COMPLIANCE TO PRENATAL SUPPLEMENT USE IN RELATION TO LOW BIRTH WEIGHT IN MALAWI

By

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ABSTRACT

COMPLIANCE TO PRENATAL SUPPLEMENT USE IN RELATION TO LOW BIRTH WEIGHT IN MALAWI

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Background: Prenatal iron and folic acid (IFA) supplements are offered free to pregnant women in Malawi to reduce maternal anemia and improve birth outcomes. We investigated the association between self-reported compliance to IFA intake and risk of low birth weight (LBW).

Methods: Pregnant women who attended Bwaila Maternity Wing of Lilongwe District Hospital for delivery were recruited (n=220). We used questionnaire to collect self-reported information on IFA use and maternal sociodemographic data. Before delivery blood samples for maternal hemoglobin (Hb) and folate status, and upon delivery, birth weight, and other newborn anthropometrics were measured. We used multivariate logistic regression to determine risk of LBW by prenatal IFA intake.

Results: The self-reported number of IFA pills taken during pregnancy was positively associated with Hb, but not serum and RBC folate concentration: <45, 45-89 and > 90 pills taken corresponded with mean (SD) Hb 10.7 (1.6), 11.3 (1.8), and 11.7 (1.6) g/dl respectively (P= 0.006). The prevalence of LBW was 20.1%, 13.5% and 5.6% for those who reported taken IFA pills < 45 , $45 – 89$, and ≥ 90 pills, respectively (P = 0.027). Taking > 60 IFA pills reduced risk of LBW delivery (OR (95% CI)= 0.11 (0.02-0.056), P = 0.008) than taking ≤ 30 pills.

Conclusion: Self-reported compliance to IFA is valid for assessing prenatal supplement program in Malawi, especially Hb status, can improve adherence and reduce LBW.

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CHAPTER ONE: INTRODUCTION

Low birth weight (LBW) is a birth outcome of importance to public health that is associated with increased morbidity and mortality in neonates and infants and cardiovascular disease risks later in life (Arnold et al., 2015; Smith et al., 2016). LBW is internationally recognized as a birth weight below 2500 g (5.5 pounds). This practical cutoff for international comparison is based on epidemiological observations that LBW babies are 20 times more likely to die than heavier infants (Wardlaw, 2004).

Over 20 million infants worldwide are born with LBW. More than half of LBW babies are born in developing countries, particularly South-Central Asia, where more than quarter (27%) of babies born weigh less than 2500 g. LBW prevalence in Sub-Saharan Africa (15%), is one of the highest burden regions similar to the level in Caribbean region (14%). The Central America and Oceania region has a LBW rate of 10% (Wardlaw, 2004). LBW is still a leading cause of neonatal and infant mortality in the U.S. and other industrialized countries, although this prevalence is still much lower than in developing countries (Lau et al., 2013).

More than half of LBW babies are born in the developing countries and Malawi is as affected as other developing countries, with a LBW prevalence of above the world average. The national prevalence of LBW in Malawi is 12%, with the highest prevalence seen among mothers younger than 20 y of age (16%) and those older than 35 y of age. The Central region has a higher prevalence of LBW than Northern and Southern regions within Malawi (NSO-ICF, 2017).

Micronutrient deficiencies during pregnancy, particularly iron and folate, contribute significantly to the prevalence of LBW (Abubaker, 2016). In a recently

reported study, correction of micronutrient deficiency in pregnancy with prenatal supplementation improved birth outcomes (Wang et al., 2016).

Deficiencies of micronutrients are a major global health problem. More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc. Pregnant and lactating women are particularly prone to micronutrient deficiencies as their requirements for the nutrients are higher than those of non-pregnant women. Multiple micronutrient supplementation (vitamin A, iron and folic acid) and food fortification are some of the recommended approaches to fighting micronutrient deficiencies (WHO, 2016).

