23, 28, 147]. Costs associated with healthcare utilization, as well as costs associated with transportation to healthcare facilities, are deterrents to accessing healthcare during pregnancy in SSA [148]. Delays in seeking treatment may result in women attempting to access healthcare facilities only after life-threatening complications develop. Decreasing delays and increasing access to emergency obstetric care, such as CS, is considered necessary to achieving Millennium Development Goal 5 [12, 13].

The obstacles women face in trying to achieve adequate prenatal care rarely exist in isolation and instead make a complex web of associations with healthcare utilization. The third delay in Thaddeus and Maine's model focuses on a woman questioning if the healthcare facility can provide her with adequate care. One way of measuring adequate care in developing countries is to look at a healthcare facility's ability to perform lifesaving obstetric measures if a complication arises [12, 13, 149]. This dissertation will focus on one type of procedure, the Cesarean Section (CS) (Aims 3 – 6).

Cesarean section

As previously mentioned, one of the factors that influence a woman's decision to access healthcare is her perception of the healthcare facility's ability to effectively treat her (see Figure 2). CS is a medical procedure during which the baby is delivered through a surgical incision in the mother's abdomen and uterus [150]. In developing countries, most CS are performed after an unexpected complication arises during delivery, including deteriorating health of the mother or fetus, breech position of the baby, or obstructed labor [150].

According to the WHO, the optimal rate of use of CS as a lifesaving intervention for women and neonates is between 5 and 15% [151-154]. Lower rates than this suggest an overall lack of access to healthcare, while higher rates suggest an overutilization of CS. High proportions of CS are a major public health concern because of increased adverse maternal and perinatal outcomes associated with elective CS [151, 155]. In the past decade, global rates of CS have been on the rise [154]; however, overutilization of CS is really only a major concern in middle- to high- income countries. In contrast, in developing countries consistently low rates of CS are reported; this is especially true among rural and poor populations [149].

Most of the continent of Africa consistently reports underutilization of CS, with regional estimates varying from 1.8% in Middle Africa, 1.9% in Western Africa, 2.3% in Eastern Africa, and 7.6% in Northern Africa, to 14.5% in Southern Africa [152]. Low percentages in middle, western and eastern Africa indicate difficulty accessing adequate maternal healthcare. As previous mentioned, socioeconomic and cultural influences often play a role in women not utilizing a healthcare facility during delivery in SSA. Gender inequalities and cultural acceptance of home deliveries are also common reasons women delay seeking treatment at a healthcare facility [2, 23, 28, 147]. Even if a woman can overcome the first two types of delays (See Figure 2), she may still be faced with a healthcare facility that does not have the means to provide her with adequate care.

Having a skilled birth attendant present during perinatal delivery has been shown to save the lives of women and neonates [5]. This is in part a result of the skilled healthcare professionals' ability to recognize the symptoms that may necessitate a CS [138]. However, even with a skilled birth attendant, there remain limitations in the availability of CS in SSA hospitals, such as a lack of appropriate operating facilities or surgical instruments [149, 156]. Doctors and nurses in SSA are often working without electrical power, water, or medication [156]. A survey of 77 hospitals in SSA found only 6% reported the ability to provide safe anesthesia for a CS and only 19% operated in facilities where electricity was always available [157]. A mere 56% of the facilities reported always having access to running water, and 23% reported having access to blood for transfusions [157]. Because of facilities' lack of water, electricity, medication, and equipment, operations—including CS— are often associated with unacceptably high rates of sepsis, hemorrhage, and death [149]. However, CS may be beneficial if preexisting medical conditions exist.

To prevent MTCT of HIV in resource unconstrained areas, evidence suggests that having a CS is beneficial if a woman's HIV-RNA level is above 1000 copies/ml near delivery [38]. Because women in resource-constrained areas are often unaware of their viral load before delivery, having a CS could be beneficial for HIV-infected women [158]. However, in resource-constrained areas, CS are often unavailable and unsafe; therefore, the WHO guidelines do not currently recommend HIV+ women in resource constrained regions have an elective CS [159].

To the authors' knowledge, no epidemiological studies have investigated the relationship between malaria parasitemia and mode of delivery. Currently, only case studies are discussed in the literature [16, 160, 161]. Therefore, the relationship between malaria parasitemia and CS warranted further attention and was studied as part of this dissertation.

The effects of anemia on mode of delivery have only recently been studied. As hemoglobin levels decreased from anemic to severe anemic, the duration of labor increased [162]. Increased time in labor is often associated with maternal and fetal distress-which is an indicator of the need for CS [162]. However, studies assessing the relationship between anemia and CS are conflicting with some studies showing an association while others have not [162-165]. The conflicting nature of these studies offers very little insight into a possible biological relationship between anemia and CS. Furthermore, the wide variability of the underlying populations in each of these studies makes comparability difficult.

One approach to decreasing maternal mortality and achieving Millennium Development Goal 5 was to increase utilization of lifesaving obstetric measures such as CS and decreasing common diseases associated with pregnancy [12, 13, 149]. This dissertation will assess the rates of CS in Nigeria, a country with one of the world's fastest-growing populations and second in the proportion of global maternal deaths (Aims 3-6).

Nigeria

Nigeria is the most populous country in SSA with a population of over 173 million [166]. By 2100, approximately 25% of Africa's population, or about 1 billion people, will reside in Nigeria [167]. This rapid population growth is at least partially attributable to Nigeria's relatively high crude birth rate, at 38.03 births per 1,000 women [1]. This translates to 6,458,000 births per year [168]. In order to achieve Millennium Development Goals 5 and 6 globally, Nigeria needed to bring about major decreases in maternal mortality, in part by increasing access to healthcare facilities during pregnancy and reducing health conditions such as malaria and HIV.

In 2013, the maternal mortality ratio in Nigeria was 560 deaths per every 100,000 live births, a 52% decrease from 1990 [7]. Although this decrease in maternal mortality ratio is a great improvement, in 2013 Nigeria still had the second largest proportion of global maternal deaths at 14% (n=40,000) [7]. The lifetime risk of maternal death from a pregnancy-related outcome in Nigeria is one of the highest in SSA at 1 in 29 [169]. This is in part because of the high prevalence of diseases such as malaria and HIV in Nigeria.

The burden of malaria in Nigeria strains an already fragile healthcare system, with nearly 110 million clinical cases occurring a year, accounting for up to 60% of outpatient visits and 30% of hospital admissions [170]. Approximately 97% of the Nigerian population lives in malaria endemic areas, making exposure to malaria during pregnancy a common occurrence [171]. Despite the WHO recommendation that all pregnant women in malaria endemic areas receive IPTp-SP to improve birth outcomes [50, 172], during 2010–2012 Nigeria had one of the lowest proportions of pregnant women receiving IPTp-SP for malaria with only 20% of pregnant women in Nigeria receiving one dose of IPTp-SP, and less than 5% receiving four doses [50, 172]. In addition, only 33.7% of pregnant women slept under an ITN [170, 173]. Because of the lack of proper treatment and utilization of preventative measures, malaria contributes to an estimated 11% of all maternal mortality within Nigeria [171].

Nigeria also has one of the highest numbers of HIV+ individuals, with 3.2 million prevalent cases and 220,000 incident cases of HIV in 2013 [174]. Of particular concern in Nigeria is the high prevalence of HIV among women of childbearing age [175]. In 2012, only 30.1% (n=57,871) of HIV+ pregnant women were receiving antiretroviral treatment to reduce the risk of MTCT [175]. Although this is almost double the proportion reported in 2011, it is still well below the levels needed to reach the Millennium Development Goal of halting the spread of HIV [175]. Furthermore, the Joint United Nations Programme on HIV and AIDS showed that Nigeria is not on target to reach their goal of eliminating new HIV infections among children, with only a 2.0% decline in three years, making it one of the slowest declines in SSA [176].

Most studies assessing mode of delivery in Nigeria focus on having a doctor present at delivery. Although having a doctor present at delivery is essential to recognizing symptoms associated with the need to have a CS, it may not correlate with his/her ability to perform a CS if fetal or maternal distress occurs. A study in Nigeria demonstrated only 1 in 21 health facilities were equipped to perform CS [138]. Therefore, even when women are utilizing healthcare facilities to deliver, this may not translate to access to a CS. Costs associated with healthcare utilization, as well as costs associated with transportation to healthcare facilities, are deterrents to accessing healthcare during pregnancy in Nigeria [148]. Some Nigerian women report that the financial burden of obstetric care is associated with a bad omen for the family; therefore, they may fail to seek seeking treatment at a healthcare facility even when complications during labor arise [148].

In order to decrease the burden of diseases during pregnancy, women must access a healthcare facility while pregnant. However, socioeconomic and demographic variables are often associated with an individual's ability and willingness to access healthcare facilities. Rates of CS in Nigeria vary according to an individual's socioeconomic status, with the richest women having better access to CS compared to the poorest women, as measured by the wealth index—a composite measure of household goods and living standards often used for research in developing countries [153]. Education and age are also a strong predictors of a woman's willingness to have a CS in Nigeria with younger and/or less educated women being more likely to refuse a CS even when it is medically necessary [14, 15]. Culture also plays a substantial role in a Nigerian woman's decision to have a CS. Symphysiotomy—a procedure used during obstructed labor that involves cutting through the cartilage and ligaments of the pelvic joint, or surgically expanding the pelvis, to allow a baby to be delivered vaginally—was preferred by two-thirds of Nigerian women [28]. Symphysiotomy was preferred over a CS because of the fears associated with having the latter procedure [28].

Low access to CS, coupled with high rates of comorbid medical conditions and low socioeconomic and cultural perceptions of healthcare facilities, are all factors that contribute to Nigeria having the second largest proportion of maternal deaths globally [7]. Therefore, women may ultimately attempt to access healthcare facilities only after life-threatening complications develop. These delays result in approximately 75% of all CS in Nigeria being linked to obstetric emergencies that could have been prevented by earlier medical interventions [147]. In order to achieve Millennium Development Goals 5 and 6, Nigeria needs to increase access to emergency obstetric services, such as access to CS, and decrease common diseases experienced during pregnancy such as malaria and HIV [149, 177].

Summary of the Introduction

The United Nations' Millennium Development Goals were ambitious, requiring improvements to basic humanitarian rights, including improved access to water, food, and healthcare, especially in areas of the world with extensive poverty such as SSA [178]. Countries within SSA present a unique opportunity to explore the progress that has been made in achieving these goals because of their historically high rates of infectious diseases and infant and maternal mortality [178]. Evaluating the progress towards achieving these goals is essential as new goals are developed. Of particular interest to this dissertation are the indicators of progress toward achieving Millennium Development Goals 5 and 6 in Nigeria. Millennium Development Goal 5 focuses on reducing maternal mortality, while Goal 6 aims to reduce infectious diseases. Nigeria contributes to the second largest proportion of the global number of maternal deaths, making access to healthcare facilities—and safe emergency obstetric services within these facilities—a priority [7]. Nigeria also has one of the highest populations of HIV+ individuals globally, as well as the highest number of malaria cases and deaths [174, 179]. Therefore, within SSA, Nigeria is a key location for assessment of both the medical and socioeconomic associations related to pregnancy complications.

CHAPTER 2

METHODS

Introduction

The methods, results and discussion of this dissertation are presented in this chapter. The three manuscripts developed based upon the six specific aims are presented in detail in Appendices A – C. Secondary analyses of two preexisting datasets were employed. Two distinct methodologies were used to complete three unique studies. The first methodology employed was a secondary analysis of a randomized control trial: The Healthy Beginnings Initiative (HBI). This randomized, controlled trial was designed to investigate the effectiveness of congregational *vs*. clinic-based HIV testing to PMTCT of HIV. This dissertation used data collected as part of a trial to assess peripheral malaria parasitemia, mode of delivery, and socioeconomic and demographic information. The second methodology also employed a primary data source: The Demographic and Health Survey (DHS). The DHS utilizes a cross-sectional survey design to attain country-specific results worldwide. Each survey collects information on socioeconomic and health related variables. The DHS is an open-source dataset that is available to any researcher; the work conducted herein included results from the Nigeria DHS dataset. The results will be presented in Chapter 3 followed by a discussion in Chapter 4.

METHODS: Healthy Beginnings Initiative

Overview

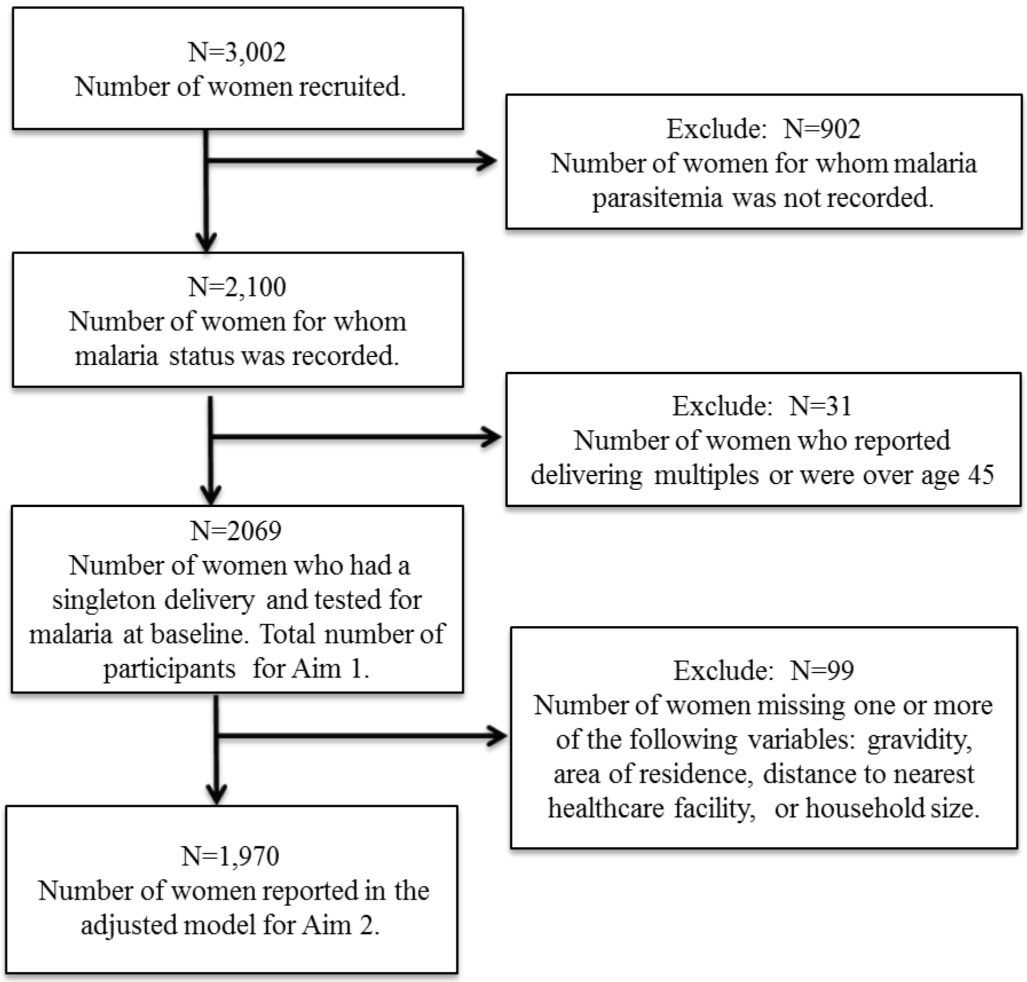
Aims 1-4 utilized data from the HBI. For Aim 1, malaria parasitemia levels were established for pregnant women in Enugu State, Nigeria. For Aim 2, person-level maternal risk factors that were associated with high malaria parasitemia were investigated. For Aim 3, the incidence of CS among pregnant women in the HBI was established; and for Aim 4, the socioeconomic and medical risk factors associated with having a CS were studied. The Methods and Results for Aims 1-4 will be described in detail in the following section; the results will be presented in Chapter 3 followed by the Discussion in Chapter 4.

Population

The Healthy Beginnings Initiative (HBI) cohort consisted of self-identified pregnant women [180]. Pregnant women were encouraged to participate in the study even when their male partner was unavailable or choose not to participate. Because of inherent risks associated with having a multiple birth, only singleton deliveries were retained for this dissertation. In total, 3002 women were recruited into the HBI cohort. Although the HBI cohort dataset was utilized to carry out Aims 1-4, two distinct outcome measures were utilized. Therefore, the total number of participants for Aims 1 and 2 differ from Aims 3 and 4 (see Figures 3 and 4).

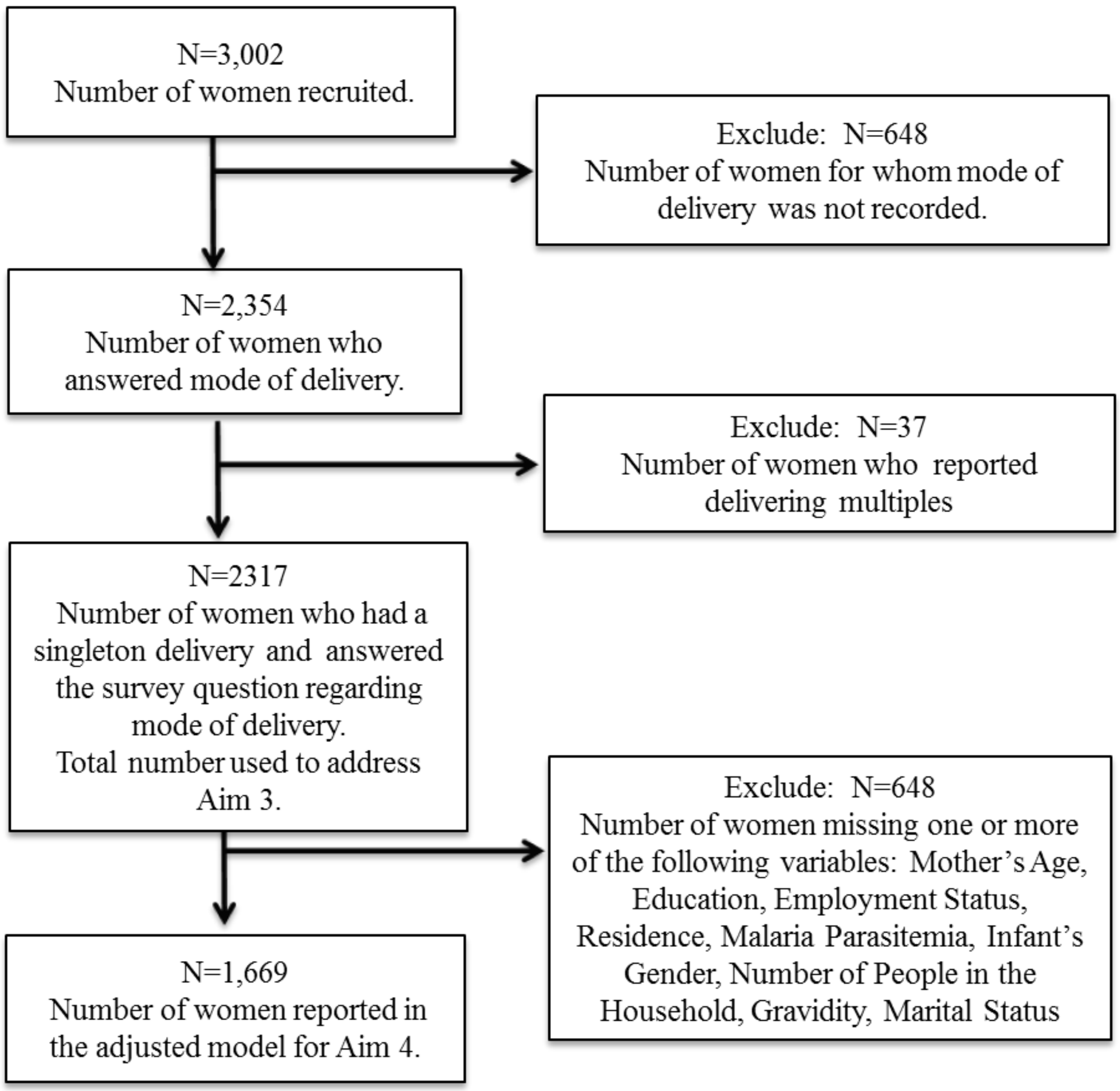
The outcome variable for Aims 1 and 2 was malaria parasitemia levels of pregnant women. As previously mentioned, 3002 women were recruited in the HBI, but information on malaria parasitemia was available for 2100 women (see Figure 3). Only singleton deliveries of women aged 17-45 were included in this analysis, therefore an additional 31 women were excluded. The total study population for Aim 1 was 2069 women. The dataset for Aim 2 included 1970 women; 99 women were excluded because they were missing one or more of the following variables: gravidity, area of residence, distance to nearest healthcare facility or household size (see Figure 3).

Figure 3: Flow chart representing the women included in Aims 1 and 2



The outcome variable for Aims 3 and 4 was mode of delivery: CS or vaginal delivery. As previously stated, 3002 women were recruited for the HBI. However, 648 lacked information on mode of delivery and an additional 37 women were pregnant with multiples and, therefore, excluded (see Figure 3). In total, Aim 3 included information on a total of 2317 pregnant women. For Aim 4, an additional 648 women were excluded because they were missing one or more of the following variables: gravidity, area of residence, distance to nearest healthcare facility, or household size-leaving the total sample size for Aim 4 at 1669 women (see Figure 4).

Figure 4: Flow chart representing the women included in Aims 3 and 4



Recruitment

Data for Aims 1 – 4 were obtained from the Healthy Beginnings Initiative (HBI) cohort [151]. Recruitment first occurred at the level of the church, then the participant level. Bishops overseeing the churches were first approached by the research team [151]. Permission for the study team to speak with each priest under the corresponding Bishop's diocese was granted by the Bishops. All priests agreed to allow recruitment within their corresponding congregation. Recruitment occurred in 40 churches from four dioceses (the Anglican diocese of Enugu, the Catholic diocese of Enugu, the Anglican diocese of Oji-River, and the Catholic diocese of Agwu) in Enugu State, Nigeria [151]. This area of Nigeria's population is more than 95% Christian [151]. Recruitment from churches was expected to provide a representative sample of pregnant woman in Enugu State, Nigeria, as church attendance approaches 90% in the country [180, 181].

Church-based volunteer health advisors (VHAs) who could read and write in English and Ibo were selected from each participating church and trained on basic research methodology, including how to obtain informed consent and complete the study's survey instruments. In total, 144 mostly female VHA were recruited and trained to administer the consent and survey [182]. The average age of the VHA was 30 years old and each VHA had a minimum of a high school education [182].

Each Sunday, priests asked pregnant women and their male partners to come to the altar so the priest could perform a prayer that included encouraging women to seek care at a healthcare facility, for the woman to have healthy pregnancy, and for successful delivery of her fetus. He then introduced the HBI study as a program that supported pregnant women during their pregnancy [151]. Women were given information on the study and, if interested, were asked to read and sign a written consent form. The VHA reported that the majority of participants were unable to read the local language (Ibo) and preferred to have the study material in English (exact count unknown). For participants who could not read, a VHA or a research assistant read the consent form aloud in English or Ibo; the participants gave their consent by affixing thumb prints or using initials.

Survey

A structured questionnaire was implemented at a 6th grade reading level (See Appendix D and E) [9]. Trained research staff and VHA administered the survey. Demographic and family structure information was utilized for this dissertation, including gravidity, area of residence, distance to the nearest healthcare facility, and household size. Other survey questions and laboratory measures not discussed in this dissertation included contraceptive use, general health questionnaire, and information about the male partner. All survey questions can be found in Appendices D and E. Post-delivery questionnaires were used to ascertain the mode of delivery, i.e., CS or vaginal birth, and the infant's gender.

Laboratory Measures

Variables assessed by laboratory tests were malaria parasitemia, hemoglobin, human immunodeficiency virus (HIV), and sickle cell disease/trait (SCD). Participants were tested for each laboratory measure either at baseline following recruitment into the study or during their prenatal visits, whereupon records were obtained from participant's corresponding hospital. Aims 1 and 2 employed the data on malaria. Aims 3 and 4 utilized information on hemoglobin, malaria, HIV, and sickle cell disease. All laboratory procedures utilized in Aims 1-4 can be found in Appendix F.

Peripheral parasitemia levels were assessed using the malaria plus system [183]. Thick blood smears were examined via oil immersion under microscopy [183]. Each slide was carefully read by two experienced laboratory technicians; a third technician was utilized to rectify any discordant results. To ensure further quality control, random slide checks were made by a hospital review panel. The malaria plus system was scored as follows: 0 for no parasites, + for 1–10 parasites per 100 high power field, ++ for 11–100 parasites per 100 high power field, +++ for 1–10 parasites per high power field, ++++ for over 10 parasites per high power field. Thus, levels of parasitemia increase as the scoring moves from 0 through ++++. For these analyses malaria parasitemia was dichotomized as high and low based on the malaria plus system. Those in the 0 and + group were classified as low parasitemia; while those in the ++ and +++ groups were classified as high parasitemia. No participants showed malaria parasitemia consistent with the ++++ group.

The following paragraphs will explain the laboratory measures exclusively used in Aims 3 and 4. Hemoglobin was assessed using the standard cyanmethemoglobin method [106]. Drabkins solution (Ranjo Medix Laboratories, Lagos, Nigeria) with a pH of 7–7.4 was measured at 5 ml and dispensed into a glass test tube. Whole blood was pipetted into the Drabkins solution and allowed to sit at room temperature away from sunlight for 5 minutes. The Drabkins fluid was then read using 540 nm in a spectrophotometer to estimate the hemoglobin concentration. WHO guidelines for anemia were employed [107], and pregnant woman were classified as anemic if they had a hemoglobin level below 11g/dl.