Many programs have been in place to fight LBW and other nutrition related problems in Malawi and other parts of the world. The World Health Organization (WHO) has put a world target of a 30% reduction in LBW by 2025. This means a 3% reduction every year from 2012 and 14 million LBW infants, down from 20 million (WHO, 2016). Malawi joined the global movement to reduce maternal and child malnutrition (major contributor of LBW) and embarked on the Scaling Up Nutrition (SUN) 1000 special days program. This new intervention called the "SUN 1000 special days" targets the first 1000 days of a child's life from conception to 24 months of age (i.e, 270 days of conception and 730 days after birth). SUN is a global programmatic approach in the fight against maternal and child undernutrition. This movement was started when the Lancet published an article that showed major economic returns when countries invest in nutrition (Black et al., 2008a; Black et al., 2008b; Bryce et al., 2008; Morris et al., 2008; Victora et al., 2008). Malawi was among the first countries to adopt and launch its SUN 1000 special days program in 2011. The program includes prenatal

micronutrient supplementation, good complementary feeding practices of children and food security initiatives targeting mainly the early days of a child's life.

There has been a micronutrient supplementation program in Malawi since the early 1990s. However, in 2011 the program was subsumed under the SUN-1000 special days program because SUN encompasses all interventions fighting maternal and child malnutrition. The SUN aims at improving birth outcomes by improving maternal nutritional status and health, especially in early pregnancy. The activities range from direct interventions that promote maternal nutrition, to much broader interventions such as production to achieve food security, infection prevention to maintain general health of the pregnant and reproductive age woman. The four major areas of focus of SUN-1000 are increasing the intake of vitamins and minerals (i.e. vitamin A, zinc, iron, folic acid and iodine oil capsules), promoting good nutrition practices (e.g., hygiene during food preparation, breast feeding and proper complementary feeding after six months), provision of micronutrients through food fortification (e.g., iron fortification of staples, vitamin A supplementation in sugar and cooking oil and the iodization of salt) and therapeutic feeding of malnourished children with special food.

The Malawian government uses prenatal supplements as a short-term solution in fighting micronutrient malnutrition (particularly iron deficiency anemia) in women of reproductive age and pregnant women to improve health status of mothers and newborns. All pregnant women, regardless of hematological status or the trimester of pregnancy receive prenatal supplements of pills of combined iron and folic acid daily from the first day of antenatal care clinic visit, throughout pregnancy to delivery. The tablet has 60 mg iron (ferrous) and 0.25 mg folic acid and taken once a day. Every month the woman gets

a new supply without checking whether she actually took the prescription given the previous month. The program is run on the assumption that women understand the need to take the supplements despite the documented evidence of poor compliance due to forgetting to take the pills and side effects (Kalimbira et al., 2009; Young et al., 2000). Monitoring prenatal supplementation intake has been continued because it is the most feasible way to fight micronutrient malnutrition and improve health outcomes at least in the short term, compared to diet, considering the social economic status of Malawians currently.

Scope of the problem

LBW increases the child mortality rate for children under the age of five, stunting levels, and chance of non-communicable disease (coronary heart disease) later in life (Smith et al., 2016). LBW contributes significantly to neonatal and infant morbidity and mortality in Malawi. Despite the Malawian government's efforts to improve birth outcomes and health status for Malawians, the prevalence of most health risk indicators remains high. Anemia prevalence has gone up in the past 6 y in reproductive age and pregnant women from 28% to 33% and 38% to 45%, from 2010 to 2016 respectively. Prevalence of LBW remains high at 12% in Malawi, with a higher than the national prevalence rate in mothers <20 y of age and >35 y and in the central part of the country. The infant mortality rate is at 42/1000 births, down from 135 per 1000 live births in 1992, neonatal mortality has dropped to 27/1000 births, down from 41 per 1000 live births in 1992 and maternal mortality ratio has improved to 439/1000 births, down from 1,123 per 100,000 live births in 2000 (NSO-ICF, 2017). The current maternal mortality rate of 439/100,000 live births indicates the need to improve maternal and child health. Maternal

and infant mortality indicates the level of quality of health care available to citizens in a country (Bhutta et al., 2008). For example a high maternal mortality rate might be from high anemia that negatively affects birth outcomes and health of newborns (Haider et al., 2013; Steer, 2000). This might indicate that there are inadequate screening and treatment services available for pregnant women. Anemia is still prevalent in reproductive age women and pregnant women in Malawi at the rate of 28% and 38%, respectively. Nutrition status of children is poor as indicated by 37% of children under the age of five being stunted (NSO-ICF, 2017).

Maternal nutrition status is a major determinant of LBW, but social and demographic factors have also been reported as major factors (Muula et al., 2011; Ngwira and Stanley, 2015). Muula et al (2008) in their study found that maternal education was associated with birth weight. Those with low attainment of education are likely to have a LBW children than those with higher education (Muula et al., 2011). It was also found that parity was a factor of low birth weight, i.e., the first delivery was likely to be LBW with later born children being heavier. The Malawi Demographic and Health Survey demonstrates that the birth orders, as well as the maternal age are factors in low birth weight. The first born child is likely to have low weight than the second, and younger mothers and those giving birth after 35 y are more likely to deliver a LBW child (NSO-ICF, 2017). In another study, it was found that the mother's height was associated with size of child at birth. The wealth index of the family had a positive association with birth weight, as well as attainment of secondary education and location of residence, for example cities vs rural areas (Ngwira and Stanley, 2015). Micronutrient supplements improve maternal nutrition status and birth outcomes (Makrides et al., 2003; Owens et

al., 2015; Preziosi et al., 1997; Rwebembera et al., 2006). The Generation R study in Rotterdam, the Netherlands, found that folic acid supplements increased weight at birth by 68g and placental weight by 13g in the supplemented group higher than those not supplemented (Timmermans et al., 2009). In another study in northern China, multiple micronutrient (MMN) supplements which included IFA increased birth weight, especially in those who had a low hemoglobin status at baseline (Wang et al., 2016). Anemia is one of the biggest contributors of low birth. In a case control study in Sudan (a developing country in Africa), hemoglobin status during antenatal was found to have modulating effects on birth outcomes, particularly LBW (Abubaker, 2016). If a woman had low hemoglobin status at baseline during pregnancy, she was likely to have a LBW child. If the low hemoglobin detected at baseline was corrected, the outcome was also positively affected).

According to the World Health Organization (WHO), iron deficiency contributes to 50% of the anemia prevalence in the world. This can be easily reversed if prenatal supplements are taken consistently, hence the recommendation by WHO that all pregnant women and women of reproductive age in some countries, take IFA supplements to reduce anemia and improve birth outcomes. Therefore compliance to prenatal micronutrient supplements is critical to achieve the desired maternal and child health outcomes. Studies conducted in Malawi using self-reports from reproductive age women have shown poor compliance to Ministry of Health universal prenatal supplement recommendations due to lack of monitoring programs (Kalimbira et al., 2009; Young et al., 2000). Compliance is as poor as less than 40% of women taking supplements for 2 months only instead on 6 months minimum, and only 2% taking the minimum

recommended amount of 180 tablets (6 months intake). Most pregnant women have complained about side effects such as nausea and vomiting, forgetfulness and the in availability of iron and folic acid tablets at the antenatal clinic as some of reasons for their non-compliance in taking supplements for the required amount and period.

The efficacy of the SUN-1000 special days program on maternal micronutrients status (IFA) and immediate birth outcomes, particularly the prevalence of LBW has not yet been documented. The prenatal supplements program progress is measured by self-reported compliance of intake of pills using questions that are not validated by biomarkers (hemoglobin and folate). Poor compliance (less than 10%) of iron supplementation prescribed during prenatal and postnatal periods has been documented in Ethiopia, Malaysia (Gebremedhin et al., 2014; Thirukkanesh and Zahara, 2010), and in a small clinical study in northern Malawi. Poor compliance was explained mainly as due to the frequent side effects and forgetting to take the pills (Young et al., 2000).

From the studies conducted in China, Zaire (DRC, central Africa), Papua New Guinea and other developing countries, Zimmermann (2011) summarized that iodine had an effect not only on cognitive development, but also on somatic growth. Maternal iodine supplementation and status were associated with children's weight, length and head circumference apart from IQ levels (Zimmermann, 2011). These studies and many others show that newborn health depends on good supply of micronutrients through the placenta. A good supply of micronutrients depends on placenta functionality, which itself depends on normal fetus development (angiogenesis and biogenesis). Efficiency of the placenta is modified by presence or absence of micronutrients during fetal development from the beginning of pregnancy (Owens et al., 2015). Therefore, placental weight and

newborn health may be used to determine maternal exposure to micronutrients early in pregnancy and to predict birth outcomes.