HIV testing was performed using the Rapid Testing Serial Algorithm II [184]. For this procedure, two concurrent HIV rapid tests, Uni-Gold Recombigen (Uni-Gold; Trinity Biotech, Inc., Wicklow, Ireland) and Stat Pak (Chembio Diagnostic Systems Inc., Medford, New York, USA) were used. Both Uni-Gold and Stat Pak are single-use immunochromatographic tests that detect HIV antibodies. Uni-Gold is used for the detection of HIV-1; while Stat Pack can detect HIV – 1 and 2. Both tests are performed by collecting whole blood from a finger stick that is placed on a test strip. Next, the test strip was placed in the testing device and a wash solution unique to each test, was added. Uni-Gold was set aside for 10–12 minutes and then read by a technician; Stat-Pak was read after 15–20 minutes. If both tests were positive for HIV, the individual was considered HIV+; if both tests were negative, the individual was considered HIV negative. When the tests showed conflicting results, they were both repeated and the results were read by another technician, who did not know the results of the first series of tests. Uni-Gold has demonstrated high sensitivity (98.5%), specificity (99.5%), positive predictive value (<99%) and negative predictive value (<99%%) in sub-Saharan African populations [185, 186]. Stat-Pak has also demonstrated high sensitivity (99.7%), specificity (96.9%), and negative predictive value (99.8%), but lower positive predictive value (94.6%) in sub-Saharan African populations[187].

EDTA-treated venous blood samples were used to screen for SCD. Cellulose acetate electrophoresis at pH 8.5 – 9.0 was used. Hemolysates were prepared by lysing saline-washed, packed red cells in 13mM EDTA, 10.7mM KCN solution using a 1:4

ratio. Tris-EDTA-borate buffer with cellulose acetate strips were used to perform electrophoresis. Each strip contained one microliter of a patient hemolysate and a microliter of a control hemolysate containing hemoglobin A, S, and C. Electrophoresis was performed at a constant power of 350V for 30 minutes or longer if maximum band separation was not observed. Each strip's band separations were compared to the control genotypes: *AA*, *AS*, *SS*, and *SC*. To decrease the chances of a false positive or negative of SCD, each sample was tested twice. If incongruent results occurred, the test was rerun.

Statistical Analysis

Two unique statistical analysis plans were utilized to complete Aims 1 and 2 and Aims 3 and 4. The outcome variable for Aims 1 and 2 was malaria parasitemia. The outcome variable for Aims 2 and 3 was delivery via. CS *vs*. vaginal delivery.

For Aims 1 and 2, gravidity was dichotomized as > 2 or ≤ 2 previous pregnancies. Associations between malaria parasitemia and continuous variables were determined using ANOVA. Pearson's Chi-square test was used to examine associations of malaria with categorical and dichotomous variables. Crude and adjusted logistic regression models were used to determine the association between participant characteristics and malaria parasitemia levels. Statistical significance was set at $p<0.05$. Data analyses were conducted using Stata version 12.0 [Stata Corporation, College Station, TX].

For Aims 3 and 4, univariate analyses were based on Pearson's Chi-square test for comparison of proportions for all variables. Fisher's exact tests for contingency tables were used to test for significance in proportions when the expected cell counts were less than 5. Chi-square analyses with $p<0.10$ were further analyzed using crude and adjusted logistic regression with CS as the main outcome. Having a CS in previous pregnancies is known to predict current CS; therefore, gravida was included in logistic regression models. Because no information on previous CS was collected, a sensitivity analysis was performed including only those experiencing their first pregnancy. Statistical significance was set at $p<0.05$. An adjusted trend in the odds ratio (OR), using the "tabodds" function in Stata [Stata Corporation, College Station, TX], to determine whether the odds of having a CS increased with participants' age and education. Participant's age was categorized as 15–24, 25–34, and 35–45. Only one women who had a CS had no formal education; therefore, education was categorized as none/primary, secondary and tertiary and above. Age and education were retained as categorical variables for inclusion in multivariable models. Birthweight was collected as part of the parent study; however, because it was self-reported and most newborns were not weighed at birth, birthweight was not deemed reliable. Therefore, birthweight was not included in this analysis. A second sensitivity analysis was done to compare those who did not complete a follow-up interview with those who did complete a follow-up interview. Data analyses were conducted using Stata version 12.0.

Ethics Review

The parent study was approved by the Institutional Review Board of the University of Nevada, Reno, and the Nigerian National Health Research Ethics Committee. This secondary analysis was considered exempt from human subjects review by the Mel and Enid Zuckerman College of Public Health Research Office.

METHODS: Demographic and Health Survey

Overview

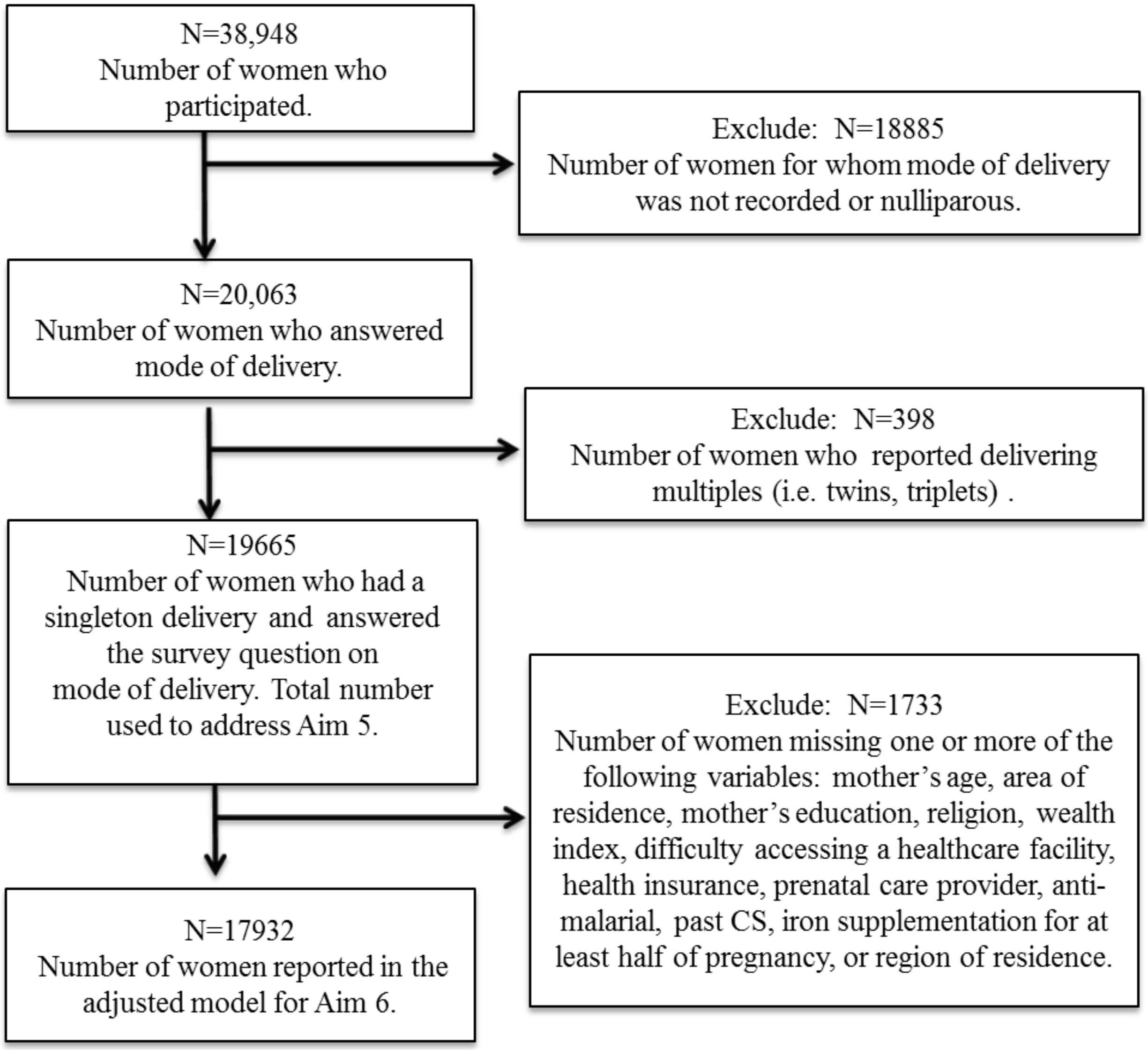
To address Aims 5 and 6 secondary analyses of The Nigerian Demographic and Health Survey were performed. Individual sampling weights were utilized as provided by the DHS to make the distribution of the data representative of all Nigeria. A weighted analysis was conducted among participants from this cross-sectional study. For Aim 5, the incidence of CS among pregnant women in Nigeria was estimated. In Aim 6, the association between socioeconomic and/or medical risk factors and mode of delivery within Nigeria was evaluated. The sample was restricted to singleton deliveries because of the risks inherent to having multiple births. A sensitivity analysis was performed among primigravida women. The Methods for each Specific Aim will be described in detail in the following section; the Results and Discussion will follow in Chapters 3 and 4.

Population

The Nigerian DHS covered the entire population of non-institutional dwelling units [188]. The 2013, households were selected as survey sites (n=40,680). In total, 38,904 households were occupied at the time of the field staff's arrival. Of these 38,522 households were successfully interviewed yielding a response rate 99% [188]. Of particular interest to this dissertation are responses from women on the individual survey.

Figure 5 illustrates the recruitment chain for women in the Nigerian DHS survey. In total, 39,902 women were interviewed, of which 38,948 (97.6%) completed the full interview. A large number of women were either nulliparous or did not provide information on the mode of delivery of their last child; therefore, they were excluded from this analysis (n=18,885). Only singleton deliveries were included in the present study. Therefore, the dataset for Aim 5 included a total of 19,655 women aged 15–49 years for whom information on the mode of delivery (vaginal *vs*. CS) of their most recent singleton pregnancy was available. For Aim 6, an additional 1,733 women were excluded from the analyses because they were missing one or more of the following variables: mother's age, area of residence, mother's education, religion, wealth index, difficulty accessing a healthcare facility, health insurance, prenatal care provider, anti-malarial, past CS, iron supplementation for at least half of pregnancy, or region of residence (see Figure 5). In total, Aim 6 included 17,932 women.

Figure 5: Flow chart representing the women included in Aims 5 and 6



Recruitment

The data for this analysis were obtained from the DHS conducted in Nigeria in 2013. The sampling frame used for Nigeria's survey was prepared using the 2006 Population Census data from the Federal Republic of Nigeria provided by the National Population Commission [188]. A total of five DHS have been conducted to date in Nigeria in the following years: 1990, 1999, 2003, 2008 and 2013. The United States

Agency for International Development (USAID), the United Nations Population Fund (UNFPA), the United Kingdom Department for International Development (DFID) (through the Partnership for Transforming Health Systems Phase II), and the government of Nigeria, provided the resources necessary to conduct the survey [188]. A technical/quality assurance team was utilized for the administration and collection of the survey. The team was headed by a project director/coordinator. Other members of the team included 18 state coordinators who were in charge of recruiting and training the field staff, monitoring the fieldwork, and assisting in any other project-related activities [188]. All field staff received a four-week-long training course from January to February 2013 [188].

Field staffs were divided up into six geographical zones: north east, north central, north west, south east, south central and south west [188]. Within these six zones, a total of 37 interview teams were created, with one for each of the 36 states and one for the Federal Capital Territory. Household interviews were conducted from February through June 2013. Eight areas faced security difficulties; therefore, the survey was not completed in these regions (four in Borno, two in Yobe, one in Nasarawa, and one in Plateau).

Survey

The DHS utilized a cross-sectional design to obtain nationally representative data [189]. The DHS was designed to provide up-to-date information on fertility levels, marriage, awareness and use of family planning methods, child feeding practices, nutritional status of women and children, adult and childhood mortality, awareness and attitudes about HIV/AIDS, and domestic violence [189]. In 2013, three questionnaires were utilized to this obtain information: the Household Questionnaire, the Woman's Questionnaire, and the Man's Questionnaire. The results reported here are form the individual Woman's Questionnaire [189]. The Women's Questionnaire contains items on the following topics: background characteristics, reproductive behavior and intentions, contraception, antenatal, delivery and postnatal care, breastfeeding and nutrition, children's health, status of women, HIV and other sexually transmitted infections, husband's background and other topics related to environmental health, tobacco use and health insurance. The DHS only collects information on any pregnancy-related outcomes only in the five years prior to the survey.

Statistical Analysis

Because select populations were oversampled, individual sampling weights provided by DHS were used as recommended [190]. Using weights allowed for adjustment for nonresponse to questions, and made the data representative of the underlying population on a national level. All data management and analyses were conducted in STATA version 12.0 [Stata Corporation, College Station, TX] using the 'svyset' command. The outcome variable used for this analysis was whether the most recent birth was reported as a CS or a vaginal delivery. A skilled birth attendant was defined using the WHO as an accredited health professional including a doctor, nurse, or midwife [191]. Otherwise, the attendant was classified as a non-skilled birth attendant.

Weighted univariate analyses utilized Pearson's Chi-square test for comparison of proportions for all variables. Weighted crude and adjusted logistic regression with mode of delivery, either vaginal or CS, as the main outcome was performed. Weighted percentages were also performed for reasons why women did not deliver at a healthcare facility. A sensitivity analysis was performed among those experiencing their first pregnancy (n=3,596). Statistical significance of all tests are presented at $p<0.05$.

Ethics Review

All protocols and survey measures are submitted for review to ensure the protection of human subjects. Nigeria's protocols for each survey were approved by the ICF International Institutional Review Board as a within country IRB board [192]. Survey data were collected by trained personnel, with participants verbally consenting at the beginning of the interview [189]. This secondary analysis was considered exempt from human subjects review by the Mel and Enid Zuckerman College of Public Health Research Office.

CHAPTER 3

RESULTS

The results of this dissertation are summarized by the outcome variable that corresponds to each Aim. Because Aims 1 and 2 have the same outcome variable (peripheral malaria parasitemia levels), they are reported together under the same results subheading. Aims 3 and 4, and 5 and 6 utilize different dataset. Therefore, the results for Aims 3 and 4 will be presented apart from Aims 5 and 6 even though the outcome variable, mode of delivery, is the same both studies. The Discussion for each Aim can be found in Chapter 4 of this dissertation.

Results: Malaria Parasitemia in Pregnancy (Aims 1 and 2)

Malaria parasitemia levels were recorded for 2,069 pregnant women. Over 99% of the women in the study tested positive for malaria parasitemia (n=2,052). Categorized according to the malaria plus system, malaria parasitemia in our sample included: < 1% in the no infection group (n=17); 62% in the + group (n=1275); 36% in the ++ group (n=737); 2% in the +++group (n=40); with none categorized in the ++++ group (n=0) (see Table 1). A total of 1,292 (62%) and 777 (38%) women were categorized as low- and high- parasitemia, respectively.

Table 1: Malaria parasitemia frequency by the Malaria Plus System

Malaria Plus System	N(%)
0	17 (<1)
+	1275 (62)
++	737 (36)
+++	40 (2)
++++	0 (0)
Total	2069 (100)

Notes: Levels of parasitemia increase as the scoring moves from 0 through ++++

As shown in Table 2, no significant differences were found between high and low malaria parasitemia and gravidity, area of residence, distance to nearest healthcare facility, household size, or age of participants.

Table 2: Comparison of participant's characteristics by malaria parasitemia low vs. high

	Parasitemia			
	Low	**High**	**Total**	***P****
Number of Participants, N(%)				
	1292(62)	777(38)	2069(100)	
Gravidity, N(%)				
Primi/secundigravida	819(65.8)	486(65.9)	1305(65.5)	*0.67*
Multigravida	425(34.2)	263(35.1)	688(34.5)	
Residence, N(%)				
Urban	326(25.3)	189(24.1)	513(24.9)	*0.54*
Rural	960(74.7)	588(75.9)	1,548(75.1)	
Distance to Healthcare Facility, N(%)				
0-5(km)	464(36.1)	255(33.0)	719(34.9)	*0.17*
5-10(km)	487(37.9)	290(37.5)	777(37.8)	
10+(km)	334(26.0)	228(29.5)	576(27.3)	
Household size, Mean (SD)[a]				
	4.51(2.0)	4.36(1.9)	4.40(1.9)	*0.07*
Age (Years), Mean (SD)[b]				
	29.10(5.8)	28.90(5.8)	29.00(5.7)	*0.44*

Notes: Numbers may not add up to 2086 due to missing data.

[a] N for each category include: n=1282 low parasitemia; n=768 high parasitemia; total n=2050

[b] No missing data

*Significance based on Chi-square p-value < 0.05

Table 3 shows the results of logistic regression analysis of the association between participant characteristics and malaria parasitemia. After controlling for confounding variables, odds for high parasitemia were lower among those who had more people in the household (For every one person increase in a household, OR=0.94, 95% CI: 0.89–0.99).

Table 3: Logistic regression models for malaria parasitemia and participant level characteristics

		Crude model	Adjusted[a]
	N	OR(95% CI)	OR(95% CI)
Gravidity			
Multigravida	1305	Ref.	Ref.
Primi/secundigravida	688	1.04 (0.86-1.26)	0.93 (0.75-1.16)
Residence			
Urban	513	Ref.	Ref.
Rural	1548	1.07 (0.87-1.31)	1.07 (0.86-1.32)
Distance to Healthcare Facility			
0-5(km)	719	Ref.	Ref.
5-10(km)	777	1.08 (0.88-1.34)	1.08 (0.87-1.34)
10+(km)	562	1.24 (0.99-1.56)	1.23 (0.98-1.56)
Household size			
	2050	0.96 (0.91-1.00)	0.94 (0.89-0.99)[b]

Notes: [a] Overall model n=1970

[b] Indicates significance at p<0.05

Results: Cesarean section in Enugu State, Nigeria (Aims 3 and 4)

As shown in Table 4, 167 (7.2%) women had CS and 2,150 (92.8%) had vaginal deliveries. A woman’s age was statistically significantly associated with having a CS (*p<0.01*), with a greater percentage of women aged 35–45 having had a CS (11.1%) than women aged 25–34 (7.5%) or 15–24 (3.2%). Education was statistically significantly associated with having a CS (*p<0.01*), with a greater percentage of women with tertiary education having had a CS (15.1%) than those with a secondary education (6.0%) or a primary education or less (4.9%). Employment status was also significantly associated with having a CS (*p=0.03*); women with full-time employment had higher percentages of CS than women who worked part time or who did not indicate they were currently employed (8.7%, 5.0, and 7.1%, respectively). Area of residence (i.e., rural vs urban), was significantly related to having a CS (*p<0.01)* with more women in urban settings having a CS (12.2%) compared to women in rural settings (5.5%). A mother's baseline malaria parasitemia was significantly associated with having a CS (*p=0.02)*; higher percentages of women who had high malaria parasitemia at baseline had a CS than women who displayed low levels of malaria parasitemia (8.9% vs 6.0%, respectively). Infant's gender was statistically associated with CS (*p=0.01*), with a higher rate of CS occurring when mothers delivered male (8.6%) than female infants (5.9%). No significant relationship was observed between CS and number of people in the household, gravidity, distance to nearest healthcare facility, marital status, HIV, sickle cell disease (SCD), or anemia. When analyses were restricted to women who had not had a prior pregnancy (n=334), 7.8% (n=26) had CS and 92.2% (n=308) had vaginal deliveries (Table 4). Among primigravida women, living in an urban environment was significantly related to having a CS (*p<0.01*) with 14.3% of urban women having a CS compared to only 5.1% of rural women. No other participant characteristics were significant predictors of having a CS among primigravida women.

Table 4: Comparison of participant baseline characteristics and infant gender with mode of delivery

	Full Sample					Primigravida				
	C-Section		Vaginal			C-Section		Vaginal		
	N	%	N	%	*P**	N	%	N	%	*P**
Total	167	7.2	2150	92.8		26	7.8	308	92.2	
Mother's Age										
15-24	16	3.2	479	96.8	*<0.01* [a*]	9	5.8	146	94.2	*0.34* [a]
25-34	107	7.5	1317	92.5		15	9.3	147	90.7	
35-45	44	11.1	354	88.9		2	11.8	15	88.2	
Education										
None /Primary	30	4.9	578	95.1	*<0.01* [a*]	3	6	47	94	*0.053* [a]
Secondary	79	6	1241	94		11	5.5	188	94.5	
Tertiary	58	15.1	327	84.9		12	14.1	73	85.9	
Employment Status										
Full-time	74	8.7	781	91.4	*0.03* [a*]	6	5.8	97	94.2	*0.64*
Part-time	28	5	536	95		6	9.4	58	90.6	
None	61	7.1	818	93.1		14	8.5	150	91.5	
Residence										
Urban	73	12.2	528	87.9	*<0.01* [a*]	14	14.3	84	85.7	*<0.01**
Rural	94	5.5	1617	94.5		12	5.1	224	94.9	
Malaria Parasitemia										
High	59	8.9	604	91.1	*0.02* [a*]	5	5.3	90	94.7	*0.36*
Low	66	6	1027	94		14	8.3	155	91.7	
Infant’s Gender										
Male	102	8.6	1085	91.4	*0.01* [a*]	13	7.8	153	92.2	*0.99*
Female	64	5.9	1028	94.1		13	7.8	152	92.2	
Number of People in Household										
1-2	31	8.7	324	91.3	*0.07*	14	7.5	174	92.6	*0.15* [a]
3-4	75	8.2	845	91.9		11	11.6	84	88.4	
5+	60	5.8	967	94.2		1	2.1	46	97.9	
Gravity										
Primigravida	26	7.8	308	92.2	*0.62*	N/A	N/A	N/A	N/A	*N/A*
Multigravida	136	7	1797	93		N/A	N/A	N/A	N/A	
Marital Status										
Married	162	7.5	2010	92.5	*0.07*	3	5	57	95	*0.59* [a]
Other	5	3.4	140	96.6		23	8.4	251	91.6	
Distance to Healthcare Facility										
0-5km	54	9.7	753	93.3	*0.52*	9	8.1	102	91.9	*0.88*
5-10km	61	7	817	93.1		10	8.5	108	90.8	
10+km	51	8.2	872	91.8		7	6.7	97	93.3	
HIV status										
Positive	2	3.3	59	96.7	*0.32*	0	0	8	100	*1.0* [a]
Negative	137	7.2	1757	92.8		21	7.4	263	92.6	
Sickle Cell Status										
AA-normal	100	7.3	1273	92.7	*0.62*	5	9.4	48	90.6	*0.55* [a]
AS/AC-carrier	25	6.5	357	93.5		14	6.6	197	93.4	
Anemia										
Yes	53	8.3	589	91.7	*0.16*	7	7.2	90	92.8	*0.99*
No	72	6.5	1042	93.5		12	7.2	155	92.8	

Notes: *Significance based on Pearson's Chi-square for Fisher's Exact, p<0.05 significant

[a] Indicates p-value based on Fishers Exact

Table 5 presents the crude and adjusted odds ratios (95% CIs) for having had a CS or a vaginal birth by participant characteristics. The adjusted models showed that, compared to women aged 15–24, the odds of having a CS were higher when the mother was aged 25–34 years (adjusted OR (aOR): 2.01; 95% CI: 1.04–3.90) and when the mother was aged 35–45 years (aOR: 2.73; 1.26–5.92; *p-trend <0.01*). Compared to those with a primary school education or less, the odds of having a CS were higher if the mother had at least a tertiary education (aOR: 2.91; 1.54–5.53), but not if she had a secondary education (aOR: 1.27; 0.73–2.30; *p-trend <0.01*). The odds of having a CS were significantly lower if participants were employed part-time compared to full-time (aOR: 0.56; 0.32–0.97). Compared to women who lived in an urban setting, those who lived in a rural setting had a significant reduction in the odds of having a CS (aOR: 0.58; 0.38–0.89). Significantly higher odds of having a CS were found among those with high peripheral malaria parasitemia compared to those with low parasitemia (aOR: 1.53; 1.03–2.27). After adjustment for confounders, no relationship was found between CS and number of people in the household, gravidity, marital status and not being employed. Among primigravida women, adjusted logistic regression models showed a significant relationship between woman living in an urban vs rural environment and having a CS (aOR: 0.27; 0.09–0.82). No other significant relationships were found among primigravida women between having a CS and a woman’s baseline characteristics or the gender of her infant.