In Malawi, assessment of nutritional status of pregnant women has not been possible due to the lack of food composition comprehensive food intake data with corresponding biomarkers. Compliance to national nutrition supplements programs targeting pregnant women has only been documented from self-reported interviews and a small study which combined pill counting (Kalimbira et al., 2009; Young et al., 2000) but not which did not use appropriate biomarkers. The placenta is a mediator for maternal nutrition and health status and the fetal growth environment and can provide the evidence of linking maternal nutritional status and newborn health. Biomarkers can also be used to validate the self-reported intake of prenatal supplements. To our knowledge, no study in has been conducted in Malawi validating the effect of self-reported intake of IFA, using biomarkers and placental weight, on immediate birth outcomes to establish the efficacy of prenatal supplementation program in Malawi. The main aim of this study is to investigate whether compliance to prenatal supplements can be used as a determinant of LBW in Malawi.

Significance of the study

Importantly, micronutrient deficiencies in childhood have been associated with a country's low GDP (gross domestic product) thereby affecting the economic development of a nation in Myanmar (Win, 2016). The significance of this study is that validation of self-reported intake of IFA with biomarkers (Hb and serum and red blood cell folate) evaluated the effectiveness of the program in general in improving the health of women and birth outcomes, and monitoring approaches used by Malawi government

for the biggest anemia program in the country. This research provides feedback to government programs and non-profit organizations working in nutrition area in Malawi on the efficacy of prenatal supplements to reduce LBW in Malawi. Overall, the research results provide evidence that self-reported intake of IFA by pregnant women can be used as a monitoring tool for compliance to prenatal supplements. The study also introduces the innovation of using the placenta, which is normally discarded in Malawi, to determine compliance to and efficacy of prenatal supplements taken during pregnancy and impact on newborn health.

Research gap

The research gap is illustrated in the Figure 1 below. There have been some clinical studies showing the efficacy of supplements in increasing biomarker status. However these were conducted in controlled environments, while in real life people take medications freely at home. There have been no studies in Malawi showing how self-reported use of prenatal supplements (while the pregnant woman is free at home) increases hemoglobin and folate status as well as birth outcomes. This in turn will validate self-reported intake of prenatal supplements as a reliable compliance monitoring method, which can be used to predict birth outcomes.

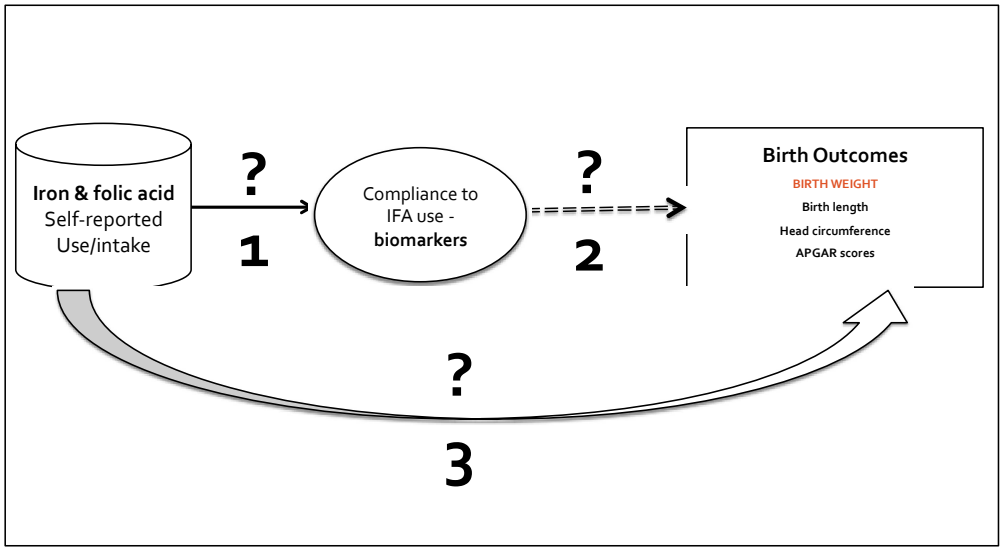

Figure 1. Diagram depicting research hypotheses.