Table 5: Crude and logistic regression models of the odds of C-Section vs vaginal birth

	Full Sample		Among Primigravida	
	Crude	Adjusted N=1669	Crude	Adjusted N=256
Mother's Age				
15-24	*Ref*	*Ref*	*Ref*	*Ref*
25-34	2.43(1.42-4.16)*	2.01(1.04-3.90)*	1.65(0.70-3.90)	0.85(0.23-3.10)
35-45	3.72(2.07-6.70)*	2.73(1.26-5.92)*	2.16(0.43-10.95)	1.54(0.22-10.75)
Education				
None/Primary	*Ref*	*Ref*	*Ref*	*Ref*
Secondary	1.22(0.80-1.89)	1.27(0.73-2.30)	0.92(0.25-3.42)	0.57(0.13-2.58)
Tertiary	3.42(2.15-5.42)*	2.91(1.54-5.53)*	2.58(0.69-9.61)	0.89(0.16-4.86)
Employment Status				
Full-time	*Ref*	*Ref*	*Ref*	*Ref*
Part-time	0.55(0.35-0.86)*	0.56(0.32-0.97)*	1.67(0.52-5.43)	1.72(0.43-6.85)
None	0.79(0.55-1.12)	0.80(0.52-1.25)	1.51(0.56-4.06)	1.42(0.42-4.87)
Residence				
Urban	*Ref*	*Ref*	*Ref*	*Ref*
Rural	0.42(0.30-0.58)*	0.58(0.38-0.89)*	0.32(0.14-0.72)*	0.27(0.09-0.82)*
Malaria Parasitemia				
Low	*Ref*	*Ref*	*Ref*	*Ref*
High	1.52(1.05-2.19)*	1.53(1.03-2.27)*	0.62(0.21-1.76)	0.67(0.22-2.06)
Infants Gender				
Female	*Ref*	*Ref*	*Ref*	*Ref*
Male	1.51(1.09-2.09)*	1.18(0.80-1.75)	0.99(0.45-2.21)	0.86(0.32-2.32)
Number of People in Household				
1-2	*Ref*	*Ref*	*Ref*	*Ref*
3-4	0.93(0.60-1.43)	0.94(0.49-1.79)	1.63(0.71-3.74)	1.38(0.49-3.87)
5+	0.65(0.41-1.02)	0.58(0.29-1.16)	0.27(0.03-2.11)	0.37(0.04-3.14)
Gravidity				
Multigravida	*Ref*	*Ref*	N/A	N/A
Primigravida	1.15(0.72-1.73)	1.03(0.55-1.96)	N/A	N/A
Marital Status				
Married	*Ref*	*Ref*	*Ref*	*Ref*
Other	0.44(0.18-1.10)	0.93(0.32-2.71)	0.57(0.17-1.98)	0.67(0.12-3.61)

Notes: Models adjusted for other variables in the table

* Indicates significance at $p<0.05$

Table 6 shows the results of the sensitivity analysis exploring the characteristics of participants who were lost to follow-up with those who completed the follow-up survey. In total, 685 (n=23%) participants did not complete the follow-up survey. There was a statistically significant relationship between completion of the follow-up interview, when mode of delivery was assessed, and the following participant's baseline characteristics: mother's age ($p=0.04$), employment status ($p<0.01$), area of residence ($p<0.01$), number of people in the household ($p=0.01$), gravidity ($p=0.03$), and anemia ($p=0.03$). A greater proportion of women aged 15-24 (24.4%) were lost to follow-up than those aged 25-34 (19.7%) and those 35-45 (20.1%). A greater proportion of unemployed women were lost to follow-up than those who worked part-time or full-time (25.7%, 14.6%, and 19.5%, respectively). In urban areas, more women were lost to follow-up compared to rural areas (28.7% and 19.5%, respectively). As the number of people in the household increased, fewer women were lost to follow-up. More anemic women were lost to follow up than those who were not-anemic (16.9% vs 13.4%, respectively).

Table 6: Sensitivity analysis of participants who completed the follow-up interview compared to those who were not present at the follow-up interview

	Follow-up		No Follow-up		
	N	**%**	**N**	**%**	***p-value***
Mother's Age					
15-24	503	75.6	162	24.4	*0.04*
25-34	1439	80.3	354	19.7	
35-45	401	79.9	101	20.1	
Education					
None /Primary	617	79.3	161	20.7	*0.29*
Secondary	1341	79.3	350	20.7	
Tertiary	390	76.2	122	23.8	
Employment Status					
Full-time	869	80.5	211	19.5	*0.00*
Part-time	573	85.4	98	14.6	
None	891	74.3	308	25.7	
Residence					
Urban	611	74.3	211	28.7	*0.00*
Rural	1736	80.5	420	19.5	
Malaria Parasitemia					
High	677	86.2	108	13.8	*0.34*
Low	1114	84.7	201	15.3	
Number of People in Household					
1-2	364	75.1	121	24.9	*0.01*
3-4	929	78.4	256	21.6	
5+	1043	81.2	241	18.8	
Gravity					
Primigravida	338	84.3	63	15.7	*0.03*
Multigravida	1959	79.7	499	20.3	
Marital Status					
Married	2208	78.6	601	21.4	*0.33*
Other	146	75.7	47	24.3	
Distance to Healthcare Facility					
0-5km	819	80.7	196	19.3	*0.24*
5-10km	893	77.7	256	22.3	
10+km	631	79.0	168	21.0	
HIV status					
Positive	63	92.7	5	7.4	*0.13*
Negative	1928	86.2	309	13.8	
Sickle Cell Status					
AA-normal	1400	85.1	246	14.9	*0.45*
AS/AC-carrier	390	86.5	61	13.5	
Anemia					
Yes	660	83.1	134	16.9	*0.03*
No	1131	86.6	175	13.4	

Results: Cesarean section in Nigeria (Aims 5 and 6)

The results of the weighted chi-square are reported in Table 7. During their last pregnancy, 2.3% of women had a CS and 97.7% had a vaginal delivery in Nigeria during 2008-2013. A statistically significant relationship between having a CS and mother's age was demonstrated ($p<0.01$), with higher percentages of women aged 34–49 having had a CS (2.7%) compared to women aged 25–34 (2.4%) and 15–24 (1.6%). Area of residence was significantly associated with having a CS ($p<0.01$), with more women in urban areas having a CS (4.4%) than women in rural settings (1.1%). Education was a significantly associated with having a CS ($p<0.01$), with women with a tertiary education or higher having higher percentages of a CS (11.1%) compared to those with secondary, primary or no formal education (3.6%, 2.0%, and 0.5% respectively). Religion was also a significant factor in having a CS ($p<0.01$), with more Catholics having a CS (4.9%) compared to Muslim (1.0%) or non-Catholic Christians (4.3%). Wealth index was also significantly associated with having a CS ($p<0.01$); as wealth index increased so too did rates of CS, with the poorest of women having the lowest percent of CS (0.5%) and the richest women having the highest percentages of CS (7.2%). Difficulty accessing a healthcare facility was also significantly associated with having a CS ($p<0.01$), with women who reported no difficulty in accessing a healthcare facility having higher percentages of a CS (2.8%) compared to women who reported difficulty accessing a healthcare facility (1.1%). Having health insurance was significantly associated with having a CS ($p<0.01$); those with health insurance displayed significantly higher percentages of having a CS (11.7%) compared to those with no insurance (2.1%). Also, demonstrated was a significant relationship betweentype of prenatal care provider and having a CS ($p<0.01$), with women who obtained prenatal care from a skilled birth attendant having higher percentages of CS (3.6%) than those who obtained prenatal care from an unskilled birth attendant (0.9%) or did not receive prenatal care (0.4%). Having a skilled birth attendant was also a significantly related to having a CS ($p<0.01$), with higher percentages in women who had a skilled birth attendant present during delivery (16.1%) than those who delivered with an unskilled birth attendant (0.6%). A significant relationship was demonstrated between having a CS if a woman took anti-malaria medication ($p<0.01$), with higher proportions of CS demonstrated among women who took anti-malaria medication (3.3%), compared to those who did not (1.3%). Being offered an HIV test as part of prenatal care was significantly related to having CS ($p<0.01$), with higher proportions of CS among women who were offered HIV testing as part of prenatal care (5.2%) compared to those who were not offered HIV testing as part of prenatal care (1.5%). Lower percentages of CS were found among those who took iron supplementation for at least half of their pregnancy (1.7%) compared to those who did not take iron supplementation for at least half of their pregnancy (2.7%; chi-square $p<0.01$). Having a CS during a previous pregnancy was also significantly associated with current CS ($p<0.01$) with more women having a current CS if they had a previous CS (50.9%) compared to those who did not (0.07%). The geographical area in which a woman lives was also related to having a CS ($p<0.01$), with women in northern regions having lower percentages of CS compared to women living in the southern regions of Nigeria. No significant association between the gender of the infant and having a CS were demonstrated ($p=0.22$).

Table 7: Weighted chi-square of the socio-economic and medical factors associated with mode of delivery

	Full sample				Primigravida			
		Vaginal	Cesarean	*P*		Vaginal	Cesarean	*P*
	N	%	%		N	%	%	
Total sample	**19665**	**97.7**	**2.3**		**3596**	**95.6**	**4.4**	
Age								
15-24	5095	98.4	1.6	*<0.01**	2521	97.3	2.6	*<0.01**
25-34	9197	97.6	2.4		1000	92.3	7.7	
34-49	5373	97.3	2.7		75	78.6	2.1	
Area of Residence								
Urban	6532	95.6	4.4	*<0.01**	1324	92.3	7.7	*<0.01**
Rural	13133	98.9	1.1		2272	97.7	2.3	
Education								
None	8997	99.5	0.5	*<0.01**	1173	98.8	1.2	*<0.01**
Primary	3989	98.0	2.0		519	97.4	2.6	
Secondary	5379	96.4	3.6		1510	94.8	5.2	
Higher	1300	88.9	11.1		394	85.8	14.3	
Religion								
Catholic	1602	95.1	4.9	*<0.01**	360	90.2	9.5	*<0.01**
Other Christian	6466	95.7	4.3		1431	93.5	6.5	
Islam	11303	99.0	1.0		1770	98.0	2.0	
Wealth index								
Poorest	4308	99.5	0.5	*<0.01**	587	98.9	1.1	*<0.01**
Poorer	4513	99.1	0.9		762	97.2	2.8	
Middle	3955	98.6	1.4		748	97.0	3.0	
Richer	3668	97.6	2.4		740	96.7	3.4	
Richest	3221	92.9	7.2		759	89.5	10.5	
Difficulty accessing a healthcare facility								
No	13327	97.2	2.8	*<0.01**	2533	94.7	5.3	*<0.01**
Yes	6259	98.9	1.1		1048	98.1	1.9	
Infant's Gender								
Male	9966	97.6	2.4	*0.2159*	1825	94.7	5.3	*0.02*
Female	9699	97.9	2.1		1771	96.6	3.4	
Health Insurance								
No	19235	97.9	2.1	*<0.01**	3505	96.0	4.0	*<0.01**
Yes	354	88.3	11.7		77	73.0	27.0	
Prenatal Care Provider								
No Prenatal Care	6496	99.6	0.4	*<0.01**	954	99.2	0.8	*<0.01**
Unskilled birth attendant	1710	99.1	0.9		291	98.6	1.4	
Skilled birth attendant	11299	96.4	3.6		2335	93.6	6.4	

Continued Table 7: Weighted chi-square of the socio-economic and medical factors associated with mode of delivery

	Full sample				Primigravida			
		Vaginal	Cesarean	*P*		Vaginal	Cesarean	*P*
	N	%	%		N	%	%	
Delivered by:								
Unskilled birth attendant	17601	99.3	0.6	*<0.01**	3069	98.8	1.2	*<0.01**
Skilled birth attendant	1974	83.9	16.1		513	77.6	22.4	
Took Anti-Malaria Medication								
No	9797	98.7	1.3	*<0.01**	1674	97.6	2.4	*<0.01**
Yes	9463	96.7	3.3		1851	93.9	6.1	
Intestinal Parasite Medication								
No	16015	97.9	2.1	*0.04**	2888	95.9	4.1	0.2
Yes	2922	97.1	2.9		571	94.5	5.5	
Offered HIV Test as part of Prenatal Care								
No	5709	98.5	1.5	*<0.01**	1028	96.9	3.1	*<0.01**
Yes	6477	94.8	5.2		1429	91.5	8.5	
Past CS								
No	19548	99.3	0.07	*<0.01**	N/A	N/A	N/A	N/A
Yes	117	49.1	50.9		N/A	N/A	N/A	
Iron Supplementation for at Least Half of Pregnancy								
No	10081	97.3	2.7	*<0.01**	1971	97.6	2.4	*<0.01**
Yes	8650	98.3	1.7		1452	93.9	6.1	
Region								
North Central	3027	97.5	2.5	*<0.01**	601	94.8	5.2	*<0.01**
North East	3946	98.9	1.1		613	98.2	1.8	
North West	6088	99.4	0.6		898	98.6	1.4	
South East	1669	96.0	4.0		377	92.9	7.2	
South Central	2436	95.4	4.6		570	92.9	7.1	
South West	2499	95.0	5.0		537	92.2	7.8	

Notes: *Significance based on weighted Chi-Square pvalue <0.05

Among primigravida women, 4.4% had a CS and 95.6% had a vaginal delivery. Age was significantly related to having a CS (*p<0.01*), with higher percentages among women 25–34 (7.7%) compared to those 15–24 (2.6%) and 34–49 (2.1%). Area of residence was also significantly related to having a CS (*p<0.01*); with higher percentages of CS occurring in urban areas (7.7%) compared to those in rural settings (2.3%). Education was significantly related to having a CS (*p<0.01*); as education increased so too did the percentage of women who had a CS (no education 1.2%; primary school education 2.6%, secondary 5.2% and tertiary or higher 14.3%). Religion was also significantly related to having a CS with higher percentages in Catholics (9.5%)

compared to other Christian denominations (6.5%) and Muslim (2.0%). Wealth index was significantly related to having a CS ($p<0.01$). As wealth index increased so too did the percent of women who had a CS (poorest 1.1%, poorer 2.8%, middle 3.0%, richer 3.4% and richest 10.5%). Expressing difficulty accessing a healthcare facility was also significantly associated with having a CS ($p<0.01$); with a higher percent of CS occurring among those who did not have difficulty accessing a healthcare facility (5.3%) compared to those who had difficulty accessing a healthcare facility (1.9%). Infants gender was significantly related to having a CS ($p<0.01$) with more women having a CS if they were pregnant with a male infant (5.3%) than a female infant (3.4%). Health insurance was also significantly related to having a CS ($p<0.01$); with more women having a CS if they had health insurance (27%) compared to those without health insurance (4%). The type of prenatal care provider was significantly associated to having a CS ($p<0.01$). Of the women who received prenatal care from a skilled birth attendant, 6.4% had a CS compared to 1.4% of those who attended an unskilled birth attendant for prenatal care and 0.8% who did not receive any type of prenatal care. Results demonstrated a significant relationship between type of provider present at delivery, skilled vs unskilled, and having a CS ($p<0.01$). Primigravida women who delivered with a skilled birth attendant had higher percentages of CS (22.4%) compared to women who delivered with an unskilled birth attendant (1.2%). Taking anti-malaria medication during pregnancy was also significantly associated with having a CS ($p<0.01$) with more women having a CS if they also took anti-malaria medication (6.1%) as opposed to women who did not take anti-malaria medication (2.4%). Among those who partook in prenatal care, a significant association between being offered HIV testing and having a CS was demonstrated (*$p<0.01$*). More women who were offered HIV testing as part of prenatal care had a CS (8.5%) compared to those who were not offered an HIV test as part of prenatal care (3.1%). A significant association was also demonstrated between taking iron supplementation for at least half of pregnancy and having a CS (*$p<0.01$*). Women who took iron supplementation for at least half or pregnancy had higher percentages of a CS (6.1%) than those who did not take iron supplementation for at least half or pregnancy (2.4%). The geographical area in which a woman lives was also related to having a CS (*$p<0.01$*), with women in northern regions having lower percentages of CS compared to women living in the southern regions of Nigeria.

Table 8 presents results from the weighted crude and adjusted odds ratios (95% CIs) for mode of delivery by participant characteristics. In the full sample, including both multigravida and primigravida births, the adjusted model demonstrated that compared to women who live in urban areas, the odds of having a CS were lower if the women lived in a rural setting (adjusted OR [aOR] 0.67: 95% 0.51–0.93). Compared to those with a no education, the odds of having a CS were higher if the mother had at least a tertiary education (aOR 2.71: 1.58–4.63) but not if she had a primary or secondary education (aOR1.20: 0.76–1.90 and 1.54: 0.98–2.42 respectively). Compared to women who practice Catholicism, lower odds of having a CS were demonstrated if the woman was Muslim (aOR 0.46: 0.28–0.73). Women who had health insurance had higher odds of having a CS compared to those without insurance (aOR 1.78: 1.18–2.67). Women who received prenatal care from a skilled birth attendant had higher odds of having a CS compared to women who did not received prenatal care (aOR 3.00: 1.51–5.96). Also, women who had a CS during previous pregnancies had higher odds of having a CS during their current pregnancy compared to women who had a previous vaginal delivery (aOR 28.97:15.31–4.81). Compared to women in North Central Nigeria, women in the North West had lower odds of having a CS (aOR 0.56:0.32–0.97). In the adjusted model, no relationship was found between CS and taking prophylaxis malaria medication and iron supplementation. Among primigravida women, compared to women who practice Catholicism, lower odds of having a CS were demonstrated if the woman was Muslim or an alternative form of Christianity (aOR 0.54: 0.30–0.98 and aOR 0.43: 0.19–0.96 respectively). Compared to those with no health insurance, those with health insurance had higher odds of having a CS (aOR 4.11: 1.97–8.54). In the adjusted models, no other significant relationships were demonstrated.

Table 8: Weighted logistic regression the socio-economic factors associated with mode of delivery

	Full Sample		Primigravida	
	Unadjusted	**Adjusted**	**Unadjusted**	**Adjusted**
		N=17932		**n=3580**
Age				
15-24	*Ref.*	*Ref.*	*Ref.*	*Ref.*
25-34	1.53 (1.59-2.03)*	0.85 (0.61-1.18)	3.03 (2.04-4.52)*	1.26 (0.74- 2.16)
34-49	1.69 (1.23-2.32)*	1.08 (0.76-1.54)	9.95 (4.86-20.36)*	4.24(1.78-10.14)
Area of Residence				
Urban	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Rural	0.25(0.19-0.33)*	0.67 (0.51-0.93)*	0.28 (0.20-0.42)*	0.55 (0.32-0.94)*
Education				
None	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Primary	3.79 (2.50-5.73)*	1.20 (0.76-1.90)	2.18 (0.96-4.94)	0.67 (0.27-1.69)
Secondary	7.06 (4.96-10.06)*	1.54 (0.98-2.42)	4.40 (2.47-7.83)*	1.15 (0.50-2.63)
Higher	23.63(16.0-34.81)*	2.71 (1.58-4.63)*	13.38 (7.19-24.90	1.54 (0.61-3.84)
Religion				
Catholic	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Other Christian	0.88 (0.64-1.20)	0.80 (0.55-1.16)	0.66 (0.39-1.12)	0.54 (0.30-0.98)*
Islam	0.19 (0.13-0.27)*	0.46 (0.28-0.73)*	0.19 (0.11-0.33)*	0.43 (0.19-0.96)*
Wealth index				
Poorest	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Poorer	1.77 (0.98-3.19)	0.83 (0.44-1.53)	2.50 (0.98-6.38)	1.37 (0.50-3.75)
Middle	2.73 (1.54-4.83)*	0.76 (0.42-1.39)	2.77 (1.09-7.01)*	0.76 (0.26-2.19)
Richer	4.58 (2.65-7.94)*	0.87 (0.46-1.65)	3.08 (1.23-7.72)*	0.55 (0.17-1.74)
Richest	14.5 (8.66-24.13)*	1.50 (0.77-3.90)	10.34 (4.45-24.16	0.87 (0.25-3.04)
Difficulty accessing a healthcare facility				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	0.37 (0.27-0.51)*	0.90 (0.62-1.30)	0.35 (0.20-0.60)*	0.66 (0.37-1.18)
Health Insurance				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	6.12 (1.29-8.73)*	1.78 (1.18-2.67)*	8.95 (4.77-16.80)*	4.11(1.97-8.54)*
Prenatal Care Provider				
No Prenatal Care	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Unskilled birth attendant	2.51(1.25-5.03)*	1.35 (0.59-3.08)	1.72 (0.56-5.28)*	0.72 (0.18-2.93)
Skilled birth attendant	10.45 (6.26-17.44)*	3.00 (1.51-5.96)*	8.53 (3.59-20.29)*	2.93 (0.96-8.90)

Continued Table 8: Weighted logistic regression the socio-economic factors associated with mode of delivery

	Full Sample		Primigravida	
	Unadjusted	**Adjusted**	**Unadjusted**	**Adjusted**
		N=17932		**n=3580**
Took Anti-Malaria Medication				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	2.49 (1.91-3.24)*	1.28 (0.96-1.70)	2.65 (0.79-3.92)	1.41 (0.94-2.13)
Past CS				
No	*Ref.*	*Ref.*	*N/A*	*N/A*
Yes	51.20 (32.00-91.90)*	28.97 (15.31-54.81)*	*N/A*	*N/A*
Iron Supplementation for at Least Half of Pregnancy				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	0.64 (0.49-0.83)*	1.20 (0.89-1.63)	0.67 (0.44-1.04)	1.07(0.68-1.68)
Region				
North Central	*Ref.*	*Ref.*	*Ref.*	*Ref.*
North East	0.45 (0.28-0.73)*	0.90 (0.54-1.50)	0.34 (0.17-0.69)*	0.68 (0.29-1.64)
North West	0.25 (0.15-0.42)*	0.56 (0.32-0.97)*	0.25 (0.22-0.52)*	0.58 (0.26-1.30)
South East	1.65 (1.11-2.44)*	0.78 (0.50-1.23)	1.40 (0.79-2.50)	0.71 (0.35-1.44)
South Central	1.89 (1.19-3.00)*	1.03 (0.67-1.59)	1.39 (0.79-2.42)	1.21 (0.65-2.27)
South West	2.07 (1.42-3.03)*	0.95 (0.65-1.37)	0.54 (0.90-2.66)	0.92(0.51-1.67)

Notes: *Significance based on weighted logistic regression p <0.05

Table 9 demonstrates the cultural beliefs and barriers that influenced women's ability to access a healthcare facility during delivery in Nigeria. Overall, 11,954 (67%) women did not deliver at a healthcare facility. Cultural beliefs influenced many women's decision not to deliver at a healthcare facility; with 34.5% of women reporting that it was not necessary to deliver at a healthcare facility; 8.1% stating that their husband's family did not allow them to access healthcare; 9.3% reporting it is not customary to deliver at a healthcare facility; 1.4% reporting distrust of the healthcare facility; 0.5% reporting that no female provider was available at the healthcare facility; and 0.2% reporting the they did not like the attitude of the healthcare professional at the health facility. Physical and financial barriers that influenced a woman's ability to deliver at a healthcare facility included: delivering too quickly (39.2%); inadequate transportation (15.5%); and costs associated with delivering at a healthcare facility (9.3%). Among primigravida women, 1,760 (49%) did not deliver at a healthcare facility (Table 8). Cultural beliefs and physical barriers were associated with accessing a healthcare facility in primigravida women in a similar manner to the full sample.

Table 9: Weighted percents of reasons why women did not healthcare facility to deliver

	Full sample (n=11954)		**Primigravida (n=1760)**	
	Yes	**No**	**Yes**	**No**
Not Necessary	34.5	65.5	30.1	69.1
Husbands family didn't allow	8.1	91.9	8.8	91.2
Not Customary	9.3	90.7	7.3	92.8
Delivered too quickly	39.2	60.8	40.4	59.7
Transportation	15.5	84.5	16.8	83.2
Cost	9.3	90.7	10.2	89.8
Facility Not Open	2.3	97.7	3.0	97.0
Distrust of healthcare facility	1.4	98.6	1.2	98.8
No female provider	0.5	99.5	0.7	99.3
Attitude of healthcare professional	0.2	99.8	0.1	99.9

Tables 10 and 11 show the reasons women did not deliver at a healthcare facility, stratified by area of residence. Both tables demonstrate that women living in rural areas endorsed both cultural and physical barriers at a greater proportion that those living in urban areas with the exception of women delivering too quickly.

Table 10: Weighted percents of reasons why women did not deliver at a healthcare facility stratified by area of resdience

	Rural		**Urban**	
	Full sample (n=9729)		**Full sample (n=2225)**	
	Yes	**No**	**Yes**	**No**
Not Necessary	35.6	64.4	30.1	69.9
Husbands family didn't allow	8.6	91.4	6.0	94.0
Not Customary	10.0	90.0	6.6	93.4
Delivered too quickly	36.5	63.5	49.6	50.4
Transportation	17.3	82.7	8.4	91.6
Cost	9.4	90.6	8.2	90.8
Facility Not Open	2.6	97.4	1.2	98.8
Distrust of healthcare facility	1.0	99.0	3.1	96.9
No female provider	0.6	99.4	0.4	99.6
Attitude of healthcare professional	0.1	99.9	0.7	99.3

Table 11: Among primigravida women, weighted percents of reasons why women did not deliver at a healthcare facility stratified by area of residence

	Rural		Urban	
	Primigravida (n=1461)		Primigravida (n=299)	
	Yes	No	Yes	No
Not Necessary	32.7	67.3	22.7	77.3
Husbands family didn't allow	9.2	90.8	7.1	92.9
Not Customary	8.1	91.9	3.4	96.6
Delivered too quickly	36.7	63.3	57.0	43.0
Transportation	18.0	92.0	11.2	88.8
Cost	10.4	89.6	9.1	90.9
Facility Not Open	3.3	96.7	1.6	98.4
Distrust of healthcare facility	0.8	99.2	2.7	97.3
No female provider	0.7	99.3	0.6	99.4
Attitude of healthcare professional	9.2	90.8	7.1	92.9

Comparison of Cesarean Section Results Across Samples

Because of the uniqueness of both datasets, different variables were included in the models used to explore the socioeconomic and medical risk factors associated with having CS (see Tables 12 and 13). However, both models included mother's age, area of residence and education levels. In the full sample, crude models demonstrated similar results between the HBI and the DHS for mother's age, area of residence, and education. As mother's age and education level increased so did her odds of having a CS. Women living in rural settings had a decrease in the odds of having a CS compared to those living in urban locations. This relationship was maintained in the adjusted model for area of residence and education but not for mother's age. In the HBI, adjusted model demonstrated an increase in the odds of having a CS as a woman aged. This relationship was not demonstrated in the DHS dataset. In the crude model of the HBI cohort, infant's gender was associated with having a CS with males having higher odds of being born via CS than females. This variable was not included in DHS analysis because this relationship was not found in the original chi-square (see Table 7).