Research objectives and hypotheses

Objectives

1. To validate self-reported compliance to prenatal supplement use by maternal biomarkers (Hb and serum and RBC folate)
2. To determine the association between maternal hemoglobin and folic acid status with the risk of LBW in Malawi
3. To determine if birth outcome (low birth weight) is predicted by self reported prenatal supplement use (IFA).

Hypotheses

1. Self-reported number of IFA intake is positively associated with biomarkers: Hb, serum and RBC folate status.
2. Hb status is inversely associated with the risk of giving LBW birth.
3. Self-reported number of IFA intake during pregnancy is inversely associated with LBW risks.

CHAPTER TWO: LITERATURE REVIEW

Geography, economy and education in Malawi

Malawi is in Southeast Africa, bordered by Tanzania to the north and northeast, Zambia to the west and Mozambique to the southwest, south and southeast. Malawi has a total size of 125 km^2; of which 90 km^2 is land and water bodies occupy the rest. The Lake Malawi spans the entire length of the country from north to south covering three quarters of the country with fertile arable land. The population grew to almost 16 million in 2016 from 4 million in 1966, as one of the fastest growing and population-dense countries in Southeast Africa. Over 85% of the population lives in the rural areas where access to clean water, electricity, hospitals, and good road network remains a challenge (NSO-ICF, 2017).

The economy of Malawi is agro-based with tobacco being the major cash crop for foreign exchange. The agriculture is rain fed and production varies every year depending on amount of rain received. Due to climate change now crop production has not been able to support the growth of the economy, rendering it unstable in the poor harvest y . Fifty three percent of the population lives below the poverty line with less than a dollar a day.

According to the Malawi Demographic and Health Survey 2010, 15% of the population received no formal education. Only 9% had finished primary school, 13% had secondary school education, and the rest of the population dropped out. Compared to boys, higher number of girls enrolls at the primary school but drop out at a higher rate. The situation leads to poor retention of nutrition education and opportunities for

knowledge of women on feeding practices of children, nutritional security for women, children and families, and malnutrition (NSO-ICF, 2017).

Nutrition and food security in Malawi and other countries

In Malawi, achievement of food and nutrition security with diversification of diets is an uphill task. Maize (corn), the staple grain comprises 50% or more of most Malawians diet and most households taking it more than once per day (Broadley et al., 2012). Consequently the major source of calories (70%) for Malawians comes from maize. It also makes a large portion of the planted and cultivated crops almost 60%. Most people in Malawi survive on their own production, they are subsistence farmers and only buy when they don't have enough food from own produce (Chirwa and Dorward, 2013).

For the past 30 y Malawi experienced food insecurity with about one third of the general population being severely affected. The country has been intermittently food insecure partly due to over reliance on the staple food (persistent use one food type) and failure to diversify the diets. In the 2000s alone Malawi experienced 3 episodes of severe food insecurity and spikes in the prices of staple food, 2001–02, 2004–05, and 2007–09 by 354%, 218%, and 395%, respectively (Ellis and Manda, 2012). Agricultural diversification problems have been partly fueled by the government's Farm Input Subsidy Program (FISP). Although aimed at achieving food security, this program ended up focusing on the staple crops and thus the communities had a single food commodity (mainly maize) with limited diversified diets that lack micronutrients. Food supply is unstable, because when maize fails due to poor rain and climate change, there is no alternative means of food production, which results in hunger throughout the country. This is because Malawi's agriculture is rain fed, and Malawi only has one rain season,

failure of one season equals hunger for the whole year. Currently one in four people are food insecure in developing countries including Malawi.

From above it can be seen that in Malawi and most developing countries availability and access to food are the major challenges. This is different with what is seen in developed countries like the U.S. where food is available throughout the year round but distribution and political will are the challenges. Unlike developing countries like Malawi where one third of the population are food insecure, only 14.3% were food insecure in 2012 in the U.S. with 85.7% having enough to eat (Coleman-Jensen et al., 2014). This is according to a U.S. food security scale, which is administered every year. The scale measures if at any time a member of a household had their food reduced or disrupted because there was not enough money for food in the household. Just like in the developing countries more vulnerable people are children or households with children. In U.S. the prevalence of severe food insecurity has been reducing but statistically insignificant over the y , and those affected only comprise 5.6% (Coleman-Jensen et al., 2014). There are two main types of access to food namely; physical and economic access to food. In the U.S., most people have physical access to food except a few, the majority of those that are food insecure it is due to lack of economic access to food.

In developing countries, both physical and economic access is a challenge. The distribution systems are poor between where food is produced in abundance and where food might be greatly needed. For example, roads to transport food might be impassable and/or difficult physical access due to armed conflict in a region that limits movement severely. Even when people have access to a place where food is available, they may not have the economic power to legitimately acquire it. Historically developing countries are

at risk of food insecurity because of mono-cropping systems of agriculture left by colonialism where countries were forced to grow one crop for export. For example tobacco in Malawi cocoa in Ghana, and tea, coffee, peanuts, cane in others countries (Nagothu, 2014).

Nutrition and health status in Malawi and developing countries

Health in Malawi is greatly affected by nutrition status of individuals. The differences with developed countries have been the types of diseases suffered by people in these countries because of the type of diet available and environment they live in. While developed countries are fighting non-communicable diseases, which mainly result from excessive intake of nutrients, the developing countries are still fighting undernutrition. Under nutrition is major cause of health burden in South Asia and Sub-Saharan Africa (Müller and Krawinkel, 2005). Undernutrition leads to a cycle of health challenges, which later lead to poor intake of nutrients and results in malnutrition again. Apart from undernutrition, developing countries are also facing increasing levels of obesity, non-communicable diseases like cancer, hypertension and diabetes which is being referred to as the double burden of malnutrition (Boutayeb, 2006). The rise in non-communicable diseases is mainly in cities due to increase in population, where demand for fast foods is also high (Dalal et al., 2011).

Obesity and overweight prevalence in Malawi has risen from 9% in 1992 to 21% in 2016 while the prevalence of thinness has remained relatively the same with just decrease from 10% to 7% in 2016, confirming the double burden of malnutrition (NSO-ICF, 2017). Overweight/obesity is higher among women in urban areas (36%) than rural areas (17%) where as thinness (BMI < 18.5) is higher among teenagers (15-19 y).

Overweight/obesity increases with increasing education and wealth, 12% in women in lowest wealth quintile and 36% in women in the highest wealth quintile. Women's nutrition status is a major concern for every country because they are major contributors to the next generation health as they do not only conceive but also nourish the fetus during the critical stages of life (Victora et al., 2008). The thin women (BMI<18) are likely to have low education, less wealth and young (15-20 y)(Victora et al., 2008; West and Christian, 2008). This partly explains the prevalence of chronic malnutrition among children born from these women which is very high (NSO-ICF, 2017)

Infections (viral, bacterial and parasites) complicate health and nutrition status of individuals in developing countries. Human immune deficiency virus (HIV) worsens nutrition status of children and women because HIV increases energy and nutrient demand which is not easy to meet in people who are food insecure. It severely reduces immunity rendering individuals prone to other infections like gastroenteritis that predispose them to malnutrition (Bradley and Mishra, 2008). In 2016, the prevalence of HIV in Malawi was 8.8%, with higher rates in those who are working, have higher education (at least secondary), higher income and in women who had an education of secondary or higher (10.8%) levels (NSO-ICF, 2017). Malaria is also the leading cause of morbidity and mortality in under-five children and pregnant women. It affects nutritional status of children and especially in pregnant women thereby affecting the outcome of pregnancy as well. Pregnant women and children suffering from malaria will be anemic since malaria parasites feed on iron in human red blood cells. Although there has been a decrease in prevalence of malaria parasitaemia in different age groups as shown in Figure1below pregnant women are still at higher risk. Malaria prevalence is supposed to

be much lower in pregnant women because they are specifically targeted with malaria control programs (Malawi Government, 2009). Pregnant women in prenatal clinics receive antimalarial prophylaxis and insecticide treated mosquito nets to sleep under with their under-five children if they have any, however, adoption has been slow (NSO-ICF, 2017). Previous studies have shown association between placental malaria and LBW hence the need to continue promotion malaria control programs (Rogerson et al., 2003; Steketee et al., 1996).