Primigravida women displayed similar results to the full sample. Both samples demonstrated statistically significant lower odds of having a CS among women living in rural settings compared to those living in urban settings. Although the crude models of HBI were not significant, they showed a similar pattern to DHS in that as age and education increased, so too did the odds of having a CS. Sample size differences between HBI and DHS dataset likely explain this difference in the statistical significance in results between these analyses.

Because of the independent nature of the studies, numerous study variables did not overlap between the HBI and DHS dataset, including: religion, wealth index, difficulty accessing a healthcare facility, health insurance, prenatal care provider, malaria parasitemia, iron supplementation, region, and employment status. Wealth index was not included as part of HBI; therefore, marital status was utilized as an attempt to control for wealth index. Likewise, past CS was not included in the HBI but was included in the DHS. Because having a CS in previous pregnancies is known to predict current CS, gravida and number of people in the household were included in the HBI as an attempt to control for past CS. Malaria parasitemia was included in the HBI. The DHS did not have information on malaria parasitemia and instead had information on whether the women took an anti-malarial during pregnancy. It is unknown if women were taking anti-malarial medication as part of preventative treatment or because they had symptoms associated with malaria. Therefore, a comparison of these two variables is difficult. Two variables included in the DHS, religion and region, were used as inclusion criteria for the HBI cohort. The HBI recruited women based on their religious affiliation with the majority of women recruited being Catholic. The DHS demonstrated lower odds of having a CS among Muslim women compared to Catholic. Women in HBI reported similar rates of CS to Catholic women in the DHS (Full sample: 7% vs 5%; Primigravida: 8% vs 9%, respectively).

Table 12: Comparison of the logistic regression models for Aim 4 and Aim 6 among the full sample

Enugu State, Nigeria (HBI)		**All Nigeria (DHS)**	
	Adjusted		**Adjusted**
	N=1669		**N=17932**
Mother's Age		**Mother's Age**	
15-24	*Ref.*	15-24	*Ref.*
25-34	2.01(1.04-3.90)*	25-34	0.85 (0.61-1.18)
35-45	2.73(1.26-5.92)*	35-49	1.08 (0.76-1.54)
Area of Residence		**Area of Residence**	
Urban	*Ref.*	Urban	*Ref.*
Rural	0.58(0.38-0.89)*	Rural	0.67 (0.51-0.93)*
Education		**Education**	
		None	*Ref.*
None/Primary	*Ref.*	Primary	1.20 (0.76-1.90)
Secondary	1.27(0.73-2.30)	Secondary	1.54 (0.98-2.42)
Higher	2.91(1.54-5.53)*	Higher	2.71 (1.58-4.63)*
Religion		**Religion**	
Catholic	N/A	Catholic	*Ref.*
Other Christian	N/A	Other Christian	0.80 (0.55-1.16)
Islam	N/A	Islam	0.46 (0.28-0.73)*
Marital Status		**Wealth index**	
Other	0.93(0.32-2.71)	Poorest	*Ref.*
Married	*Ref.*	Poorer	0.83 (0.44-1.53)
		Middle	0.76 (0.42-1.39)
		Richer	0.87 (0.46-1.65)
		Richest	1.50 (0.77-3.90)
Difficulty Accessing a Healthcare Facility		**Difficulty Accessing a Healthcare Facility**	
No	N/A	No	*Ref.*
Yes	N/A	Yes	0.90 (0.62-1.30)
Health Insurance		**Health Insurance**	
No	N/A	No	*Ref.*
Yes	N/A	Yes	1.78 (1.18-2.67)*
Prenatal Care Provider		**Prenatal Care Provider**	
No Prenatal Care	N/A	No Prenatal Care	*Ref.*
Unskilled birth attendant	N/A	Unskilled birth attendant	1.35 (0.59-3.08)
Skilled birth attendant	N/A	Skilled birth attendant	3.00 (1.51-5.96)*

Continued Table12: Comparison of the logistic regression models for Aim 4 and Aim 6 among the full sample

Enugu State, Nigeria (HBI)		All Nigeria (DHS)	
	Adjusted N=1669		**Adjusted N=17932**
Malaria Parasitemia		**Took Anti-Malaria Medication**	
High	1.53(1.03-2.27)*	No	*Ref*.
Low	*Ref*	Yes	1.28 (0.96-1.70)
Gravidity		**Past CS**	
Primigravida	1.03(0.55-1.96)	No	*Ref*.
Multigravida	*Ref*.	Yes	28.97 (15.31-54.81)*
Iron Supplementation for at Least Half of Pregnancy		**Iron Supplementation for at Least Half of Pregnancy**	
No	N/A	No	*Ref*.
Yes	N/A	Yes	1.20 (0.89-1.63)
Region		**Region**	
North Central	N/A	North Central	*Ref*.
North East	N/A	North East	0.90 (0.54-1.50)
North West	N/A	North West	0.56 (0.32-0.97)*
South East	N/A	South East	0.78 (0.50-1.23)
South Central	N/A	South Central	1.03 (0.67-1.59)
South West	N/A	South West	0.95 (0.65-1.37)
Infant Gender		**Infant Gender**	
Male	1.18(0.80-1.75)	Male	N/S
Female	*Ref*.	Female	N/S
Number of People in Household		**Number of People in Household**	
1-2	*Ref*.	1-2	N/I
3-4	0.94(0.49-1.79)	3-4	N/I
5+	0.58(0.29-1.16)	5+	N/I
Employment status		**Employment status**	
Full-time	*Ref*.	Full-time	N/A
Part-time	0.56(0.32-0.97)*	Part-time	N/A
None	0.80(0.52-1.25)	None	N/A

Notes: *Significance based on weighted logistic regression pvalue <0.05

N/A=not available in dataset; N/S=not significant based on Pearson's chi-square or Fisher's exact therefore not included in logistic regression; N/I=not included-variable was used in Aim 4 to control for previous C-section

Table 13 : Comparison of the logistic regression models for Aim 4 and Aim 6 among primigravida women

Enugu State, Nigeria (HBI)		All Nigera (DHS)	
	Adjusted N=256		**Adjusted N=3580**
Mother's Age		**Mother's Age**	
15-24	*Ref.*	15-24	*Ref.*
25-34	0.85(0.23-3.10)	25-34	1.26 (0.74- 2.16)
35-45	1.54(0.22-10.75)	34-49	4.24(1.78-10.14)*
Area of Residence		**Area of Residence**	
Urban	*Ref.*	Urban	*Ref.*
Rural	0.27(0.09-0.82)*	Rural	0.55 (0.32-0.94)*
Education		**Education**	
		None	*Ref.*
None/Primary	*Ref.*	Primary	0.67 (0.27-1.69)
Secondary	0.57(0.13-2.58)	Secondary	1.15 (0.50-2.63)
Higher	0.89(0.16-4.86)	Higher	1.54 (0.61-3.84)
Religion		**Religion**	
Catholic	N/A	Catholic	*Ref.*
Other Christian	N/A	Other Christian	0.54 (0.30-0.98)*
Islam	N/A	Islam	0.43 (0.19-0.96)*
Marital Status		**Wealth index**	
Other	0.67(0.12-3.61)	Poorest	*Ref.*
Married	*Ref.*	Poorer	1.37 (0.50-3.75)
		Middle	0.76 (0.26-2.19)
		Richer	0.55 (0.17-1.74)
		Richest	0.87 (0.25-3.04)
Difficulty Accessing a Healthcare Facility		**Difficulty Accessing a Healthcare Facility**	
No	N/A	No	*Ref.*
Yes	N/A	Yes	0.66 (0.37-1.18)
Health Insurance		**Health Insurance**	
No	N/A	No	*Ref.*
Yes	N/A	Yes	4.11(1.97-8.54)*
Prenatal Care Provider		**Prenatal Care Provider**	
No Prenatal Care	N/A	No Prenatal Care	*Ref.*
Unskilled birth attendant	N/A	Unskilled birth attendant	0.72 (0.18-2.93)
Skilled birth attendant	N/A	Skilled birth attendant	2.93 (0.96-8.90)

Continued Table 13: Comparison of the logistic regression models for Aim 4 and Aim 6 among primigravida women

Enugu State, Nigeria (HBI)		All Nigeria, (DHS)	
	Adjusted N=256		**Adjusted N=3580**
Malaria Parasitemia		**Took Anti-Malaria Medication**	
High	0.67(0.22-2.06)	No	*Ref.*
Low	*Ref*	Yes	1.41 (0.94-2.13)
Iron Supplementation for at Least Half of Pregnancy		**Iron Supplementation for at Least Half of Pregnancy**	
	N/A	No	*Ref.*
	N/A	Yes	1.07(0.68-1.68)
Region		**Region**	
North Central	N/A	North Central	*Ref.*
North East	N/A	North East	0.68(0.29-1.64)
North West	N/A	North West	0.58(0.26-1.30)
South East	N/A	South East	0.71(0.35-1.44)
South Central	N/A	South Central	1.21(0.65-2.27)
South West	N/A	South West	0.92(0.51-1.67)
Infant Gender		**Infant Gender**	
Male	0.86(0.32-2.32)	Male	N/S
Female	*Ref.*	Female	N/S
Number of People in Household		**Number of People in Household**	
1-2	*Ref.*	1-2	N/I
3-4	1.38(0.49-3.87)	3-4	N/I
5+	0.37(0.04-3.14)	5+	N/I
Employment status		**Employment status**	
Full-time	*Ref.*	Full-time	N/A
Part-time	1.72(0.43-6.85)	Part-time	N/A
None	1.42(0.42-4.87)	None	N/A

Notes: *Significance based on weighted logistic regression pvalue <0.05

N/A=not available in dataset; N/S=not significant based on Pearson's chi-square or Fisher's exact therefore not included in logistic regression; N/I=not included-variable was used in Aim 4 to control for previous C-section

CHAPTER 4

DISCUSSION

In the following sections I will discuss each of the Specific Aims. Aims 1 and 2 of this dissertation were designed to establish the prevalence of malaria parasitemia during pregnancy and explore the person-level risk factors associated with malaria parasitemia in the Enugu State, Nigeria. Aims 3-6 focused on estimating the incidence of CS among pregnant women and determining the socioeconomic and medical risk factors associated with having a CS in Nigeria. The HBI and DHS datasets were utilized to analyze these Aims and develop three unique manuscripts. The results will be discussed in 3 separate sections, and an overall discussion will follow.

Malaria Parasitemia in Pregnancy

This dissertation demonstrated that over 99% of pregnant women in Enugu State, Nigeria showed at least low levels of malaria parasitemia, with 38% showing high levels of malaria parasitemia. For each additional person in the household, pregnant women in Enugu State had 6% lower odds of having high malaria parasitemia. Estimates presented in this paper are consistent with hospital-based estimates of malaria in pregnant women in the southeastern region of Nigeria [193]. With nearly 110 million clinical cases of malaria occurring a year, this disease places a heavy burden on Nigeria’s already fragile healthcare system and accounts for approximately 60% of outpatient visits and 30% of hospital admissions [170]. Malaria is estimated to cost Nigeria $8.6 billion USD per year in hospital care and lost wages [194].

In order to reach Millennium Development Goal 6, improved access to prenatal care is needed [12, 13]. The Roll Back Malaria program recommends that pregnant women receive intermittent preventative treatment with the inclusion of sulphadoxine-pyrimethamine (IPTp-SP) as part of antenatal care, and recommends that pregnant women sleep under insecticide-treated nets (ITN). Although Nigeria has adopted these recommendations, with only 13.2% of pregnant women receiving IPTp-SP, and 33.7% of pregnant women sleeping under an ITN, more work is required to achieve these goals [170, 173]. Based on the high prevalence of malaria in this study, education about best practices to prevent malaria during pregnancy and resources in support of these practices are urgently needed. Increases in obstetric services during delivery are also needed; this will be discussed further in the following two sections.

Cesarean section in Enugu State, Nigeria

Information presented in this part of the discussion was collected in Enugu State, Nigeria, located in the southeastern part of Nigeria. Increasing access to emergency obstetric care, including CS, decreases maternal and infant morbidity and mortality [12, 13]. However, women in SSA struggle to obtain adequate obstetric care during pregnancy. Nigeria, one of the fastest growing populations in the world with a crude birth rate of 38.03 births per 1,000 women, is a key location to study access to healthcare in pregnancy [1]. This dissertation showed that 7.2% of women in Enugu State, Nigeria had a CS, while 92.8% had a vaginal delivery. Compared to women who had full-time positions, women who worked part-time had 44% lower odds of having a CS after adjusting for potential confounders. Likewise, significantly lower odds of having a CS was observed among women who lived in rural settings compared to those who resided in urban settings. This was true for both the full sample and for the sample of primigravida women. This dissertation demonstrated 53% higher odds of having a CS if participants had high peripheral malaria parasitemia compared to those with lower peripheral malaria parasitemia, after adjusting for potential confounding.

The present dissertation showed that utilization of CS increased with maternal age and education, such that in older women and women with more education were more likely to have had a CS. In SSA, education has been shown to be a strong predictor of using professionally assisted delivery services [130, 137]. Older and more educated women in SSA are considered more confident and influential in their household decision-making, including the use of healthcare services [130, 142]. Likewise, women with more education or who are employed often have greater control over family resources and play a larger part in reproductive decision-making [23, 142, 143]. Maternal age and education may be proxies of a woman's ability to access healthcare, thereby increasing her chances of having had a CS.

The relationship between infant gender and having a CS has been well-documented in developed countries, but has only recently been studied in Africa [195]. In Libya, male fetuses were associated with higher odds of maternal diabetes mellitus, preterm delivery, needing instruments when a vaginal delivery was performed, and having CS compared to female fetuses [196]. In the present study, being pregnant with a boy may have been associated with these pregnancy complications; however, sex was unknown during gestation, as women in this part of Nigeria were unlikely to have an ultrasound to determine the gender of their infants. Therefore, the finding of the current work that male infants were associated with a higher odds of CS suggests that the relationship between CS and male infants is biologically and not culturally based [196]. The biological basis of the relationship between being pregnant with a male fetus and having a CS is unclear and warrants further attention in future research [196].

Although the analyses herein did not find an association between distance to nearest health facility and having had a CS, a statically significant relationship was demonstrated between living in rural vs urban environments and having had a CS. In rural settings, distance has consistently been an important barrier to seeking healthcare [2, 4, 197]. It is possible that in this self-report data, area of residence (i.e., rural vs urban) was an indirect assessment of the ease of reaching a healthcare facility for childbirth. Like women in other rural areas, women living in rural parts of Enugu State, Nigeria may have had difficulty accessing facilities that can perform a CS because of limited transportation options, poor road conditions, and poverty [2, 4, 23]. In order to reach Millennium Development Goal 5 by end of 2015, improved access to emergency obstetric care, such as CS, is needed [12, 13].

The relationship between malaria parasitemia and the need for emergency obstetric care has not been well established. To the authors' knowledge, this dissertation is the first epidemiological investigation to report that high malaria parasitemia is associated with higher odds of CS; although there are some case studies [16, 160, 161]. A biological pathway assessing the relationship between malaria parasitemia and the increased need to have a CS has not been established; however, there is some evidence to indicate that malaria parasitemia puts stress on the fetus. Malaria parasitemia is believed to produce adverse fetal outcomes via systemic effects such as maternal anemia,[26, 64, 65] and local effects such as placental infection [65-68]. Maternal anemia decreases erythropoiesis[30, 69] and increases red blood cell apoptosis[30, 70], ultimately leading to a maternal hypoxic state. During this hypoxic state, impaired growth and vascularization occur within the pregnant woman, which in turn can lead to fetal hypoxia [71]. Decreased maternal vascularization leads to a reduction in the exchange of important nutrients and gases across the placenta, including oxygen, which can also lead to a fetal hypoxic state [66, 72, 73]. Due to fetal hypoxia and decreased nutrient uptake, intrauterine growth retardation (IUGR) can occur [72]. Malaria infection is also thought to disrupt cytokine activity, resulting in an increase in placental infection; this is especially seen during the first or second pregnancy and can result in preterm infants with IUGR [67, 68, 74-80]. The maternal hypoxia and inflammatory response often caused by maternal malaria puts stress on the fetus and makes it difficult for the fetus to get the nutrients needed to grow often leading to a preterm delivery of a LBW infant [63]. Therefore, an increased need to have a CS may be due to an increase in fetal stress caused by high rates of malaria parasitemia. The relationship between malaria parasitemia and CS warrants further attention.

The relationship between sickle cell disease (SCD) and CS also warrants further attention. Some evidence has suggested that women with sickle cell disease (SCD) are more likely to have a CS in SSA [198, 199]. However, because both SCD and CS are related to high risk of adverse maternal and neonatal outcomes in SSA, it is difficult to determine whether women with SCD would benefit from having a CS [199-201]. Also, SCD and malaria are both associated with anemia during pregnancy [18, 30, 69, 202]. This further complicates the relationship between SCD and CS as anemia is related to an increase in maternal morbidity and mortality in developing countries [114]. Also related to an increase in maternal morbidity and mortality is HIV [36].

This dissertation is consistent with a meta-analysis that found HIV+ women were no more likely to have a CS than those not infected [36]. Evidence from resource-unconstrained areas suggests that having a CS is beneficial if a woman's HIV–RNA level is above 1000 copies/ml near delivery [38]. However, women in resource-constrained areas are often unaware of their viral load before delivery; thus, having a CS may or may not be beneficial for HIV-infected women [158]. Nonetheless, in resource-constrained areas, CS are often unavailable and unsafe; therefore, the WHO guidelines do not currently recommend HIV+ women in resource constrained regions have an elective CS [159]. Instead the WHO recommends that HIV+ women take 3 or more antiretroviral medications in order to decrease mother-to-child transmission of HIV [93]. The WHO also recommends that infants born to HIV+ mothers receive antiretrovirals during the post-natal period [93].

The sensitivity analysis assessing the association between participant's baseline characteristics and being lost to follow-up demonstrated that testing positive for HIV did not deter women from completing a follow-up questionnaire. As stigma related to HIV testing has been demonstrated to deter women from partaking in HIV testing, it is surprising that this dissertation did not demonstrate a statistically significant relationship between HIV status and being lost to follow-up [203]. Women who were younger and unemployed had higher proportions of being lost to follow-up than older and employed women. This may occur because older and employed women in SSA may be more influential in household decision-making, including whether to attend follow-up interviews [23, 130, 142, 143]. A larger proportion of urban dwelling women were lost to follow-up than those living in rural areas. This may be reflective of a difference in consistent church attendance in rural *vs*. urban areas in SSA; however, this cannot be confirmed in this dissertation, and remains understudied in the literature. Lastly, a larger proportion of primigravida women were lost to follow-up than multigravida. Perhaps this difference in being lost to follow-up reflects an adjustment to having a newborn child; therefore primigravida women were less likely to attend church.

Information presented in this part of the discussion was collected in Enugu State, Nigeria, located in the southeastern part of Nigeria. The following section will discuss data obtained from the Nigerian Demographic and Health Survey, a cross-sectional study conducted throughout Nigeria.

Cesarean Sections in Nigeria

Nigeria has the second highest maternal mortality ratio in the world, in part because of barriers women encounter accessing adequate healthcare during pregnancy. In order to reach Millennium Development Goal 5 by end of 2015, improved access to emergency obstetric care—such as CS—needed to be provided [12, 13]. The aims for this study were to estimate the incidence of CS in Nigeria and to determine the socioeconomic, demographic, and medical risk factors associated with having a CS in Nigeria. In the overall sample, including both multi- and primigravida births, only 2.3% of women had a CS. Women living in rural areas had 33% lower odds of having a CS compared to women living in urban areas. Religion was also significantly associated with having had a CS; Muslim women (54%) had significantly lower odds of having a CS compared to Catholic women. Women with health insurance had a 78% higher odds of having a CS compared to women without health insurance; while those offered HIV testing as part of prenatal care had a 96% increase in the odds of having a CS compared to women who were not.

Lower frequencies of CS among women living in rural environments have consistently been reported because of barriers to accessing treatment, such as limited transportation options and poor road conditions [2, 4, 23, 197]. Poverty plays a substantial role in a woman's ability to access a healthcare facility during delivery. In rural areas, costs associated with having a CS, as well as costs associated with transportation are common reasons for not accessing a healthcare facilities during delivery [2, 28, 147]. In this sample, approximately 10% of all women reported costs as a deterrent to accessing a healthcare facility during delivery. Furthermore, 17.3% of rural Nigerian women sampled indicated that they did not deliver at a healthcare facility because of transportation difficulties, compared to less than 8.4% of urban women. It is likely that women in rural settings do not actively choose to deliver outside of healthcare facilities—thereby decreasing their rates of CS—but are instead forced to deliver outside of healthcare facilities because of the barriers associated with living in a rural setting.

The present work also demonstrated higher odds of having a CS if women had a tertiary education or more compared to those with no education. Although no relationship was observed in the odds of having a secondary education or higher and having a CS, increasing education in woman was an important step in achieving Millennium Development Goal 5. Generally, as education increases, access to healthcare facilities grows, and maternal mortality tends to decrease [2, 204, 205]. Women with more education possess greater control over family resources and play a larger role in their reproductive decision-making [23, 142, 143]. Karlsen et al., [205] demonstrated higher maternal mortality among those with low education levels. This relationship persisted even after controlling for potential confounders [205]. The importance of increasing access to primary school education (Millennium Development Goal 2) and eliminating gender inequalities in education (Millennium Development Goal 3) are both substantial steps toward achieving Millennium Development Goal 5.

To improve healthcare, the Nigerian government implemented the National Health Insurance Scheme in 1999 [206]. However, numerous studies have demonstrated that Nigerians continue to rely on direct payment to finance their healthcare needs [207]. Less than 1% of women in this sample had health insurance. It is possible that those who stated they had health insurance were more likely to have privately funded health insurance and, therefore, possessed the ability to financially afford a CS even if the their insurance did not cover all the associated costs.

Ethnic and cultural diversity in Nigeria often vary by geographical region [208]. The interconnectedness of geographical region and religion in Nigeria is difficult to tease apart as the northern areas of Nigeria are predominately Muslim, while the middle and southern regions are predominately Christian [208]. This dissertation demonstrated lower odds of having a CS among Muslim women compared to Catholic women, and that women in the northwestern part of Nigeria had lower odds of having had a CS compared to the north-central area. This may be in part because of a lack of access to skilled health providers in northern regions of Nigeria [23, 208]. Having a skilled birth attendant available at delivery is necessary to perform a CS. Furthermore, previous research has demonstrated that women living in northern parts of Nigeria have lower rates of prenatal care and higher rates of home delivery [209]. With the percentage of home deliveries ranging from 12% in southwest Nigeria to 86% in northwest Nigeria, women in northern areas of Nigeria receive significantly less support from a skilled birth attendant than their counterparts in southern Nigeria [209]. The vast majority of these women have friends or relatives assisting with their delivery [209]. Cultural and religious norms may dictate this relationship—as some women in northern areas have restrictions in seeking health-related assistance during childbirth, especially from male providers [208]. Attaining the Millennium Development Goals is especially difficult in the Northern parts of Nigeria where poverty, illiteracy, and early marriage rates remain high among women, and reproductive health and family planning are not historically women's decisions [208]. Therefore, it is likely that women in the northern areas of Nigeria need better access to reproductive health education and trained birth attendants and community health workers, so the symptoms associated with potential obstetric complications can be detected before infant and/or maternal mortality ensues.

It is well documented in the literature that skilled birth attendants are associated with a decrease in maternal mortality [210]. This dissertation demonstrated that compared to women who received no prenatal care, the odds of having a CS were increased if a women received prenatal care from a skilled birth attendant. No significant relationship was demonstrated between receiving prenatal care from an unskilled birth attendant and having a CS. Additional research has indicated that traditional birth attendants can be effective at implementing interventions that reduce neonatal mortality in rural areas [211-214]. Further developing the skills of traditional births attendants and community health workers in recognizing early warning signs of life threatening obstetric complications could reduce maternal mortality in Nigeria, especially in Northern Nigeria.

Overall Discussion

The results of this dissertation indicate that Nigeria is not on target to achieve Millennium Development Goals 5 or 6 by the end of 2015. First, this dissertation demonstrated high rates of peripheral malaria parasitemia (>99%) in pregnant women living in Enugu State, Nigeria (Aim 1). Previous reports estimated that malaria is responsible for 11% of all maternal deaths within Nigeria [194]. Therefore, decreasing malaria in Nigeria is essential to reducing its high rates of maternal mortality. Next, we demonstrated that Nigeria has 2–3 times lower rates of CS than the WHO estimated is needed within Nigeria to decrease maternal mortality (Aims 3 and 5) [154]. Therefore, high rates of malaria parasitemia and low rates of CS are expected to continue to contribute a large portion to Nigeria's maternal mortality.

These results were consistent with previous research in Nigeria. Aim 1 demonstrated high rates of malaria parasitemia (>99%) during pregnancy in women from southeastern Nigeria. Agan, et al. [193] demonstrated that 95.4% of pregnant women in a hospital-based population in southeastern Nigeria tested positive for malaria parasitemia. The parent study for Aims 1 and 2 took place approximately five years later, but high rates of malaria parasitemia in pregnant women remained. Thus, little to no effective improvements had been made to reduce the burden of malaria in pregnant women in this region of Nigeria during that time period.

Aims 3 and 5 both estimated the incidence of CS among pregnant women in Nigeria. The WHO estimated that 15.5% of pregnancies in Nigeria need to be delivered by CS [154]. Both Aims 3 and 5 demonstrated low rates of CS in Nigeria compared to the need estimated by the WHO (7% and 2%, respectively). Geographical location may have resulted in the different rates of CS reported in this dissertation. The HBI was a congregational-based randomized control trial in southeastern Nigeria, while the DHS was a country wide cross-sectional study. Because data for both aims were collected no more than two years apart, it is less likely that the different rates of CS demonstrated between these two studies are a result of time trend differences. The HBI solely relied on data collected in southeastern Nigeria, which have become relatively politically stable in the last few years [208]. Political stability often offers economic growth as well as educational and infrastructure improvements—such as hospitals [208]. The northern parts of Nigeria have not displayed political stability in recent years and continually distort national trends—because of the region's high poverty, high illiteracy, low family planning, and other reproductive health measures—compared to the rest of the county [208, 215]. This may explain the different rates of CS demonstrated in the HBI and the DHS.

Both studies demonstrated that women living in rural areas struggled to access a health care facility during delivery, as demonstrated in lower odds of having a CS among women living in rural areas compared to urban areas. With the DHS dataset, this dissertation demonstrated that women did not deliver in a healthcare facility because they faced cultural, economic and physical barriers while attempting to access perinatal care. Tables 9 and 10 both demonstrated that women living in rural areas struggle with finding adequate transportation to a healthcare facility to deliver. Decreasing the barriers women face when attempting to access both prenatal care and services for delivery are imperative to decreasing maternal mortality in Nigeria and achieving Millennium Development Goal 5 and 6.

Strengths and Limitations

There are numerous strengths and limitations to this dissertation. Over 99% of the pregnant women in the HBI tested positive for malaria parasitemia. This information is essential to advocating for malaria prevention programs in Enugu State, Nigeria. The HBI cohort was also used to explore the relationship between CS and socio/demographic variables as well as different disease statuses. This allowed us to capture a holistic understanding of risk factors associated with CS in Enugu State, Nigeria. However, the HBI was not without limitations. It was not possible to establish the effects of malaria parasitemia on the fetus because adequate birthweights and gestational age were not able to be established. This information would have been an important contribution to the literature, as the relationship between malaria parasitemia levels and perinatal outcomes are not well established. Also in the HBI, the reasons a woman had a CS were not established, nor was prior use of CS. An attempt was made to control for prior CS by using the number of people in the household as well as gravidity as proxies for prior CS; however, it is unknown if these control methods were adequate. A sensitivity analysis was also performed using only primigravida women; however, the number of women (n=26) that had a CS for their first pregnancy was small. Therefore, significant relationships between CS and other variables may not have been present in this analysis because of lack of power. Also, no follow-up occurred when women did not attend post-natal interviews; therefore, it was not possible to determine the rate of maternal mortality. In the HBI, 3002 people filled out the initial baseline questionnaire; however, only 2317 (77.2%) individuals returned for follow-up and completed information regarding mode of delivery. This relatively high rate of being lost to follow-up may have resulted in an inadequate estimate of the CS in Enugu State, Nigeria. If all women who were lost to follow-up had a CS, than the estimate for CS in Enugu State would be much higher (28.4%); likewise, if all women who were lost to follow-up had a vaginal delivery, the estimate for CS in Enugu State would be lower (5.6%). Therefore, women lost to follow-up may have had substantial influences on the CS estimates in this dissertation. Finally, it is unknown from the HBI data whether women in rural settings truly had difficulty accessing a healthcare facility. It is possible that more women in urban settings elected to have a CS. Answering these questions is essential to understanding the barriers that women face when seeking adequate perinatal care in Nigeria. Some of these questions were answered by utilizing the DHS dataset, as it provided information on previous CS and the reasons why women did not attend a healthcare facility for delivery.

Like the HBI, the results from the DHS are unique to the literature in that the relationships between CS and socio/demographic variables, as well as medical risk factors, were explored. As the DHS includes data across Nigeria, I also controlled for geopolitical region. The DHS contained information regarding the reasons women did not deliver at a healthcare facility. Therefore, I was able to get a better understanding of the barriers women face while attempting to access healthcare while in labor. A sensitivity analysis was performed using only primigravida women. In the unadjusted models, primigravida women showed similar trends in the OR as the full sample. However, after adjustment, statistical significance in the primigravida model diminished. It is possible that this analysis lacked statistical power because either over-adjustment occurred in the primigravida model, or a relatively small number of women (n=170) had a CS for their first pregnancy.

The Aims based on the DHS are not without their limitations. Although more women who were offered HIV testing as part of prenatal care had a CS, the data do not permit us to determine if this was from high rates of HIV among these women, or if it was from access to robust prenatal care. Likewise, it is unknown if women were taking iron supplementation because of anemia or because they attended prenatal care. Many socio/demographic and economic variables were significantly correlated but did not have high correlation coefficients. Although this indicates no collinearity between these variables in this dataset, it is still difficult to determine if all models were over adjusted by including variables that are typically related—such as education and wealth. Although all Aims had their own unique strengths and limitations, they all added to the literature indicating that women in Nigeria face numerous barriers to accessing healthcare during pregnancy and delivery.

Practice and Policy

This dissertation demonstrated that >99% of pregnant women in Enugu State, Nigeria had malaria parasitemia. This is aligned with other research indicating that in Nigeria, only13.2% of pregnant women receive IPTp-SP and 33.7% of pregnant women sleep under an ITN [170, 173]. Based on the high prevalence of malaria in the HBI data, education on best practices to prevent malaria during pregnancy, and resources in support of these practices are urgently needed. Increases in obstetric services during delivery are also needed.

When medically necessary, CS is frequently a lifesaving procedure; however, risks associated with CS are often highest within African countries where medical personnel may lack the training or proper equipment and supplies [4, 155]. In general, better perinatal health outcomes have been associated with CS rates between 5%–15% [151-154, 216], and the WHO has estimated that in 15.5% of pregnancies in Nigeria, a CS is medically necessary [154]. This is 2-3 times the rate found in the HBI and the DHS (7% and 2% respectively). Therefore, unlike in other parts of the world where discussion centers on overutilization of CS [154], it is likely that in this area of Nigeria an overall underutilization of CS occurs. This may explain why Nigeria accounts for half of the global burden of incident obstetric fistulas, which is caused by prolonged labor and can be prevented with access to emergency obstetric services such as a CS [204].

Although many countries in SSA have healthcare facilities that can perform CS, the quality of care within these clinics is neither consistent nor reliable [217]. It is estimated that less than 1% of individuals in western SSA have access to surgical care that is safe, affordable and can be performed in a timely manner [218]. Countries in SSA suffer from an overall shortage of facilities equipped to perform such specialized treatment; additionally, countries in SSA also suffer from a lack of skilled workers capable of performing specialized medicine [2]. Because previous research within Nigeria showed that only 1 in 21 health facilities was equipped to perform CS [138], it is likely that even if access to health clinics was increased, most clinics would not be equipped to perform CS. In a survey of 77 hospitals in SSA only 6% reported the ability to provide safe anesthesia for a CS [157]. The anesthesiologist in these facilities reported that only 19% operated in facilities were electricity was always available [157]. Only 56% of the facilities reported always having access to running water and only 23% reported having access to blood for transfusion [157]. Because many facilities lack water, electricity, medication, equipment and trained personnel to perform CS, the operation is often associated with unacceptably high rates of sepsis, hemorrhage, and maternal death [149]. Therefore, increasing access to healthcare facilities alone would not improve medical care for pregnant women, vast improvements in infrastructure and training of skilled obstetricians needs to be made within SSA.

Future Directions

The Millennium Development Goals were developed to reduce poverty, hunger, illiteracy, gender inequality, and diseases while also attempting to increase environmental sustainability and access to healthcare [14]. Although no goal has been achieved thus far, great improvements have been demonstrated. It is this author's opinion that continuing to develop global goals emphasizes our responsibility as global citizens. However, change within individual countries should reflect local customs and be sustainable from within.

Results from this dissertation indicate that there are both needs and opportunities for improvements in access to proper prenatal care in Nigeria. This will not be an easy task to achieve. The infrastructure needed to achieve the Millennium Development Goals, such as schools and hospitals, remains underdeveloped in Nigeria and most of SSA [208]. Additionally, previous research has demonstrated that even if access to health clinics were increased, most skilled birth attendants would not have the proper equipment to perform a lifesaving obstetric procedures [149]. Because many healthcare facilities in SSA lack water, electricity, medication, equipment and trained personnel, it is likely that in Nigeria malaria during pregnancy will persist at high rates and most women will delay seeking treatment at healthcare facilities until dire complications develop [149]. Further education of traditional birth attendants and community health workers in the administration of prenatal medications and recognizing signs and symptoms of pregnancy complication may decrease the burden of diseases associated with pregnancy in Nigeria, ultimately leading to a decrease in maternal mortality. This approach has been successful in other parts of the world and may prove to be beneficial in Nigeria [211-214]. By utilizing preexisting infrastructure, such as the congregational based infrastructure utilized by the HBI, women who would not regularly attend a healthcare facility for prenatal care or delivery services, could be screened for early warning signs of high risk pregnancy and for disease such as HIV and malaria. Women who are told they have a high risk pregnancy could then be encouraged to attend a healthcare facility or a mobile clinic prior to expected delivery date. Mobile clinics in SSA have demonstrated a decrease in the burden of diseases in rural areas by: increasing adherence to medications for both communicable and non-communicable disease; providing antenatal care; providing child immunizations; and decreasing the burden of costs associated with attending a healthcare facility outside of women's local region [219, 220]. More innovated programs that aim to decrease the burden of malaria and increase access to obstetric services in Nigeria are needed. Programs, such as *Riders for Health*[221], that utilize motorbikes to provide healthcare to individuals living in rural places in SSA may be beneficial. Studies assessing the efficacy of programs targeting increasing access to care in rural villages are also needed.

Other studies that address the questions raised by this dissertation are needed. More in-depth studies that establish the causes of maternal mortality in Nigeria are essential. Based on Nigeria's maternal mortality ratio of 560 maternal deaths per 100,000 live births, it is estimated that 17 women in the HBI died [2]. Knowing the reasons women die is essential to understand how to decrease maternal mortality in Nigeria. Also, a future study assessing the relationship between having a CS in Nigeria and future maternal and infant morbidity and mortality is essential to understanding the safety of having a CS in Nigeria. As neither the HBI nor the DHS could answer questions pertaining to causes of maternal mortality or the efficacy of having a CS, future studies are essential.

Conclusion

In summary, the goals of this dissertation were to investigate common pregnancy complications of Nigerian women. This dissertation demonstrated substantially high rates of malaria during pregnancy and lower rates of CS in Nigeria than have been recommended, indicating an overall lack of access to proper obstetric care and demonstrates that Millennium Development Goal 5 has not been achieved in Nigeria [154].

The results from this dissertation are concordant with other research establishing the prevalence of malaria in Nigeria. In hospital samples, Agan et al, demonstrated 95% of pregnant women in southeastern Nigeria had malaria parasitemia [193]; in a community sample, this dissertation demonstrated that over 99% of pregnant women had malaria parasitemia. The results should call attention to the need for further effective strategies to reduce the burden of malaria in southeastern part of Nigeria [193]. Malaria places a heavy burden on Nigeria's healthcare system, accounting for up to 60% of outpatient visits, 30% of hospital admissions and accounts for 11% of all maternal deaths in Nigeria [170]. As post-Millennium Development Goals are created, reducing the burden of malaria in Nigeria is essential. The prevention and treatment of malaria is crucial for the further development of Nigeria and any other country with high malaria transmission rates.

This dissertation verified that women in Nigeria continue to face barriers that delay their seeking perinatal care. Two unique datasets were analyzed and both demonstrated lower rates of CS than the WHO recommends within Nigeria to decrease maternal mortality [154]. Both datasets also demonstrated that women living in rural areas struggled to access health care facilities during delivery, and that as education increased, so too did the odds of having a CS. The increase in CS as education increased further calls to attention the need to promote gender equality in access to education(Millennium Development Goal 2 and 3). This dissertation also demonstrated that women did not deliver in a healthcare facility because they face cultural, economic and physical barriers while attempting to access perinatal care. It is apparent that women in Nigeria are delaying seeking treatment, not out of ignorance, but because they encounter real barriers that delay seeking treatment, such as cost and limited transportation. This was particularly important in rural areas where women had consistently lower odds of having a CS compared to women in urban settings.

Furthermore, these studies revealed that women in this part of SSA are not following global trends and over-utilizing CS, but instead are struggling to obtain adequate perinatal healthcare, ultimately perpetuating the cycle of high maternal mortality and gross health deficiencies that are common in SSA. Ultimately, results from this dissertation indicate that in Nigeria there exists great need for improvement in access to proper perinatal care.

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APPENDIX A

MANUSCRIPT 1

TITLE: Population based prevalence of malaria among pregnant women in Enugu State, Nigeria: The Healthy Beginning Initiative Cohort Study.

ABSTRACT

Background: Malaria adversely affects pregnant women and their fetuses or neonates. Estimates of the malaria burden in pregnant women based on health facilities often do no present a true picture of the problem due to the low proportion of women delivering at these facilities in malaria endemic regions. Data for this study were obtained from the Healthy Beginnings Initiative using community based sampling. Self-identified pregnant women between the ages of 17-45 years were recruited from churches in Enugu State, Nigeria. Malaria parasitemia was classified as high and low based on the malaria plus system.

Findings: Of the 2069 pregnant women for whom malaria parasitemia levels were recorded, over 99% tested positive for malaria parasitemia; 62% showed low parasitemia and 38% high parasitemia. After controlling for confounding variables, odds for high parasitemia were lower among those who had more people in the household (for every one person increase in a household, OR=0.94, 95% CI 0.89-0.99).

Conclusion: Results of this study are consistent with hospital-based estimates of malaria during pregnancy in southeastern Nigeria. Based on the high prevalence of malaria parasitemia in our sample, education on best practices to prevent malaria during pregnancy, and resources in support of these practices are urgently needed.

Key Words: Prevalence, pregnancy, malaria, Nigeria

BACKGROUND

Nigeria accounts for roughly 25% of the malaria burden in sub-Saharan Africa [1,2]. Often undetected and untreated, malaria adversely affects pregnant women and their fetus or neonate [2-4]. Current estimates of malaria parasitemia in Nigerian pregnant women vary greatly among geographic regions. Hospital-based prevalence percentages range from 5% in the northwestern region [5], 17% in the southwestern region [6], to 95% in the southeastern region where Nigeria borders the Gulf of Guinea [7].

In Nigeria, only an estimated 35% of pregnant women deliver at a healthcare facility [8]. Therefore, health facility based estimates of the malaria burden in pregnant women often do no present a true picture of the problem. This cohort study of pregnant women in the community is likely to be a more representative sample and, therefore, a more accurate estimate of the burden of malaria during pregnancy in southeastern Nigeria. The aims of this study were two-fold, (1) to investigate the population-based malaria parasitemia burden during pregnancy in Enugu State, Nigeria (2) to explore person-level maternal risk factors that are associated with high malaria parasitemia.

METHODS

Data for this study were obtained from the Healthy Beginnings Initiative (HBI), which has previously been described in detail [9]. Briefly, self-identified pregnant women between the ages of 17-45 years were recruited from 40 churches of varying denominations in Enugu State, Nigeria where the population is more than 95% Christian. Recruitment from churches was expected to provide a representative sample of pregnant woman in Enugu State, Nigeria, as church attendance approaches 90% in the country [9,10]. Pregnant women were given information on the study and, if interested, were asked to read and sign a written consent form. Majority of participants were unable to read the local language (Ibo) and preferred to have the study material in English. For participants who could not read, a church-based health advisor or a research assistant read the consent form aloud in English or Ibo; the participants gave their consent by affixing thumb prints or using initials.

A structured questionnaire consisting of both validated and not validated measures was implemented at a 6th grade reading level [9]. Trained research staff and church-based health advisors administered the survey. Participants had the option of reading the survey themselves or having study personnel read the questions to them in either English or Ibo. Other survey questions and laboratory measures not discussed in this paper can be found in the study protocol [9].

Parasitemia levels were assessed using the malaria plus system [11]. Thick blood smears were examined using microscopy under oil immersion [11]. To ensure quality control, each slide was examined by multiple laboratory technicians and random checks were made by a hospital review panel. The malaria plus system was scored as follows: 0 for no parasites, + for 1-10 parasites per 100 high power field, ++ for 11-100 parasites per 100 high power field, +++ for 1-10 parasites per high power field, ++++ for over 10 parasites per high power field. Thus, levels of parasitemia increase as the scoring moves from 0 through ++++.

Malaria parasitemia was classified as high and low based on the malaria plus system. Those in the 0 and + group were classified as low parasitemia; while those in the ++ and +++ groups were classified as high parasitemia. No participants showed malaria parasitemia consistent with the ++++ group. Gravidity was dichotomized as > 3 previous pregnancies and <3 pregnancies. Associations between malaria parasitemia and continuous variables were determined using ANOVA. Pearson's Chi-square test was used to examine associations of malaria with categorical and dichotomous variables. Crude and adjusted logistic regression models were used to determine the association between participant characteristics and malaria parasitemia levels. Statistical significance was set at $p < 0.05$. Data analyses were conducted using Stata version 12.0 [Stata Corporation, College Station, TX]. The parent study was approved by the Institutional Review Board of the University of Nevada, Reno, and the Nigerian National Health Research Ethics Committee. This secondary data-analysis was considered exempt from human subjects review by the Mel and Enid Zuckerman College of Public Health Research Office.

FINDINGS

Malaria parasitemia levels were recorded for 2069 pregnant women. Over 99% of the women in the study tested positive for malaria parasitemia (n=2052). Categorized according to the malaria plus system, malaria parasitemia in our sample included: less than < 1% no infection (n=17); 62% in the + group (n=1275); 36% in the ++ group (N=737); 2% in the +++group (n=40); with no one falling into the ++++ group (n=0) (see Table 1). When malaria was categorized as low and high parasitemia, 62% (n=1292) were classified as low parasitemia and 38% (n=777) as high parasitemia. As shown in Table 2, no significant differences were found between high and low malaria parasitemia and gravidity, area of residence, distance to nearest healthcare facility, household size, or age of participants. Table 3 shows the results of logistic regression analysis of the association between participant characteristics and malaria parasitemia. After controlling for confounding variables, odds for high parasitemia were lower among those who had more people in the household (For every one person increase in a household, OR=0.94, 95% CI 0.89-0.99).

DISCUSSION

The results of our study demonstrated that over 99% of pregnant women in Enugu, Nigeria showed some level of malaria parasitemia, with 38% showing high levels of parasitemia. For each additional person in the household, a 6% lower odds of high malaria parasitemia was found. Estimates presented in this paper are consistent with hospital-based estimates of malaria during pregnancy in the southeastern region of Nigeria [7].

Malaria places a heavy burden on Nigeria’s already fragile health care system with nearly 110 million clinical cases occurring a year, accounting for up to 60% of outpatient visits and 30% of hospital admissions[1]. The Roll Back Malaria program recommends that pregnant women receive intermittent preventative treatment with the inclusion of sulphadoxine-pyrimethamine (IPTp) as part of antenatal care and recommends that pregnant women sleep under insecticide-treated nets (ITN). Although Nigeria has adopted these recommendations, it has a long way to go in achieving these goals with only 13.2% of pregnant women receiving IPTp and 33.7% of pregnant women sleeping under an ITN [1,4]. Based on the high prevalence of malaria in our sample, education on best practices to prevent malaria during pregnancy, and resources in support of these practices are urgently needed.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

JG wrote and conceptualized the frame work for the paper. EE is the principal investigator for the grant. MO was the local investigator for HBI. CE assisted in the conceptualization and development of the research protocol. AO was the research coordinator in charge of participant recruitment, trial implementation and data collection. JE, EJ, KE, SP, LK, SH, and EE provided input and feedback during the planning, analyses and framework for the paper. All authors read and approved the final version of the manuscripts.

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conduct without the support of PeTR-GS (our PEPFAR-supported partner) staff and volunteers.

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Table 1: Malaria parasitemia frequency by the Malaria Plus System

Malaria Plus System	N(%)
0	17 (<1)
+	1275 (62)
++	737 (36)
+++	40 (2)
++++	0 (0)
Total	2069 (100)

Notes: Levels of parasitemia increase as the scoring moves from 0 through ++++

Table 2: Comparison of participant's characteristics by malaria parasitemia low vs. high

	Parasitemia			
	Low	**High**	**Total**	***P****
Number of Participants, N(%)				
	1292(62)	777(38)	2069(100)	
Gravidity, N(%)				
Primi/secundigravida	819(65.8)	486(65.9)	1305(65.5)	*0.67*
Multigravida	425(34.2)	263(35.1)	688(34.5)	
Residence, N(%)				
Urban	326(25.3)	189(24.1)	513(24.9)	*0.54*
Rural	960(74.7)	588(75.9)	1,548(75.1)	
Distance to Healthcare Facility, N(%)				
0-5(km)	464(36.1)	255(33.0)	719(34.9)	*0.17*
5-10(km)	487(37.9)	290(37.5)	777(37.8)	
10+(km)	334(26.0)	228(29.5)	576(27.3)	
Household size, Mean (SD)[a]				
	4.51(2.0)	4.36(1.9)	4.40(1.9)	*0.07*
Age (Years), Mean (SD)[b]				
	29.10(5.8)	28.90(5.8)	29.00(5.7)	*0.44*

Notes: Numbers may not add up to 2086 due to missing data.

[a] N for each category include: n=1282 low parasitemia; n=768 high parasitemia; total n=2050

[b] No missing data

*Significance based on Chi-square p-value < 0.05

Table 3: Logistic regression models for malaria parasitemia and participant level characteristics

	N	Crude model OR(95% CI)	Adjusted[a] OR(95% CI)
Gravidity			
Multigravida	1305	*Ref.*	*Ref.*
Primi/secundigravida	688	1.04(0.86-1.26)	0.93(0.75-1.16)
Residence			
Urban	513	*Ref.*	*Ref.*
Rural	1548	1.07(0.87-1.31)	1.07(0.86-1.32)
Distance to Healthcare Facility			
0-5(km)	719	*Ref.*	*Ref.*
5-10(km)	777	1.08(0.88-1.34)	1.08(0.87-1.34)
10+(km)	562	1.24(0.99-1.56)	1.23(0.98-1.56)
Household size			
	2050	0.96(0.91-1.00)	0.94(0.89-0.99)[b]

Notes: [a] Overall model n=1970

[b] Indicates significance at $p<0.05$

APPENDIX B

MANUSCRIPT 2

The need for increased access to life saving obstetric procedures: A cohort study of Caesarean-section in Enugu State, Nigeria

ABSTRACT:

Background: In order to meet the Millennium Development Goals to decrease maternal mortality an increase in access to lifesaving obstetric measures such as Cesareans is needed. Nigeria contributed the second largest percent of the global number of maternal deaths in 2013. In this analysis, we aim to establish the rates of Cesareans and determine socioeconomic or medical risk factors associated with having a Cesarean in Enugu State, Nigeria.

Methods: Data for this study were derived from the Healthy Beginnings Initiative cohort. Participant demographic characteristics were obtained from 2317 women at baseline via a semi-structured questionnaire. Only singleton deliveries of women between the ages of 17-45 at baseline were retained for this analysis. Post-delivery questionnaires were used to ascertain the mode-of-delivery. Crude and adjusted logistic regressions with Cesareans as the main outcome are presented.

Results: In this sample, 7.2% women had Cesarean. Adjusted logistic regression models demonstrated that compared to women aged 15-24, the odds of having a CS were higher when the mother was aged 25-34 years (adjusted OR (aOR): 2.01; 95% CI: 1.04-3.90) and when the mother was aged 35-45 years (aOR: 2.73;1.26-5.92). Compared to those with a primary school education or less, the odds of having a CS were higher if the mother had a tertiary education (aOR: 2.91; 1.54-5.53), but not if she had a secondary education (aOR: 1.27; 0.73-2.30). The odds of having a CS were significantly lower if participants were employed part-time compared to full-time (aOR: 0.56; 0.32-0.97). Compared to women who lived in an urban setting, those who lived in a rural setting had a significant reduction in the odds of having a CS (aOR: 0.58; 0.38-0.89). Significantly higher odds of having a CS were seen among those with high peripheral malaria parasitemia compared to those with low parasitemia (aOR: 1.53; 1.03-2.27).

Conclusion: Findings from this study reveal that women in this part of Nigeria are not following global trends and over-utilizing Cesareans, but are instead, struggling to obtain adequate perinatal healthcare. Increasing access to safe and affordable obstetric services is needed in this area of Nigeria to reduce the high maternal mortality.