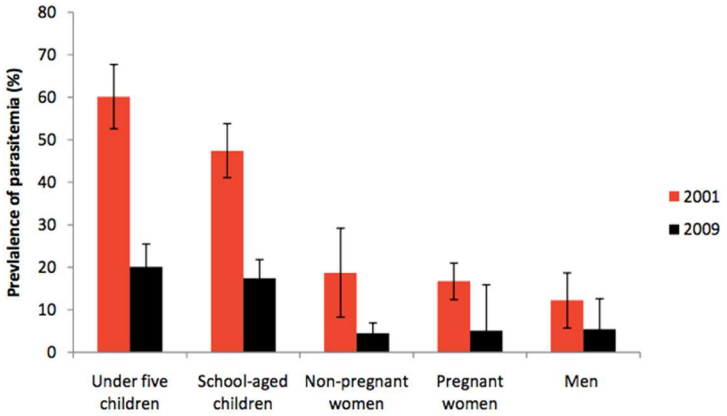

Figure 2. Prevalence of malaria parasitaemia by age/sex groups (Source: Malawi micronutrient report 2009).

Maternal and child health in Malawi

The United Nations Millennium Development Goals (MDGs) that would impact on prevalence of LBW are 4 and 5 (MDG 4 and 5) in 2015 focused on children and women respectively. MDG 4 was aimed at reducing child mortality while MDG 5 aimed at improving maternal health by the year 2015. The suggested indicators for MDG 4 were; reducing under-five mortality and infant mortality per 1,000 live births as well as improving the proportion of children under one year who are vaccinated. The indicators

for MDG 5 were increasing access to family planning and skilled health care, antenatal and perinatal care and reduced maternal mortality ratios. In MDHS 2017 the maternal mortality ratio was 439/100,000 live births compared to 984/100,000 live births in 2004, which is still a very high number of women dying in this age. In 2010, about one out of seven (14%) of babies are delivered in the villages attended by unskilled traditional birth attendants, 9% assisted by a relative, and 3% delivered with no assistance (NSO-ICF, 2017).

Table 1. Maternal and child health indicators in selected developing countries.

Country	MMR	IMR	Stunting (%)	Underweight (%)	Wasting (%)
Malawi	439	42	37.1	11.4	2.7
Tanzania	530	43	34	13.5	4.4
Rwanda	253	32	37.9	9.3	2.2
Mali	368	56	38	25.5	12.7
Nepal	281	46	40.5	28.8	10.9
Afghanistan	1,291	45			
Indonesia	313	32			
Cambodia	170	28	32.4	23.9	9.6

Infant mortality (IMR): the probability of dying before the first birthday (rate per 1,000 live births). MMR= maternal mortality ratio per 100,000 live births.

Malawi faces many other public health concerns that lead to poor health indicators such as high neonatal, infant, under-five, and maternal mortality. According to MDHS 2016, the prevalence of LBW (LBW < 2.5 kg) was 12% with the highest rates (16%) in central region and among newborns born to young mothers (<20 y) and older mothers (ages 35- 49 y). Infant and child mortality rates, basic indictors of a country's socioeconomic status and quality of life (UNDP 2007) are high. In 2017, the mortality rates (per 1,000 live births) of neonates, infants and children under-five were 27, 42, and

63 from 41, 135, and 234 in 1992 respectively. Although there has been a decrease in child mortality, the numbers has have not met the international policies like MDGs. The Millennium Development Goal number 4 was to reduce child mortality by two thirds by the year 2015, however, from Table 1 above it can be seen that Malawi did not meet the goal (NSO-ICF, 2017).

In Malawi antenatal care is given to all women and is free in all public health care institutions. Prenatal care is an important opportunity for women to learn about hygiene, child feeding and nutrition for pregnant women. Antenatal care visit occur monthly in first and second trimester and every two weeks in the final trimester or as determined by health care professional. All pregnant women receive iron and folate supplements (60 mg and 0.25 mg), anti-helminthes, vaccines for tetanus, and screening for HIV, syphilis, and urinary tract infections. In 2010, 50% of pregnant women had antenatal visit once or twice only while 2% did not have prenatal care at all. The reduced number of antenatal care visits was mostly due to long distance to the health facility. Hence majority of pregnant women did not receive iron and folate supplements, nutrition education and screening for disease, which are some of the services given at the antenatal care clinic.