Keywords: Nigeria, Cesarean, maternal mortality, malaria, education

BACKGROUND

Globally, the number of Caesarean sections (CS) has been on the rise over the last decade (1, 2). The adverse maternal and perinatal outcomes when a CS is not medically necessary have become a major public health concern and the associated expenses decrease the resources available for other issues (3, 4). According to the World Health Organization (WHO), CS rates between 5-15% are considered optimal as a lifesaving intervention for mothers and their neonates (1, 5, 6). Lower rates suggest an overall lack of access to health care, and higher rates indicate an overutilization of the procedure (1). Of particular concern in most of Africa is underutilization of CS, with regional estimates varying from 1.8% in Central Africa, 1.9% in Western Africa, 2.3% in Eastern Africa, 7.6% in Northern Africa, and 14.5% in Southern Africa (5). Low percentages in central, western, and eastern Africa indicate difficulty accessing adequate maternal health care.

Although most African countries have hospitals with surgical services available to perform CS, a plethora of individual and health system characteristics impede access and contribute to the delay in women seeking services during pregnancy. Thaddeus and Maine (1994) developed the “three-delay” model that has been widely accepted as a framework to explain the obstacles in obtaining adequate healthcare during pregnancy (7). This three-tiered framework includes: delay in decisions to seek care, delays in arrival at a healthcare facility, and delays in receiving adequate treatment for obstetric complications.

In sub-Saharan Africa (SSA), delayed access to healthcare services during pregnancy and delivery can be influenced by multiple factors. Lack of knowledge of the importance of perinatal care and an inability to pay for healthcare services are common reasons for delaying healthcare utilization (7). Women also delay seeking treatment during pregnancy because of poverty, gender inequalities in household decision-making, cultural barriers, and geographical and transport barriers (7). Increased age, education and wealth are all positively associated with deciding to have a doctor present at delivery within SSA (8, 9). When life threatening complications occur during labor, delays in seeking adequate care can increase maternal mortality even when lifesaving CS is utilized.

In 2013, Nigeria contributed the 2nd largest proportion of the global number of maternal deaths and had the 11th highest crude birth rate- making it an important country in which to study the barriers to obtaining adequate obstetric care (10, 11). More than 75% of all CS in Nigeria are linked to obstetric emergencies that could have been prevented by earlier medical care (12). Even with birth plans in place, many Nigerian women opt to deliver with an unskilled birth attendant in a setting other than a hospital because of barriers to seeking treatment, such as cost and geographical/transportation difficulties (7, 12, 13). Cultural factors such as gender inequalities and the cultural acceptance of home deliveries compared to hospital deliveries also influence a woman delivering at a healthcare facility (14). Delays in seeking treatment results in women attempting to access care at healthcare facilities only after life-threatening complications develop. Increasing access to obstetrics care, such as CS, decreases maternal and infant morbidity and mortality (15, 16). However, fears associated with having a CS further delay a woman's decision to seek treatment. Common fears associated with having a CS in Nigeria include: cultural beliefs that vaginal birth is a confirmation of womanhood, stigma from fear of being mocked by other women, death, violation of religious beliefs, post-operative pain, future infertility, expense, and medical incompetence (17-19). A woman's socioeconomic status also influences access to CS, with the richest women, as measured by wealth index, having better access to CS compared to the poorest women(6). Education and age are also strong predictors of a woman's willingness to have a CS in Nigeria [14, 15]. Women who are either younger or less educated are more likely to refuse a CS even when it is medically necessary from concerns about the expense (9, 20, 21).

Even women who do attempt to access healthcare facilities during delivery, often encounter untrained personnel and a lack of proper equipment and supplies (9). Most studies evaluating pregnancy outcomes in Nigeria equate having a doctor present at delivery to having access to quality healthcare. However, this metric may not be an accurate predictor of the facility's ability to perform a CS. Many healthcare facilities within Nigeria cannot offer a CS, and ambulance services are virtually non-existent (22). In fact, one study demonstrated that only 1 in 21 health facilities in Nigeria is equipped to perform CS (23). This complicates the ability of pregnant women to obtain adequate healthcare during pregnancy.

In 2013, Nigeria contributed 14% (n=40,000) of the global number of maternal deaths (10). In order to meet the Millennium Development Goals to decrease maternal mortality an increase in access to lifesaving obstetric measures such as CS is needed (15, 16, 24). Examining factors associated with having a CS will help provide insight into ways to increase access to healthcare during pregnancy. In addition, socioeconomic and comorbid conditions are often not examined together when exploring the factors associated with CS in Nigeria. Therefore, the aims of this paper were two-fold: 1) to establish the rates of CS in Enugu State, Nigeria; and 2) to determine socioeconomic or medical risk factors associated with having a CS in Enugu State, Nigeria.

METHODS

Survey: Data for this study were derived from the Healthy Beginnings Initiative (HBI) cohort, which has been described in detail elsewhere (25). In Nigeria, approximately 35% of pregnant women deliver at a healthcare facility; therefore, a community-based sampling technique was employed to obtain a more representative sample of pregnant women (26). Pregnant women were recruited from churches in Enugu State, Nigeria. This strategy was expected to provide a representative sample of pregnant women in the state, as the population is more than 95% Christian and church attendance approaches 90% (25). Women interested in the study were asked to read and sign a consent form in either English or the local language, Ibo. If the participant was illiterate, the consent form was read aloud to her in the local language; then, the participant gave her consent by affixing her thumb print, as an indication of consent to participate in the study (25). Participant demographic characteristics were obtained from 2317 women at baseline via a semi-structured questionnaire written at a 6th grade reading level (25). Trained research staff and church-based health advisors administered the survey. Participants had the option of reading the survey themselves or having study personnel read to them. Because of inherent risks associated with having multiples (i.e. twins, triplets etc.), only singleton deliveries of women between the ages of 17-45 at baseline were retained for this analysis. Post-delivery questionnaires were used to ascertain the mode of delivery, i.e., CS or vaginal birth, and the infant's gender. Gravidity was dichotomized as primigravida and multigravida.

Laboratory Measures: Variables assessed by laboratory tests were hemoglobin, malaria parasitemia, human immunodeficiency virus (HIV), and sickle cell disease/trait

(SCD). Participants were tested for each laboratory measure either at baseline following recruitment into the study or during their prenatal visits, whereupon records were obtained from the participant's corresponding hospital.

Hemoglobin was assessed using the standard cyanmethemoglobin method (27). Drabkins solution (Ranjo Medix Laboratories, Lagos, Nigeria) with a pH of 7-7.4 was measured at 5 ml and dispensed into a glass test tube. Whole blood was pipetted into the Drabkins solution and allowed to sit at room temperature away from sunlight for 5 minutes. The Drabkins fluid was then read using 540 nm in a spectrophotometer to estimate the hemoglobin concentration. WHO guidelines for anemia were employed (28), and pregnant woman were classified as anemic if they had a hemoglobin level below 11g/dl.

Peripheral parasitemia levels were assessed using the malaria plus system (29). Thick blood smears were examined via oil immersion under microscopy (29). Each slide was carefully read by two experienced laboratory technicians; a third technician was utilized to rectify any disparate results. Because results indicated that 99% of this sample showed malaria parasitemia, malaria parasitemia was reclassified as low and high based on the malaria plus system with those in the 0 and + group classified as low parasitemia and those in the ++ and +++ groups classified as high parasitemia.

HIV testing was performed using the Rapid Testing Serial Algorithm II (30). For this procedure, two concurrent HIV rapid tests, Uni-Gold Recombigen (Uni-Gold; Trinity Biotech, Inc., Wicklow, Ireland) and Stat Pak (Chembio Diagnostic Systems Inc., Medford, New York, USA) were used. Both Uni-Gold and Stat Pak are single-use immunochromatographic tests that detect HIV antibodies. Uni-Gold is used for the detection of HIV-1; while Stat Pack can detect HIV-1 and -2. Both tests are performed by collecting whole blood from a finger stick that is placed on a test strip. Next, the test strip was placed in the testing device and a wash solution that is unique to each test, was added. Uni-Gold was set aside for 10-12 minutes and then read by a technician; Stat-Pak was read after 15-20 minutes. If both tests were positive for HIV, the individual was considered HIV positive; if both tests were negative, the individual was considered HIV negative. When the tests showed conflicting results, they were both repeated and the results were read by another technician, who did not know the results of the first series of tests.

EDTA-treated venous blood samples were used to screen for SCD. Cellulose acetate electrophoresis at pH 8.5 – 9.0 was used. Hemolysates were prepared by lysing saline-washed, packed red cells in 13mM EDTA, 10.7mM KCN solution using a 1:4 ratio. Tris-EDTA-borate buffer with cellulose acetate strips were used to perform electrophoresis. Each strip contained one microliter of a patient hemolysate and a microliter of a control hemolysate containing hemoglobin A, S, and C. Electrophoresis was performed at a constant power of 350V for 30 minutes or longer if maximum band separation was not observed. Each strip's band separations were compared to the control genotypes: AA, AS, SS, and SC. To decrease the chances of a false positive or negative of SCD, each sample was tested twice. If incongruent results occurred, the test was rerun.

Statistical Methods: Univariate analyses were based on Pearson's Chi-square test for comparison of proportions for all variables. Fisher's exact tests for contingency tables were used to test for significance in proportions when the expected cell counts were less than 5. Chi-square analyses with $p<0.10$ were further analyzed using crude and adjusted logistic regression with CS as the main outcome. Having a CS in previous pregnancies is known to predict current CS; therefore, gravida was included in logistic regression models. Because no information was collected specifically regarding previous CS, a sensitivity analysis was performed among those experiencing their first pregnancy. Statistical significance was set at $p< 0.05$. An adjusted trend in the Odds Ratio (OR) was conducted to determine whether an increasing trend in the odds of having a CS occurred as a participant's age and education level increased by using the "tabodds" function in Stata [Stata Corporation, College Station, TX]. Participant's age was categorized as 15-24, 25-34, and 35-45. Only one women who had a CS had no formal education; therefore, education was categorized as none/primary, secondary and tertiary and above. Age and education were retained as categorical variables for inclusion in multivariable models. Birthweight was collected as part of the parent study; however, because it was self-reported and most newborns were not weighed at birth, birthweight was not deemed reliable. Therefore, birthweight was not included in this analysis. Data analyses were conducted using Stata version 12.0. The parent study was approved by the Institutional Review Board of the University of Nevada, Reno, and the Nigerian National Health Research Ethics Committee. This secondary data-analysis was considered exempt from human subjects review by the Mel and Enid Zuckerman College of Public Health Research Office.

RESULTS

As shown in Table 1, 167 (7.2%) women had CS and 2150 (92.8%) had vaginal deliveries. A woman's age was statistically associated with having a CS ($p<0.01$), with a greater percentage of women aged 35-45 having had a CS (11.1%) than women aged 25-34 (7.5%) or 15-24 (3.2%). Education was statistically associated with having a CS ($p<0.01$), with a greater percentage of women with tertiary education having had a CS (15.1%) than those with a secondary education (6.0%) or a primary education or less (4.9%). Employment status was also statistically associated with having a CS ($p=0.03$); women with full-time employment had higher percentages of CS than women who worked part time or who did not indicate they were currently employed (8.7%, 5.0, and 7.1%, respectively). Area of residence (i.e., rural vs urban), was significantly related to having a CS ($p<0.01$) with more women in urban settings having a CS (12.2%) compared to women in rural settings (5.5%). A mother's baseline malaria parasitemia was significantly associated with having a CS ($p=0.02$); higher percentages of women who had high malaria parasitemia at baseline had a CS than women who displayed low levels of malaria parasitemia (8.9% vs 6.0%, respectively). Infant's gender was statistically associated with CS ($p=0.01$), with a higher rate of CS occurring when mothers delivered male (8.6%) than female infants (5.9%). No significant relationship was observed between CS and number of people in the household, gravidity, distance to nearest healthcare facility, marital status, HIV, sickle cell disease (SCD), or anemia. When analyses were restricted to women who had not had a prior pregnancy (n=334), 7.8% (n=26) had CS and 92.2% (n=308) had vaginal deliveries (Table 1). Among primigravida women, living in an urban environment was significantly related to having a CS ($p<0.01$)

with 14.3% of urban women having a CS compared to only 5.1% of rural women. No other participant characteristics were significant predictors of having a CS among primigravida women.

Table 2 presents the crude and adjusted odds ratios (95% CIs) for having had a CS or a vaginal birth by participant characteristics. The adjusted models showed that, compared to women aged 15-24, the odds of having a CS were higher when the mother was aged 25-34 years (adjusted OR (aOR): 2.01; 95% CI: 1.04-3.90) and when the mother was aged 35-45 years (aOR: 2.73; 95% CI: 1.26-5.92; p-trend <0.01). Compared to those with a primary school education or less, the odds of having a CS were higher if the mother had at least a tertiary education (aOR: 2.91; CI: 1.54-5.53), but not if she had a secondary education (aOR: 1.27; CI: 0.73-2.30; p-trend <0.01). The odds of having a CS were significantly lower if participants were employed part-time compared to full-time (aOR: 0.56; CI: 0.32-0.97). Compared to women who lived in an urban setting, those who lived in a rural setting had a significant reduction in the odds of having a CS (aOR: 0.58; CI: 0.38-0.89). Significantly higher odds of having a CS were seen among those with high peripheral malaria parasitemia compared to those with low parasitemia (aOR: 1.53; CI: 1.03-2.27). After adjustment for confounders, no relationship was found between CS and number of people in the household, gravidity, marital status and not being employed. Among primigravida women, adjusted logistic regression models showed a significant relationship between woman living in an urban vs rural environment and having a CS (aOR: 0.27; CI: 0.09-0.82). No other significant relationships were found among primigravida women between having a CS and a woman's baseline characteristics or the gender of her infant.

DISCUSSION

Women in SSA continually face struggles in obtaining adequate obstetric care during pregnancy. Increasing access to emergency obstetrics care, such as CS, decreases maternal and infant morbidity and mortality (15, 16). Nigeria has one of the fastest growing populations in the world, making it a key location to study access to healthcare in pregnancy. Overall results indicate that 7.2% of women in Enugu State, Nigeria had a CS while 92.8% had a vaginal delivery. Percentages of CS increased as maternal age and/or education increased. Compared to women who had a full-time position, women who worked part-time had 44% lower odds of having a CS after adjustment for potential confounders. Likewise, significantly lower odds of having a CS was observed among women who live in a rural setting compared to those who reside in an urban setting in both the full sample and among primigravida women. After adjustment for confounding, this study demonstrated 53% higher odds of having a CS if participants had high peripheral malaria parasitemia compared to those with lower peripheral malaria parasitemia.

The present work demonstrated higher percentages of CS in older women and those with more education. In SSA, education has been shown to be a strong predictor of using professionally-assisted delivery services (8, 31). Older and more educated women in SSA are thought to be more confident and influential in their household decision-making, including the use of healthcare services (8, 32). Likewise, women with more education and/or women who are employed often have greater control over family resources and play a larger part in reproductive decision-making (14, 32, 33). These variables may be a proxy of a woman's ability to access healthcare, thereby increasing her chances of having had a CS.

The relationship between infant gender and having a CS has been well documented in developed countries and has only recently been studied in Africa (34). In Libya, male fetuses were associated with higher odds of maternal diabetes mellitus, preterm delivery, needing instruments when a vaginal delivery was performed, and having CS compared to female fetuses (35). In our study, being pregnant with a boy may have been associated with these pregnancy complications; however, these variables were not measured in our survey. Nonetheless, because women in this part of Nigeria were unlikely to have an ultrasound to determine the gender of their infants, the results of our study add to the literature that suggests that the relationship between CS and male infants is not culturally based (35). The biological basis of the relationship between being pregnant with a male fetus and higher odds of having a CS is unclear and warrants further attention (35).

Although the results herein did not demonstrate an association between distance to nearest health facility and having had a CS, a statically significant relationship between living in rural vs urban environments was demonstrated. In rural settings, distance has consistently been an important barrier to seeking healthcare (7, 9, 36). It is possible that in this self-report study, area of residence (i.e., rural vs urban) was an indirect assessment of the ease of reaching a healthcare facility for childbirth. Women living in rural parts of Enugu State, Nigeria - like women in other rural areas - may have had increased difficulty accessing facilities that can perform a CS because of limited transportation options, poor road conditions and poverty (7, 9, 14).

To the authors' knowledge, this study is the first epidemiological investigation to report that high malaria parasitemia is associated with higher odds of CS. The literature and current guidelines are based on case studies (37-39). It is not known if a biological pathway exists underlying this relationship or if women with higher malaria parasitemia lack adequate health care overall, which inherently makes their pregnancies higher risk. The relationship between malaria parasitemia and CS warrants further attention.

In SSA, some evidence has suggested that women with SCD are more likely to have a CS (40, 41). However, it is difficult to determine whether those with SCD receive any benefits from having a CS, because both SCD and CS are related to high risk of adverse maternal and neonatal outcomes in SSA (41-43). It has been established that malaria and SCD are associated with anemia during pregnancy (44-47). There has been much debate in the literature whether anemia is related to an increase in maternal morbidity and mortality in the context of developing countries (48). Having a CS would complicate this relationship and warrants further attention.

Our study is consistent with a meta-analysis that found HIV-infected women were no more likely to have a CS than those not infected (49). Evidence from resource unconstrained areas suggests that having a CS is beneficial if a woman's HIV-RNA level is above 1000 copies/ml near delivery (50). Because women in resource-constrained areas are often unaware of their viral load before delivery, having a CS could be beneficial for HIV-infected women (51). However, in resource-constrained areas, CS are often unavailable and unsafe; therefore, the WHO guidelines do not currently recommend HIV positive women in resource constrained regions have an elective CS (52). Instead the WHO recommends that HIV positive women take 3 or more antiretroviral medications in order to decrease mother to child transmission of HIV (53). The WHO also recommends that infants receive antiretrovirals during the post-natal period if their mother is HIV positive(53).

Practice and Policy

When medically necessary, CS are frequently lifesaving procedures; however, risks associated with CS are often highest within African countries as medical personnel may lack the training to perform a safe CS and lack proper equipment and supplies (4, 9). In general, better perinatal health outcomes have been associated with CS rates between 5%-15% (1, 3, 5, 6, 54). Although the rate of CS in our sample, 8.8%, fell within the lower end of these parameters, the WHO has estimated that in 15.5% of pregnancies in Nigeria, a CS is medically necessary (1). This is almost double the rate found in our sample. Therefore, unlike in other parts of the world where discussion centers on overutilization of CS (1), it is likely that in this area of Nigeria an overall underutilization of CS occurs, particularly in rural settings where only 5.5% of all births were delivered via CS.

Although many countries in SSA have healthcare facilities that can perform CS, the quality of care within these clinics is neither consistent nor reliable (55). It is estimated that less than 1% of individuals in western SSA have access to surgical care that is safe, affordable and can be performed in a timely manner (56). Countries in SSA suffer from an overall shortage of facilities equipped to perform such specialized treatment; additionally, countries in SSA also suffer from a lack of skilled workers capable of performing specialized medicine (7). Because previous research within

Nigeria showed that only 1 in 21 health facilities was equipped to perform CS (23), it is likely that even if access to health clinics was increased, most clinics would not be equipped to perform CS. Increasing access to doctors and nurses who can identify and perform life-saving obstetric procedures is needed, especially in rural areas of Nigeria.

Strengths, Weaknesses and Future Research

This study is unique to the literature on CS in SSA, in that it explored the relationship between CS and socio/demographic variables as well as different disease statuses. This allowed us to capture a holistic understanding of risk factors associated with CS in Nigeria. This study was not without its limitations. In the overall sample, the reasons a woman had a CS were not established, nor was prior use of CS. An attempt was made to control for prior CS by using the number of people in the household as well as gravidity status as proxies for prior CS; however, it is unknown if this control method was adequate. A sensitivity analysis was also performed using only primigravida women; however, the number of women (N=26) that had a CS for their first pregnancy was small. Therefore, significant relationships between CS and other variables may not have been present in this study because of lack of power. Also, no follow-up occurred when women did not attend post-natal interviews; therefore, it was not possible to determine the rate of maternal mortality. Finally, it is unknown whether women in rural settings truly had difficulty accessing a healthcare facility; perhaps more women in urban settings elected to have a CS. Answering these questions is essential to understanding the barriers that women face when seeking adequate perinatal care in Nigeria.

In conclusion, rates of CS remain substantially lower in Nigeria than WHO estimates is needed (1). Findings from this study reveal that women in this part of SSA are not following global trends and over-utilizing CS, but are instead, struggling to obtain adequate perinatal healthcare, ultimately perpetuating the cycle of high maternal mortality and gross health disparities seen in SSA.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JG wrote and conceptualized the frame work for the paper. EE is the principal investigator for the grant. MO was the local investigator for HBI. CE assisted in the conceptualization and development of the research protocol. AO, AO and NM were research coordinators in charge of participant recruitment, trial implementation and data collection. JE, EJ, KE, SP, and EE provided input and feedback during the planning, analyses and framework for the paper. All authors read and approved the final version of the manuscripts.

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Table1: Comparison of a participants baseline characterstics and infants gender with mode of delivery

	Full Sample					Primigravida				
	C-Section		Vaginal Birth			C-Section		Vaginal Birth		
	N	**%**	**N**	**%**	*P**	**N**	**%**	**N**	**%**	*P**
Total	167	7.2	2150	92.8		26	7.8	308	92.2	
Mother's Age										
15-24	16	3.2	479	96.8	*0.00* [a*]	9	5.8	146	94.2	*0.34* [a]
25-34	107	7.5	1317	92.5		15	9.3	147	90.7	
35-45	44	11.1	354	88.9		2	11.8	15	88.2	
Education										
None /Primary	30	4.9	578	95.1	*0.00* [a*]	3	6	47	94	*0.053* [a]
Secondary	79	6.0	1241	94.0		11	5.5	188	94.5	
Tertiary	58	15.1	327	84.9		12	14.1	73	85.9	
Employment Status										
Full-time	74	8.7	781	91.4	*0.03* [a*]	6	5.8	97	94.2	*0.64*
Part-time	28	5.0	536	95.0		6	9.4	58	90.6	
None	61	7.1	818	93.1		14	8.5	150	91.5	
Residence										
Urban	73	12.2	528	87.9	*0.00* [a*]	14	14.3	84	85.7	*0.00**
Rural	94	5.5	1617	94.5		12	5.1	224	94.9	
Malaria Parasitemia										
High	59	8.9	604	91.1	*0.02* [a*]	5	5.3	90	94.7	*0.36*
Low	66	6.0	1027	94.0		14	8.3	155	91.7	
Infants Gender										
Male	102	8.6	1085	91.4	*0.01* [a*]	13	7.8	153	92.2	*0.99*
Female	64	5.9	1028	94.1		13	7.8	152	92.2	
Number of People in Household										
1-2	31	8.7	324	91.3	*0.07*	14	7.5	174	92.6	*0.15* [a]
3-4	75	8.2	845	91.9		11	11.6	84	88.4	
5+	60	5.8	967	94.2		1	2.1	46	97.9	
Gravidy										
Primigravida	26	7.8	308	92.2	*0.62*	N/A	N/A	N/A	N/A	*N/A*
Multigravida	136	7.0	1797	93.0		N/A	N/A	N/A	N/A	
Marital Status										
Married	162	7.5	2010	92.5	*0.07*	3	5	57	95	*0.59* [a]
Other	5	3.4	140	96.6		23	8.4	251	91.6	
Distance to Healtcare Facility										
0-5km	54	9.7	753	93.3	*0.52*	9	8.1	102	91.9	*0.88*
5-10km	61	7.0	817	93.1		10	8.5	108	90.8	
10+km	51	8.2	872	91.8		7	6.7	97	93.3	
HIV status										
Postive	2	3.3	59	96.7	*0.32*	0	0	8	100	*1.0* [a]
Negative	137	7.2	1757	92.8		21	7.4	263	92.6	
Sickle Cell Status										
AA-normal	100	7.3	1273	92.7	*0.62*	5	9.4	48	90.6	*0.55* [a]
AS/AC-carrier	25	6.5	357	93.5		14	6.6	197	93.4	
Anemia										
Yes	53	8.3	589	91.7	*0.16*	7	7.2	90	92.8	*0.99*
No	72	6.5	1042	93.5		12	7.2	155	92.8	

Notes: *Significance based on Pearson's Chi-square for Fisher's Exact, p<0.05 significant
a: Indicates p-value based on Fishers Exact

Table 2: Crude and logistic regression models of the odds of C-Section vs vaginal birth

	Full Sample		Among Primigravida	
	Crude	Adjusted N=1669	Crude	Adjusted N=256
Mother's Age				
15-24	*Ref*	*Ref*	*Ref*	*Ref*
25-34	2.43(1.42-4.16)*	2.01(1.04-3.90)*	1.65(0.70-3.90)	0.85(0.23-3.10)
35-45	3.72(2.07-6.70)*	2.73(1.26-5.92)*	2.16(0.43-10.95)	1.54(0.22-10.75)
Education				
None/Primary	*Ref*	*Ref*	*Ref*	*Ref*
Secondary	1.22(0.80-1.89)	1.27(0.73-2.30)	0.92(0.25-3.42)	0.57(0.13-2.58)
Tertiary	3.42(2.15-5.42)*	2.91(1.54-5.53)*	2.58(0.69-9.61)	0.89(0.16-4.86)
Employment Status				
Full-time	*Ref*	*Ref*	*Ref*	*Ref*
Part-time	0.55(0.35-0.86)*	0.56(0.32-0.97)*	1.67(0.52-5.43)	1.72(0.43-6.85)
None	0.79(0.55-1.12)	0.80(0.52-1.25)	1.51(0.56-4.06)	1.42(0.42-4.87)
Residence				
Urban	*Ref*	*Ref*	*Ref*	*Ref*
Rural	0.42(0.30-0.58)*	0.58(0.38-0.89)*	0.32(0.14-0.72)*	0.27(0.09-0.82)*
Malaria Parasitemia				
High	1.52(1.05-2.19)*	1.53(1.03-2.27)*	0.62(0.21-1.76)	0.67(0.22-2.06)
Low	*Ref*	*Ref*	*Ref*	*Ref*
Infants Gender				
Male	1.51(1.09-2.09)*	1.18(0.80-1.75)	0.99(0.45-2.21)	0.86(0.32-2.32)
Female	*Ref*	*Ref*	*Ref*	*Ref*
Number of People in Household				
1-2	*Ref*	*Ref*	*Ref*	*Ref*
3-4	0.93(0.60-1.43)	0.94(0.49-1.79)	1.63(0.71-3.74)	1.38(0.49-3.87)
5+	0.65(0.41-1.02)	0.58(0.29-1.16)	0.27(0.03-2.11)	0.37(0.04-3.14)
Gravidity				
Primigravida	1.15(0.72-1.73)	1.03(0.55-1.96)	N/A	N/A
Multigravida	*Ref*	*Ref*	N/A	N/A
Marital Status				
Other	0.44(0.18-1.10)	0.93(0.32-2.71)	0.57(0.17-1.98)	0.67(0.12-3.61)
Married	*Ref*	*Ref*	*Ref*	*Ref*

Notes: Models adjusted for other variables in the table

* Indicates significance at p<0.05

APPENDIX C

MANUSCRIPT 3

ABSTRACT

Background: Millennium Development Goal (MDG) 5 aims to reduce maternal mortality. Nigeria has the second highest number of maternal deaths in the world. Increasing access to emergency obstetric care, such as Cesarean Section (CS) , is largely considered necessary to achieving MDG 5. Therefore, this paper aimed to estimate the incidence of CS in Nigeria and to determine the socioeconomic and medical risk factors that are associated with having a CS in Nigeria.

Methods: The Nigerian Demographic and Health Survey was utilized to carry out this analysis. Weighted crude and adjusted logistic regression models were used to explore factors that influence CS in Nigeria. Two separate analysis were performed, one among all women in the dataset and one including only primigravida women.

Results: In the full sample, 2.3% of Nigerian women had CS; this rate was almost double when the data was restricted to primigravida women (4.4%). In both groups, women living in rural areas had significantly lower odds of having had a CS compared to women living in urban areas. Also in both groups, Muslim women showed significantly lower odds of CS compared to Catholic women (full sample OR 0.43: 0.28-0.73; primigravida aOR 0.43: 0.19-0.96). Overall, 11,954 (67%) women did not deliver at a healthcare facility. Cultural beliefs and barriers often influenced women not access a healthcare facility during delivery in Nigeria.

Discussion: Rates of CS remain substantially lower in Nigeria than is recommended by the World Health Organization, indicating an overall lack of access to proper obstetric care. Our study demonstrated that women often delay seeking treatment because of an inability to reach healthcare facilities, poverty, gender inequality and cultural beliefs. Overcoming these delays in seeking treatment is needed to reach the Millennium Development Goals.

BACKGROUND

The United Nations' Millennium Development Goal (MDG) 5 sets out to improve maternal health [1]. Specifically, world leaders aimed to reduce maternal mortality by 75% from 1990 to 2015 and achieve worldwide access to reproductive health services by 2015 [1]. Although great strides have been made in reducing global maternal mortality from 546,000 deaths in 1990, an estimated 289,000 women still died in 2013 from pregnancy and childbirth complications that are largely considered preventable [2, 3]. This is in addition to nearly 15-20 million that suffer various debilitating consequences of pregnancy [4].

Sub-Saharan Africa (SSA) has historically carried a disproportionate burden of adverse maternal and child health related outcomes [1], and has the highest maternal mortality ratio (MMR) in the world. The region recorded an estimated 510 deaths for every 100,000 live births among women aged 15-49 years in 2013 [2]. Although this was a vast improvement from the 1990 estimate of 990 deaths per 100,000 live births, it is still more than double the ratio for any other region of the world [2]. In order to meet MDG5 in SSA, vast improvements in facilities, infrastructure, and training of skilled attendants were needed.

In 2013, Nigeria comprised40,000 (14%))of the global number of maternal deaths, trailing only India (17 %) [2]. In the same year, the MMR in Nigeria was 560 deaths per 100,000 live births, a 52% decrease in MMR from 1990 [2]. Although this decrease in MMR was a remarkable progress towards the MDG, in 2013 the lifetime risk of a pregnancy-related maternal death in Nigeria was 1 in every 29 women [5].

Women in Nigeria face a variety of obstacles while trying to obtain adequate healthcare during pregnancy. Thaddeus and Maine (1994) describe a "three delay" framework to explain the obstacles women face, including: delay in decision to seek care, delay in reaching a healthcare facility, and delay in receiving adequate treatment for obstetric complications [6]. These delays are mediated by a range of sociodemographic, cultural, and economic factors. For example, gender inequality and cultural acceptance of home deliveries are common reasons women delay seeking treatment at a healthcare facility [6-9]. Costs associated with use of healthcare as well as costs associated with transportation to healthcare facilities, are deterrents to accessing healthcare during pregnancy in Nigeria [10]. Some Nigerian women report that the financial burden of obstetric care is associated with a bad omen for the family; therefore, they may fail to seek treatment at a healthcare facility even when complications during labor arise [10]. Therefore, women may ultimately attempt to access healthcare facilities only after life-threatening complications develop. As a result of these delays approximately 75% of all Cesarean sections (CS) in Nigeria result from obstetric emergencies that could have been prevented by early intervention [7].

Decreasing delays and increasing access to emergency obstetric care, such as CS, was considered necessary for achieving MDG 5 [11, 12]. However, in Nigeria, fears associated with having a CS— death, violation of religious beliefs, post-operative pain, future infertility, cost, and medical incompetence —are commonly reported as deterrents to seeking obstetric care during pregnancy [13, 14]. Even when life-threatening pregnancy complications occurred, two-thirds of Nigerian women preferred a symphsiotomy over a CS because of the fears associated with complications of a CS [8].

Symphysiotomy is a procedure that involves making an incision through the cartilage and ligaments of a pelvic joint or in some cases, cutting the pelvis bone to widen it and allow a baby to be delivered unobstructed. Socioeconomic, demographic, and cultural variables coupled with high rates of comorbid medical conditions and cultural perceptions of hospitals are all reasons why Nigeria has the second largest proportion of the global maternal deaths [2] . In developing countries such as Nigeria, rates of CS are a vital measure of a woman's access to emergency obstetric services as they are often associated with a decrease in maternal mortality [15, 16]. The aims of this paper were twofold: 1) to estimate the incidence of CS among pregnant women in Nigeria, and 2) to assess the socioeconomic and/or medical risk factors that are associated with having a CS in Nigeria.

METHODS

Data Source and population: The data from this study were obtained from the Demographic and Health Survey (DHS) conducted in Nigeria in 2013. Information regarding the collection and sampling techniques employed by the DHS data have been previously published in detail [17]. In brief, DHS utilized a cross-sectional design to obtain nationally representative data [17]. The sampling frame used for Nigeria's survey was prepared using the 2006 Population Census data from the Federal Republic of Nigeria provided by the National Population Commission [18]. The DHS is designed to collect information on demographic, health and family planning variables. Data were collected on a standard core set of questions; however, some surveys were individually tailored for a specific country. Survey data were collected by trained personnel, with participants verbally consenting. In total, 98% of the eligible women in the sampling households were interviewed [18]. Three main datasets are available from DHS: individual survey data, HIV test results and geographical data [19]. The results reported here were form the individual survey data. The DHS collects information regarding any pregnancy in the five years prior to the survey. In total, 19,655 women aged 15-49 years had information regarding the mode of delivery (vaginal vs. CS) of their most recent pregnancy. Because of the inherent risks associated with multiple births, only singleton deliveries were retained for this analysis. A skilled birth attendant was defined using the World Health Organization's standard as an accredited health professional including a doctor, nurse, or midwife [20]. Otherwise, the attendant was classified as an unskilled birth attendant.

Statistical Analysis. Because select populations were oversampled, individual weights provided by DHS were used as recommended [21]. Using weights allowed for adjustment for nonresponse to questions, and made the data representative of the underlying population on a national level. All data manipulation and analyses were conducted in STATA version 12.0 [Stata Corporation, College Station, TX] using the 'svyset' command. The outcome variable used for this analysis was whether the most recent birth was reported as a CS or a vaginal delivery. Weighted univariate analyses were based on Pearson's Chi-square test for comparison of proportions for all variables. Weighted crude and adjusted logistic regression with mode of delivery, i.e., vaginal or CS-as the main outcome was performed. Weighted percentages were also performed for reasons why women did not deliver at a healthcare facility. A sensitivity analysis was performed among those experiencing their first pregnancy in order to determine if there were any major differences in accessing care during a woman's first pregnancy (n=3596). Statistical significance of all tests are presented at $p<0.05$.

RESULTS

The results of the weighted chi-square are reported in Table 1. During their last pregnancy, 2.3% of women had a CS and 97.7% had a vaginal delivery in Nigeria during 2008-2013. A statistically significant relationship between having a CS and the following variables was demonstrated: and mother's age ($p<0.01$), area of residence ($p<0.01$), education ($p<0.01$), religion($p<0.01$) , wealth index, difficult accessing a healthcare facility($p<0.01$), health insurance($p<0.01$), prenatal care provider ($p<0.01$), skill level of birth attendant ($p<0.01$), taking antimalarial medication ($p<0.01$), taking medication of an intestinal parasite ($p<0.04$), being offered an HIV tests as part of prenatal care ($p<0.01$), having a previous CS ($p<0.01$), taking iron supplements for at least half of pregnancy ($p<0.01$), and geographical region ($p<0.01$). No significant association between the gender of the infant and having a CS ($p=0.22$). Similar relationships were found among primigravida women with the exception that no significant association was demonstrated between taking medication for a parasite and having had a CS ($p=0.20$).

Table 2 presents results from weighted crude and adjusted odds ratios (95% CIs) for mode of delivery by participant characteristics. In the full sample including both multigravida and primigravida births, the adjusted model demonstrated that compared to women who live in urban areas, the odds of having a CS were lower if women who lived in rural settings (adjusted OR [aOR] 0.67: 95% 0.51-0.93). Compared to those with a no education, the odds of having a CS were higher if the mother had at least a tertiary education (aOR 2.71: 1.58-4.63) but not if she had a primary or secondary education (aOR1.20: 0.76-1.90 and 1.54: 0.98-2.42 respectively). Compared to women who practice Catholicism, lower odds of having a CS were demonstrated if the woman was

Muslim (aOR 0.46: 0.28-0.73). Women who had health insurance had higher odds of having a CS compared to those without insurance (aOR 1.78: 1.18-2.67). Women who received prenatal care from a skilled birth attendant had higher odds of having a CS compared to women who did not received prenatal care (aOR 3.00: 1.51-5.96). Also, women who had a CS during previous pregnancies had higher odds of having a CS during their current pregnancy compared to women who had a previous vaginal delivery (aOR 28.97:15.31-54.81). Compared to women in North Central Nigeria, women in the North West had lower odds of having a CS (aOR 0.56:0.32-0.97). In the adjusted model, no relationship was found between CS and taking prophylaxis malaria medication and iron supplementation. Among primigravida women, compared to women who practice Catholicism, lower odds of having a CS were demonstrated if the woman was Muslim or practiced an alternative form of Christianity (aOR 0.54: 0.30-0.98 and aOR 0.43: 0.19-0.96 respectively). Compared to those with no health insurance, those with health insurance had higher odds of having a CS (aOR 4.11: 1.97-8.54). In the adjusted models, no other significant relationships were demonstrated.

Table 3 demonstrates the cultural beliefs and barriers that influenced women not access a healthcare facility during delivery in Nigeria. Overall, 11,954 (67%) women did not deliver at a healthcare facility. Cultural beliefs influenced many women's decision not to deliver at a healthcare facility with 34.5% of women reporting that it was not necessary to deliver at a healthcare facility;8.1% reported their husbands family did not allow them to access healthcare; 9.3% reported it is not customary to deliver at a healthcare facility; 1.4% reported distrust of the healthcare facility; 0.5% reported that no female provider was available at the healthcare facility; and 0.2% reported the they did not like the attitude of the healthcare professional at the health facility. Physical and financial barriers that influenced a woman's ability to deliver at a healthcare facility included: delivering too quickly (39.2%).;inadequate transportation (15.5%); and costs associated with delivering at a healthcare facility (9.3%). Among primigravida women, 1760 (49%) did not deliver at a healthcare facility (Table 3). Cultural beliefs and physical barriers were associated with accessing a healthcare facility in primigravida women in a similar manner to the full sample.

DISCUSSION

Nigeria has the second highest MMR in the world in part because of barriers women encounter in accessing adequate healthcare during pregnancy. In order to reach MDG 5 by end of 2015, improved access to emergency obstetric care such as CS should be provided to women who need it [11, 12]. The aims of this study were to estimate the incidence of CS in Nigeria and to determine the socioeconomic, demographic and medical risk factors associated with having a CS in Nigeria. In the overall sample including both multi- and primigravida births, only 2.3% of women had a CS. Women living in rural areas had 33% lower odds of having a CS compared to women living in urban areas. After adjusting for potential confounders, women with a tertiary education or higher had greater odds of having a CS compared to those with no education. Religion was also significantly associated with having had a CS; Muslim women had a statistically significant 54% lower odds of having a CS compared to Catholics. Women with health insurance had a 78% increase in the odds of having a CS compared to women without health insurance; while those offered HIV testing as part of prenatal care had a 96% increase in the odds of having a CS compared to women who were not.

Lower rates of CS among women living in rural environments have consistently been reported because of barriers to seeking treatment, such as limited transportation options and poor road conditions [6, 9, 22, 23]. Poverty plays a substantial role in a woman's ability to access a healthcare facility during delivery. In rural areas, costs associated with having a CS as well as costs associated with transportation are common reasons for not accessing a healthcare facilities during delivery [6-8]. In our study, approximately 10% of all women reported costs as a deterrent to accessing a healthcare facility during delivery. Furthermore, in this study, 17.3% of rural Nigerian women indicated that they did not deliver at a healthcare facility because of transportation difficulties, compared to less than 8.4% of urban women It is likely that women in rural settings do not actively choose to not deliver at a healthcare facility-thereby decreasing their rates of CS-but are instead forced to deliver outside of a healthcare facilities because of the barriers associated with living in a rural setting.

The present work also demonstrated higher odds of having a CS if women had a tertiary education or higher compared to those with no education. Increasing education in woman is an important step in achieving MDG 5. As education increases, access to healthcare facilities grows, and maternal mortality often decreases [6, 24, 25]. Women with more education possess greater control over family resources and play a larger role in their reproductive decision-making [9, 26, 27]. Karlsen et al.,[25] demonstrated that among women who delivered at a healthcare facility, a relationship between low education levels and high maternal mortality existed even after controlling for potential confounders [25]. The importance of increasing access to primary school education (MDG2) and eliminating gender inequalities in education (MDG 3) are both substantial parts to achieving MDG 5.

To improve healthcare, the Nigerian government implemented the National Health Insurance Scheme in 1999 [28]. However, numerous studies have demonstrated that Nigerians continue to rely on direct payment to finance their healthcare needs [29]. Less than 1% of women in this sample had health insurance. It is thought that those who stated they had health insurance were more likely to have privately funded health insurance and therefore, possessed the ability to financially afford a CS even if the their insurance did not cover all the associated costs.

Ethnic and cultural diversity in Nigeria often vary by geographical region[30]. The inner connectedness of geographical region and religion in Nigeria is difficult to tease apart as the northern areas of Nigeria are predominately Muslim, while the middle and southern regions are predominately Christian[30]. Our study demonstrated lower odds of having a CS among Muslim women compared to Catholic women. Our study showed that women in the northwestern part of Nigeria had lower odds of having had a CS compared to the north-central area. This may be in part due to a lack of access to skilled health providers in northern regions of Nigeria [9, 30]. Having a skilled birth attendant available at delivery is necessary to perform a CS. Furthermore, previous research has demonstrated that women living in northern parts of Nigeria have lower rates of prenatal care and higher rates of home delivery[31]. With the percentage of home deliveries ranging from 12% in southwest Nigeria to 86% in northwest Nigeria, women in northern areas of Nigeria receive significantly less support from a skilled birth attendant than their counterparts in southern Nigeria [31]. The vast majority of these women have friends or relatives assisting with their delivery[31]. Cultural and religious norms may dictate this relationship as some women in northern areas have restrictions in seeking health-related assistance during childbirth, especially from male providers[30]. Attaining the Millennium Development Goals is especially difficult in the Northern parts of Nigeria where poverty, illiteracy, and early marriage rates remain high among women, and reproductive health and family planning are not historically women's decisions [30]. Therefore, it is likely that women in the northern areas of Nigeria need better access to reproductive health education and trained birth attendants and community health workers, so that the symptoms associated with potential obstetric complications can be detected before infant and/or maternal mortality ensues.

It is well documented in the literature that skilled birth attendants are associated with a decrease in maternal mortality [32]. This study demonstrated that compared to women who received no prenatal care, the odds of having a CS were increased if a women received prenatal care from a skilled birth attendant but not if she received prenatal care from an unskilled birth attendant. Training unskilled birth attendants to recognizing some symptoms associated with the need for future emergency obstetric services is essential. This is far from a novel idea, other research has indicated that traditional birth attendants can be effective at implementing interventions that reduce neonatal mortality in rural areas [33-36]. Further developing traditional births attendants and community health workers skills could be effective at reducing recognizing early warning signs to life threatening obstetric complications. It may be an essential way of reducing mortality, especially in Northern Nigeria.

Practice and Policy

In order to meet the Millennium Development Goals, a decrease in maternal mortality and worldwide access to reproductive health needed to be obtained [1]. However, this study demonstrated that 62% of all vaginal births occurred outside of a healthcare facility. Delivering outside of a health facility and with an unskilled birth attendant increases a woman's chance of maternal mortality. The World Health Organization has estimated that 15.5% of pregnancies in Nigeria need to have a CS

[37]—more than triple the rates found in this study. Therefore, unlike in other parts of the world where discussion centers on overutilization of CS [37], this study demonstrated an overall gross underutilization of the procedure in Nigeria. This may explain why Nigeria accounts for half of the global burden of incident obstetric fistulas, which is caused by prolonged labor and can be prevented with access to emergency obstetric services such as a CS [24].

Although having access to health facility decreases maternal mortality, it does not always equate to having adequate healthcare. Countries in SSA suffer from acute shortage of facilities equipped to perform specialized treatment. In a survey of 77 hospitals in SSA only 6% reported the ability to provide safe anesthesia for a CS [38]. The anesthesiologist in these facilities reported that only 19% operated in facilities were electricity was always available [38]. Only 56% of the facilities reported always having access to running water and only 23% reported having access to blood for transfusion [38]. Among individuals in western Africa, it is estimated that less than 1% have access to surgical care that is safe, affordable and can be performed in a timely manner [39]. In Nigeria, previous research demonstrated that only 1 in 21 health facilities were equipped to perform CS [40]. Therefore, even if access to health clinics were increased, most skilled birth attendants would not have the proper equipment to perform a safe CS. Because many facilities lack water, electricity, medication, equipment and trained personnel to perform CS, the operation is often associated with unacceptably high rates of sepsis, hemorrhage and maternal death [15]. Therefore, increasing access to healthcare facilities alone would not negate the problem, vast improvements in infrastructure and training of skilled obstetricians needs to be made within SSA.

Strengths and Limitations

This study is unique to the literature in that it explored the relationship between CS and socio/demographic variables as well as diseases while controlling for geopolitical region. A sensitivity analysis was performed using only primigravida women. In the unadjusted models, primigravida women showed similar trends in the OR as the full sample. However, after adjustment, statistical significance in the primigravida model diminished. It is possible that this study lacked statistical power because either over-adjustment occurred in the primigravida model, or because a relatively small number of women (N=170) had a CS for their first pregnancy; therefore, significant relationships between CS and other variables may not have been found in this study.

This study is however not without its limitations. Although more women who were offered HIV testing as part of prenatal care had a CS, the data does not permit us to determine if this was from high rates of HIV among these women or if it was from access to robust prenatal care. Likewise, it is unknown if women were taking iron supplementation because of anemia or because they attended prenatal care. Many socio/demographic and economic variables were significantly correlated but not highly correlated. Although this indicates no collinearity between these variables in this dataset, it is still difficult to determine if all models were over adjusted by including variables that historically are related such and education and wealth index.

Conclusion

In conclusion, rates of CS remain substantially low in Nigeria than needed, indicating an overall lack of access to adequate obstetric care [37]. Our study demonstrated that women often delay seeking treatment because of an inability to reach healthcare facilities, poverty, gender inequality and cultural beliefs. Overcoming these delays in seeking treatment was needed to reach the Millennium Development Goals. However, it is still apparent that women in Nigeria are not delaying seeking treatment out of ignorance but are encountering real barriers that delay seeking treatment.

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Table 1: Weighted chi-square of the socio-economic and medical factors associated with mode of delivery

	Full sample				Primigravida			
		Cesarean	Vaginal	*P*		Cesarean	Vaginal	*P*
	N	Weighted %	Weighted %		N	Weighted %	Weighted %	
Total sample	**19665**	**2.3**	**97.7**		**3596**	**4.4**	**95.6**	
Age								
15-24	5095	1.6	98.4	*<0.01**	2521	2.6	97.3	*<0.01**
25-34	9197	2.4	97.6		1000	7.7	92.3	
34-49	5373	2.7	97.3		75	2.1	78.6	
Area of Residence								
Urban	6532	4.4	95.6	*<0.01**	1324	7.7	92.3	*<0.01**
Rural	13133	1.1	98.9		2272	2.3	97.7	
Education								
None	8997	0.5	99.5	*<0.01**	1173	1.2	98.8	*<0.01**
Primary	3989	2.0	98.0		519	2.6	97.4	
Secondary	5379	3.6	96.4		1510	5.2	94.8	
Higher	1300	11.1	88.9		394	14.3	85.8	
Religion								
Catholic	1602	4.9	95.1	*<0.01**	360	9.5	90.2	*<0.01**
Other Christian	6466	4.3	95.7		1431	6.5	93.5	
Islam	11303	1.0	99.0		1770	2.0	98.0	
Wealth index								
Poorest	4308	0.5	99.5	*<0.01**	587	1.1	98.9	*<0.01**
Poorer	4513	0.9	99.1		762	2.8	97.2	
Middle	3955	1.4	98.6		748	3.0	97.0	
Richer	3668	2.4	97.6		740	3.4	96.7	
Richest	3221	7.2	92.9		759	10.5	89.5	
Difficulty accessing a healthcare facility								
No	13327	2.8	97.2	*<0.01**	2533	5.3	94.7	*<0.01**
Yes	6259	1.1	98.9		1048	1.9	98.1	
Infant's Gender								
Male	9966	2.4	97.6	*0.2159*	1825	5.3	94.7	*0.02*
Female	9699	2.1	97.9		1771	3.4	96.6	
Health Insurance								
No	19235	2.1	97.9	*<0.01**	3505	4.0	96.0	*<0.01**
Yes	354	11.7	88.3		77	27.0	73.0	
Prenatal Care Provider								
No Prenatal Care	6496	0.4	99.6	*<0.01**	954	0.8	99.2	*<0.01**
Unskilled birth attendant	1710	0.9	99.1		291	1.4	98.6	
Skilled birth attendant	11299	3.6	96.4		2335	6.4	93.6	
Delivered by:								
Unskilled birth attendant	17601	0.6	99.3	*<0.01**	3069	1.2	98.8	*<0.01**
Skilled birth attendant	1974	16.1	83.9		513	22.4	77.6	
Took Anti-Malaria Medication								
No	9797	1.3	98.7	*<0.01**	1674	2.4	97.6	*<0.01**
Yes	9463	3.3	96.7		1851	6.1	93.9	
Intestinal Parasite Medication								
No	16015	2.1	97.9	*0.04**	2888	4.1	95.9	0.2
Yes	2922	2.9	97.1		571	5.5	94.5	
Offered HIV Test as part of Prenatal Care								
No	5709	1.5	98.5	*<0.01**	1028	3.1	96.9	*<0.01**
Yes	6477	5.2	94.8		1429	8.5	91.5	
Past CS								
No	19548	0.07	99.3	*<0.01**	N/A	N/A	N/A	N/A
Yes	117	50.9	49.1		N/A	N/A	N/A	
Iron Supplementation for at Least Half of Pregnancy								
No	10081	2.7	97.3	*<0.01**	1971		97.6	*<0.01**
Yes	8650	1.7	98.3		1452		93.9	
Region								
North Central	3027	2.5	97.5	*<0.01**	601	5.2	94.8	*<0.01**
North East	3946	1.1	98.9		613	1.8	98.2	
North West	6088	0.6	99.4		898	1.4	98.6	
South East	1669	4.0	96.0		377	7.2	92.9	
South Central	2436	4.6	95.4		570	7.1	92.9	
South West	2499	5.0	95.0		537	7.8	92.2	

Notes: *Significance based on weighted Chi-Square p<0.05

Table 2: Weighted logistic regression the socio-economic factors associated with mode of delivery

	Full Sample		Primigravida	
	Unadjusted	**Adjusted**	**Unadjusted**	**Adjusted**
		N=17932		**n=3580**
Age				
15-24	*Ref.*	*Ref.*	*Ref.*	*Ref.*
25-34	1.53 (1.59-2.03)*	0.85 (0.61-1.18)	3.03 (2.04-4.52)*	1.26 (0.74- 2.16)
34-49	1.69 (1.23-2.32)*	1.08 (0.76-1.54)	9.95 (4.86-20.36)*	4.24(1.78-10.14)*
Area of Residence				
Urban	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Rural	0.25(0.19-0.33)*	0.67 (0.51-0.93)*	0.28 (0.20-0.42)*	0.55 (0.32-0.94)*
Education				
None	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Primary	3.79 (2.50-5.73)*	1.20 (0.76-1.90)	2.18 (0.96-4.94)	0.67 (0.27-1.69)
Secondary	7.06 (4.96-10.06)*	1.54 (0.98-2.42)	4.40 (2.47-7.83)*	1.15 (0.50-2.63)
Higher	23.63(16.0-34.81)*	2.71 (1.58-4.63)*	13.38 (7.19-24.90	1.54 (0.61-3.84)
Religion				
Catholic	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Other Christian	0.88 (0.64-1.20)	0.80 (0.55-1.16)	0.66 (0.39-1.12)	0.54 (0.30-0.98)*
Islam	0.19 (0.13-0.27)*	0.46 (0.28-0.73)*	0.19 (0.11-0.33)*	0.43 (0.19-0.96)*
Wealth index				
Poorest	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Poorer	1.77 (0.98-3.19)	0.83 (0.44-1.53)	2.50 (0.98-6.38)	1.37 (0.50-3.75)
Middle	2.73 (1.54-4.83)*	0.76 (0.42-1.39)	2.77 (1.09-7.01)*	0.76 (0.26-2.19)
Richer	4.58 (2.65-7.94)*	0.87 (0.46-1.65)	3.08 (1.23-7.72)*	0.55 (0.17-1.74)
Richest	14.5 (8.66-24.13)*	1.50 (0.77-3.90)	10.34 (4.45-24.16	0.87 (0.25-3.04)
Difficulty accessing a healthcare facility				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	0.37 (0.27-0.51)*	0.90 (0.62-1.30)	0.35 (0.20-0.60)*	0.66 (0.37-1.18)
Health Insurance				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	6.12 (1.29-8.73)*	1.78 (1.18-2.67)*	8.95 (4.77-16.80)*	4.11(1.97-8.54)*
Prenatal Care Provider				
No Prenatal Care	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Unskilled birth attendant	2.51(1.25-5.03)*	1.35 (0.59-3.08)	1.72 (0.56-5.28)*	0.72 (0.18-2.93)
Skilled birth attendant	10.45 (6.26-17.44)*	3.00 (1.51-5.96)*	8.53 (3.59-20.29)*	2.93 (0.96-8.90)
Took Anti-Malaria Medication				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	2.49 (1.91-3.24)*	1.28 (0.96-1.70)	2.65 (0.79-3.92)	1.41 (0.94-2.13)
Past CS				
No	*Ref.*	*Ref.*	*N/A*	*N/A*
Yes	51.20 (32.00-91.90)*	28.97 (15.31-54.81)*	*N/A*	*N/A*
Iron Supplementation for at Least Half of Pregnancy				
No	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Yes	0.64 (0.49-0.83)*	1.20 (0.89-1.63)	0.67 (0.44-1.04)	1.07(0.68-1.68)
Region				
North Central	*Ref.*	*Ref.*	*Ref.*	*Ref.*
North East	0.45 (0.28-0.73)*	0.90 (0.54-1.50)	0.34 (0.17-0.69)*	0.68 (0.29-1.64)
North West	0.25 (0.15-0.42)*	0.56 (0.32-0.97)*	0.25 (0.22-0.52)*	0.58 (0.26-1.30)
South East	1.65 (1.11-2.44)*	0.78 (0.50-1.23)	1.40 (0.79-2.50)	0.71 (0.35-1.44)
South Central	1.89 (1.19-3.00)*	1.03 (0.67-1.59)	1.39 (0.79-2.42)	1.21 (0.65-2.27)
South West	2.07 (1.42-3.03)*	0.95 (0.65-1.37)	0.54 (0.90-2.66)	0.92(0.51-1.67)

Notes: *Significance based on weighted logistic regression pvalue <0.05

Table 3: Weighted percents of reasons why women did not healthcare facility to deliver

	Full sample (n=11954)		**Primigravida (n=1760)**	
	Yes	**No**	**Yes**	**No**
Not Necessary	34.5	65.5	30.1	69.1
Husbands family didn't allow	8.1	91.9	8.8	91.2
Not Customary	9.3	90.7	7.3	92.8
Delivered too quickly	39.2	60.8	40.4	59.7
Transportation	15.5	84.5	16.8	83.2
Cost	9.3	90.7	10.2	89.8
Facility Not Open	2.3	97.7	3.0	97.0
Distrust of healthcare facility	1.4	98.6	1.2	98.8
No female provider	0.5	99.5	0.7	99.3
Attitude of healthcare professional	0.2	99.8	0.1	99.9

APPENDIX D

MOTHER QUESTIONNAIRE

Program ID ______________________

Study Location: ______________________ Date: ______________

PRE-DELIVERY QUESTIONS (collect during enrollment)

		Demographics			
	Date of birth	DD / MM / YYYY			
	Sex	☐ Female			
	Telephone number				
	Telephone number of close relative				
	Marital status	☐Married	☐Single	☐Divorced	☐Separated
	Education level	☐None	☐Primary	☐Secondary	☐Tertiary
	Employment status	☐Full time	☐Part time	☐Unemployed	
	Occupation				
	Household income				
	Number of people in household				
	Area of residence	☐Urban		☐Rural	
	Distance to closest health facility	☐0-5 km	☐5-10 km	☐10-15 km	☐15+ km

		Background Information			
	Have you ever been tested for HIV?	☐Yes		☐No	
	If yes: date DD / MM / YYYY				
	If no: Are you willing to get tested?	☐Yes		☐No	
	If you are unwilling to get tested for HIV what are your reasons? (Check all that apply)	☐Fear of knowing result	☐Fear of spousal rejection	☐Fear of spousal abuse	
		☐Social stigma	☐Recently tested	☐Perception of no risk	
		☐Fear of associated shame if positive		☐Other	
	Are you aware of your sickle cell trait status?	☐Yes		☐No	
	If yes: what is your trait?	☐AA (Normal)	☐AS (Carrier)	☐SS (Disease)	

Mother Questionnaire

Program ID _______________ Study Location: _________________Date: _____________

General Health Questionnaire

We want to know how your health has been in general over the last few weeks. Please read the questions below and each of the four possible answers. Circle the response that best applies to you. Thank you for answering all the questions.

Have you recently:	**1**	**2**	**3**	**4**
1. been able to concentrate on what you're doing?	better than usual	same as usual	less than usual	much less than usual
2. lost much sleep over worry?	Not at all	No more than usual	Rather more than usual	much more than usual
3. felt that you are playing a useful part in things?	more so than usual	same as usual	less so than usual	much less than usual
4. felt capable of making decisions about things?	more so than usual	same as usual	less than usual	much less than usual
5. felt constantly under strain?	Not at all	No more than usual	Rather more than usual	much more than usual
6. felt you couldn't overcome your difficulties?	Not at all	No more than usual	Rather more than usual	much more than usual
7. been able to enjoy your normal day to day activities?	more so than usual	same as usual	less so than usual	much less than usual
8. been able to face up to your problems?	more so than usual	same as usual	less than usual	much less than usual
9. been feeling unhappy or depressed?	Not at all	No more than usual	Rather more than usual	much more than usual
10. been losing confidence in yourself?	Not at all	No more than usual	Rather more than usual	much more than usual
11. been thinking of yourself as a worthless person?	Not at all	No more than usual	Rather more than usual	much more than usual
12. been feeling reasonably happy, all things considered?	more so than usual	same as usual	less so than usual	much less than usual
13. been optimistic about the future?	Not at all	No more than usual	Rather more than usual	much more than usual

Mother Questionnaire

Program ID ______________ Study Location: ________________Date: __________

Mother Specific Questions (Only ask female participants)						
	Age at first pregnancy					
	Number of previous pregnancies					
	Past breastfeeding	☐Yes		☐No		
	Last menstrual period	DD / MM / YYYY				
	Are you receiving antenatal care?	☐Yes		☐No		
	What is your due date?	DD / MM / YYYY				
	Are you aware of the types of female contraception	☐Yes		☐No		
	If yes: Are you interested in using contraception in the future?	☐Yes		☐No		
	If yes: What type	☐Oral	☐Injections	☐IUD	☐Other	
	If other: Please specify					
	Are you planning to circumcise your baby if it is a boy?	☐Yes		☐No		

Mother Questionnaire

Program ID ______________ Study Location: ________________Date: ______________

Laboratory Testing					(office use only)
	Hemoglobin level		☐ Normal	☐ low	☐ Unknown
	Malaria		☐ Positive	☐Negative	☐ Unknown
	RPR (Syphilis)		☐ Positive	☐Negative	☐ Unknown
	Hepatitis B		☐ Positive	☐Negative	☐ Unknown
	Hepatitis C		☐ Positive	☐Negative	☐ Unknown
	Sickle Cell Genotype	☐AA	☐AS	☐SS	☐Other
	If other: Please specify				
	HIV Test	☐ Positive		☐Negative	☐ N/A
	Repeat HIV Test	☐ Positive		☐Negative	☐ N/A
	ART Therapy Received during Pregnancy	☐Yes		☐No	☐ N/A
	If yes, When initiated	☐1st Trimester		☐2nd Trimester	☐3rd Trimester
	ART Therapy Received during Labor	☐Yes		☐No	☐ N/A
	ART Therapy Received during Breastfeeding	☐Yes		☐No	☐ N/A

Recorded in Access Database: ☐ Yes ☐ No

Date Recorded: ____

Recorded by: ____

Verified by: ____

APPENDIX E

POST-DELIVERY QUESTIONNAIRE

Mother's Name: ___________________________ Date of Birth: ____________

Church ID: ___________________

Mother Information						
Mothers Phone Number:						
	Did mother receive antenatal care		☐Yes	☐No	☐ Unknown	
	If Yes, Where		☐TBA	☐Health Center	☐Hospital	
	How many antenatal visits		☐Only one	☐less than 4	☐More than 4	
	Was blood drawn for HIV test?		☐Yes	☐No	☐ Don't know	
	If yes: Where was screening done					
	Pregnancy Outcome	☐Miscarriage	☐Normal Birth	☐Mother Died	☐Mother Alive	
	Total Number of Children Alive	1 2 3 4 5 6 7 8 9 10				
	HIV Test Result:	☐Positive	☐Negative	☐Unknown		

POST-DELIVERY QUESTIONNAIRE

Mother's Name: ________________________ Date of Birth: ___________ Church ID: _________________

Infant Information					
	Name of infant				
	Date of Birth				
	Location of birth	☐ Your Home	☐ TBA	☐ Health Center	☐ Hospital
	Mode of delivery	☐Vaginal		☐C/S	
	Infant's gender	☐Male		☐Female	
	Infant's weight	Kg			
	Gestational weeks at birth	☐ Full-term		☐ Pre-term	
	Was infant screened for HIV?		☐Yes	☐No	☐ Don't know
	Baby Outcome	☐Single	☐Twin	☐Alive	☐Dead
	Comments:				
Father Name:			Date of Birth:		
Fathers Phone Number:					
	Was HIV test done?		☐ Yes	☐ No	☐ Don't know
	If yes: Where was screening done				
	HIV Test Result:	☐Positive	☐Negative	☐Unknown	

APPENDIX F

LABORATORY MANUAL

PeTR UNIQE GOLBAL SERVICES/ANNUNCIATION SPECIALIST HOSPITAL,EMENE ENUGU LABORATORY DEPARTMENT

TEST NAME; One Step Hepatitis B Surface antigen test strip(serum/plasma)	
DATE DUE FOR REVIEW: 15/5/2014	DATE OF SOP ISSUE: 15/5/2013
AUTHORISED BY: REV SR CHUKWU C.C	AUTHOR(S):CHIDI,BENEDICTA, SYLVIA, SUSSAN, OGECHUKWU
STATUS:CONTRO LLED DOCUMENT	NUMBER OF PAGES: 2
OPERATORS:BML S, MLT, MLA	LOCATION:MICROBIOLOGY (SEROLOGY)

PRINCIPLE: The HBsAg one Step Hepatitis B surface Antigen test strip (serum/plasma) is a qualitative lateral flow immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the strip. During testing, the serum or plasma specimen reacts with the particle coated with anti-HBsAg antibody. The mixtures migrate upwards on the membrane and generate a coloured line.

TEST PROCEDURE:

1. Allow test strip, serum/plasma and controls to equilibrate to room temperature (15 -30^{O}C) prior to testing.
2. Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
3. Immerse the test strip vertically in the serum/plasma for at least 10 – 15 seconds.
   Note: do not pass the maximum line (max) on the test strip when immersing the strip.

4. Place the test strip on a non- absorbent flat surface.
5. Start the timer and wait for the red line(s) to appear.
   Note: the result should be read at 15minutes.
   Do not interpret results after 30 minutes.

INTERPRETATION OF RESULTS:

1. POSITIVE two distinct red lines appear, one line should be in the control region (C) and another line should be in rest region (T)

Note: the intensity of the red colour in the test line region (T) will vary depending on the concentration of HBsAg present in the specimen. Therefore any shade of red in the test region (T) should be considered positive.

2. NEGATIVE: One red line appears on the control region (C), no apparent red or pink line appears in the test region (T).
3. INVALID: Control line fails to appear.

Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.
Note: Review the procedure and repeat the test with a new strip.
If the problem persists, discontinue using the test kit immediately. Report to the HOD.

PeTR GLOBAL UNIQE SERVICES / ANNUNCIATION SPECIALIST HOSPITAL, EMENE ENUGU LABORATORY DEPARTMENT	
TEST NAME: Syphilis ultra rapid test strip(whole blood/serum/plasma)	
DATE DUE FOR REVIEW:15/5/2014	DATE OF SOP ISSUE: 15/5/2013
AUTHORISED BY: CHUKWU C.C	AUTHOR(S):REV.SR.CHUKWU.C.C.CHIDI,BENEDICTA, SYLVIA, SUSSAN, OGECHUKWU
STATUS:CONTR	NUMBER OF PAGES: 2

OLLED DOCUMENT	
OPERATORS: BMLS, MLT, MLA	LOCATION:MICROBIOLOGY (SEROLOGY)

PRINCIPLE: The syphilis ultra rapid test strip (whole blood/serum/plasma) is a qualitative membrane strip based immunoassay for the detection of Treponema Pallidum TP antibodies (igG and igM) in whole blood, serum or plasma. In this test procedure, recombinant syphilis antigen is immobilized in the test line region of the strip. After a specimen is added to the specimen pad it reacts with syphilis antigen coated to the particles that have been applied to the specimen pad. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized syphilis antigen. The double antigen test format can detect both igG and igM in specimen

PROCEDURES:

1. Remove the test strip from the sealed foil pouch.
2. Use it as soon as possible.

   (Best result will be obtained if the assay is performed within one hour)
3. Peel off the tape from the test card.
4. Stick the test strip in the middle of the test card with the arrows pointing downwards.
5. Transfer 2 drops (approximately 50ml) of serum/plasma onto the specimen pad of the test strip.
6. Add 40ul (1 drop) of buffer.
7. Start the timer.
8. Wait for the red line(s) to appear.
9. Read the result at 10minutes.

Note: do not interpret result after 30minutes.

INTERPRETATION OF RESULT

1. Positive: Two distinct red lines appear. One line should be in the control line region (C) and another line should be in the test line region (T).

Note: The intensity of the red colour in the test line region (T) will vary depending on the concentration of Treponema Pallidum antibodies present in the specimen. Therefore, any shade of red in the test line region (T) should be considered positive.

2. Negative: One red line appears in the control line region(C). No apparent red or pink line appears in the test region (T).

3. Invalid: Control line fails to appear.

Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

Note: Review the procedure and repeat the test with a new test kit immediately and report to the HOD.

PeTR UNIQUE GLOBAL SERVICES/ANNUNCIATION SPECIALIST HOSPITAL, ENUGU LABORATORY DEPARTMENT	
TEST: Examination of Blood for Malaria Parasite in Thick Film USING GIEMSA STAIN (CAPILLARY BLOOD, WHOLE BLOOD)	
DATE FOR REVIEW: 12/4/2014	DATE OF SOP ISSUE: 12/4/2013
AUTHORISED BY: REV SR CHUKWU C.C	AUTHOR(S): REV SR CHUKWU C.C, CHIDI BENEDICTA, FRANCISCA.
STATUS:CONTROLLED DOCUMENT	LOCATION: HAEMATOLOGY
OPERATORS: BMLS, MLT,MLA	NUMBER OF PAGES: 2

PRICIPLE OF THE TEST

Malaria parasites in thick blood film require staining at PH 7.1 – 7.2 using Rowmanwsky stain. (Contains azure dyes and eosin) e.g Giemsa stain. Thick film helps to concentrate the malaria parasite more than thin film.

Giemsa Stain is alcohol – based Rowmanwsky stain. It should be diluted to PH 7.1 -7.2 buffered water before use. It stains thick film well provided they are completely dry (overnight drying is recommended). The concentration of the Giemsa should be increased to reduce the staining time.

PROCEDURE

BLOOD COLLECTION:

1. (a) collect venous blood using vacutainer needle into EDTA tube, mix gently and thoroughly or if using a capillary blood then.

   (b) Cleanse the lobe of the finger (or heel if an infant). Using a swab moistened with 70% alcohol, allow the area to dry.

   (c) Use a sterile lancet, Prick the finger or heel.

   (d) Squeeze gently to obtain a large drop of blood.

THICK FILM MAKING:

2. Using a completely clean frosted end grease free microscope slide, add a large drop of blood at the centre of the slide.
3. Without delay, (using the end of a stick/tube) spread the large drop of blood to make thick smear.

   Note: cover evenly an area about 15x15mm.

   It should just be possible to see (but not read) newsprint through the film.
4. Using a grease pencil or black lead pencil, label the slide with the patient lab number/name.
5. Allow the blood film to air-dry with slide in a horizontal position and placed in a safe place.

THICK FILM STAINING:

1. Immediately before use, dilute the Giemsa stain.

Note (i) 3% SOLUTION FOR 30 MINUTES STAINING

Measure 50ml of buffered water (PH 7.1-7.2)

Add 1.5ml of Giemsa stain and mix gently.

(ii) 10% solution for 10minutes staining

Measure 45ml of buffered water (PH 7.1 – 7.2) in a 50ml cylinder.

Add 5ml of Giemsa stain (to 50ml mark) and mix gently.

2. Place the slides in a staining rack.
3. Flood the thick film with diluted Giemsa stain

   (10minutes if using 10% stain solution)
4. Wash off the stain using clean water
   (Need not be distilled water)
   Note: flushing the stain from the slides is necessary to avoid the films being covered with a fine deposit of stain.
5. Wipe the back of each slide clean and place in a draining rack for the preparation to air-dry.

REPORTING OF BLOOD FILM FOR MALARIA PARASITE:

1. When the thick film is completely dry, apply a drop of immersion oil to an area of the film which appears mauve coloured (usually around the edges)
2. Spread the oil to cover an area about 10mm in diameter, (there is no need to add a cover glass).
3. First use 10x and 40x as preliminary objectives, these will guide you on where to focus 100x objectives.

Note: Blood films should be examined microscopically using 100x oil immersion objectives because; these give a brighter and cleaner image.

4. Examine for malaria parasites. Use the chart placed on the wall to help identify the parasites.
5. Using the plus sign scheme given, report approximate numbers of parasites (trophpzites, schizontes and gametocytes).

PLUS SIGN SCHEME:

1 -10 Parasite per 100 High power field +

11 – 100 Parasite per 100 High power field	++
1 – 10 Parasite per High power field	+++
Over 10 parasite per High power field	++++

PeTR UNIQUE GLOBAL SERVICES /ANNUNCIATION SPECIALIST HOSPITAL, ENUGU. LABORATORY DEPARTMENT	
TEST:Hemoglobin Estimation (Cyanmethemoglobin Method) Whole (Blood in EDTA)	
DATE FOR REVIEW: 12/4/2014	DATE OF SOP ISSUE: 12/4/2013
AUTHORISED BY: REV SR CHUKWU C.C	AUTHOR(S):REV SR CHUKWU C.C, CHIDI ,BENEDICTA, GERALDINE
STATUS:CONTROLLED DOCUMENT	LOCATION: HAEMATOLOGY
OPERATORS: BMLS, MLT, MLA	NUMBER OF PAGES: 1

PRINCIPLE OF THE TEST

Whole blood is diluted 1 in 201 in a modified Drabkins solution, which contains potassium ferricyanide and potassium cyanide. The red cells are haemolysed and the haemoglobin is oxidized by the ferry cyanide to methaemoglobin. This is converted by the cyanide to stable haemiglobincyanide

(HiCN). Absorbance of the HiCN solution is read in a spectrophotometer at wavelength 540mm or in a colorimeter using a yellow-green filter. The absorbance obtained is compared with that of a reference HiCN standard solution.

TEST PROCEDURES:

1. Measure 5ml of Drabkins solution and dispense into a 7ml or 10ml very clean glass test tube.
2. Measure carefully by pipetting 20ul (0.02ml) of well mixed venous whole blood and dispense into the Drabkins solution.
3. Stopper the tube, mix and leave the diluted blood at room temperature protected from sunlight for 5minutes.

   Note: this time is adequate for conversion of hemoglobin to HiCN when using a neutral PH (7.0 – 7.4) Drabkins reagents up to 20minutes is required when using an alkaline Drabkins reagent.
4. Set the wavelength of the spectrophotometer at 540mm or place a yellow-green filter in the colorimeter.
5. Zero the spectrophotometer/colorimeter with Drabkins fluid and read the absorbance of the patient's sample.
6. Using the table prepared from calibration graph read off the patient's hemoglobin value.

PeTR GLOBAL UNIQE SERVICES/ANNUNCIATION SPECIALIST HOSPITAL, ENUGU LABORATORY DEPARTMENT	
TEST:HEMOGLOBIN Electrophoresis (Hb genotype) using washed and lyzed Red cells	
DATE FOR REVIEW: 12/4/2014	DATE OF SOP ISSUE: 12/4/2013
AUTHORISEDBY: REV SR	AUTHOR(S): REV SR CHUKWU C.C BENEDICTA,

LABORATORY MANUAL

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STATUS:CONTROLLED DOCUMENT	LOCATION: HAEMATOLOGY
OPERATORS: BMLS, MLT,MLA	NUMBER OF PAGES: 2

PRINCIPLE OF THE TECHNIQUE:

The most important primary screening method for the presence of clinically significant hemoglobin variants is cellulose acetate electrophoresis at PH 8.5 – 9.0. Hemoglobin electrophoresis exploits the observation that due to differences in the amino acid composition of their Globin chains, hemoglobin variants differ in their rate of travel across a cellulose acetate support when an electric current is applied.
Hemoglobin electrophoresis does not provide unequivocal identification of hemoglobin variants, it merely indicates their presence.
The position of hemoglobin variant band relative to HbA when compared provides sufficient evidence for a likely diagnosis.

TEST PROCEDURE:

1. Add equal volumes about 100ml of TEB (Tris-EDTA-borate) buffer to the anode and cathode compartments of an electrophoresis tank. Set the bridge gap to about 7cm and place a thoroughly wetted filter paper wick so that it rests on each support, but still is dripping into the buffer.

2. Place the cellulose acetate strip on the surface of the buffer and leave to soak for several minutes.

   Blot to remove excess buffer and set in place between the two supports and resting on the wicks.

   Ensure that the strip is taut.

3. Apply 2ul of the test and control haemolysates to the origin (about 2cm from the cathode end of the cellulose acetate strip). This is best achieved using an applicator designed for this purpose.

   The control haemolysate(s) should include HbA, HbS, Cover the lid firmly.

4. Electrophoresis at approximately 350V for 30 minutes. The strips should be monitored during this period and the time altered to maximum separation.

5. Note the presence, location of the abnormal bands his may provide sufficient information for a presumptive diagnosis, but confirmatory tests are usually required.

Preparation of haemolysate from EDTA (Anticoagulated blood)

When testing a haemolysate within 1-2days

1. Lyse 1 volume of saline washed packed red cells in 4 volume of lysing reagent (water).

Note: Lysing reagent

Dissolve 3.8g EDTA (ethylenediamine tetra acetic acid) and 0.7g potassium cyanide in 1 Liter of distilled water.

For long term storage of haemolysate:

1. Centrifuge sample, remove the plasma and wash the red cells three times in physiological saline.

   After each wash, centrifuge the red cells at about 1000g for 5 minutes.

   Remove the saline.

2. Lyze the red cells with 1.5 volume distilled water and 1 volume of toluene.

   Shake well for several minutes in a stopper tube or preferably vortex at high speed for 1 minute. Centrifuge at about 1000g for 20minutes.

3. Transfer the clear supernatant haemolysate into a tube. Adjust the haemoglobin content to about 100g/L (10g/dl) by adding distilled water. Label clearly.

   Note: HAEMOLYSATE WILL BE STABLE FOR SEVERAL WEEKS AT 4-8^{0}C AND FOR UP TO 3 MONTHS WHEN STORED FROZEN.

BLOOD COLLECTION TEAM

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