Nutrition status of children reflects socio-economic of a population and can predict the economy of a country in the future. This is so because the stunted children will make a large proportion of the work force in the next 2 decades and if not well developed they will not be productive. High proportion of stunted children indicates that the population is likely going to underperform in future in terms of productive capacity for economic development (Bryce et al., 2008; Morris et al., 2008; Win, 2016). In 2016 a large percentage of children under-five were undernourished as indicated by stunting

(37%), underweight (11.4%) and wasting (2.7%). Figure 3 shows that prevalence of underweight and wasting in under-five children has been decreasing. On the other hand stunting remained stagnant for 2 decades before making a significant drop in the last five y , indicating the existence of challenges in reducing malnutrition in Malawi (NSO-ICF, 2017).

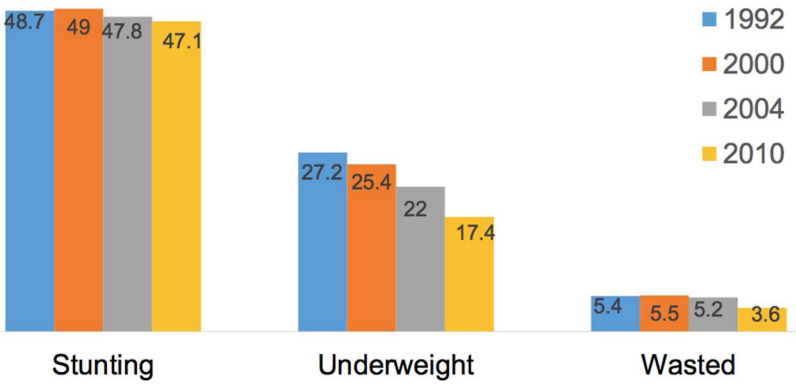

Figure 3. Prevalence of malnutrition in under-five children in Malawi (MDHS: 1992, 2000, 2004 and 2010).

Prevalence of LBW and consequences in later life

The national prevalence of LBW is 12% with the central region having the highest (16%) than the other parts of the country. Birth weight is an important indicator for assessing child health in terms of early exposure to childhood morbidity and mortality. Children whose birth weight is less than 2500 g, or children reported to be 'very small' or 'smaller than average' are considered to have a higher-than-average risk of early childhood death , (Arnold et al., 2015). The national prevalence of LBW directly contributes to the 37% prevalence of stunting levels in Malawi. Young mothers aged 20 or less are more likely to give birth to a LBW baby followed by older mothers aged 35 y and above. The least number of LBW babies is observed in mothers aged at least 20 y to

34 y old (NSO-ICF, 2017).

Children born to mothers below the age of 20 y will not have the necessary support in their upbringing because most teen mothers are financially unstable. These children will be underdeveloped, when they grow up, they may end up in early marriages, which increase the risk of giving birth to LBW babies as well, continuing the vicious cycle. Probably this occurs because teen pregnancies are associated with complications in the family structure and support for the children is low as teenagers are not economically stable to provide for their children (Victora et al., 2008).

The central region has high prevalence of LBW compared to the south or the northern regions (NSO-ICF, 2017). For this reason, Lilongwe district in central Malawi was chosen as a research site.

Micronutrient deficiency of iron and iodine in Malawi

Along with the general health and nutrition problems, Malawi faces nutrition problems associated with micronutrient deficiencies. Most prevalent ones with great concerns at the global level are iodine (2 billion at risk) iron (2 billion) zinc (estimated to be very high in developing countries) and vitamin A (254 million preschool children). According to the WHO World Health Report, almost one third of the world's population is affected by them, the majority living in developing countries (Ahmed et al., 2013).

Iron

Iron is the micronutrient that is of great concern almost worldwide, although the severity differs from region to region and country to country. However, the developing world is disproportionately affected. Malawi is not spared and the pregnant

mothers are affected by anemia. WHO reports that half of the anemia cases worldwide are caused by iron deficiency anemia, and occur in resource-poor areas. Malaria, HIV/AIDS, hookworm infestation, schistosomiasis, and other infections such as tuberculosis are particularly important factors contributing to the high prevalence of anemia in some areas (WHO, 2016). In Malawi the national prevalence of anemia is 13.6%. This anemia prevalence reported in the Malawi government National Micronutrient survey report 2009, could have been reduced by half if iron deficiency had been eradicated and improve birth outcomes (Allen, 2000; Malawi Government, 2009). However,

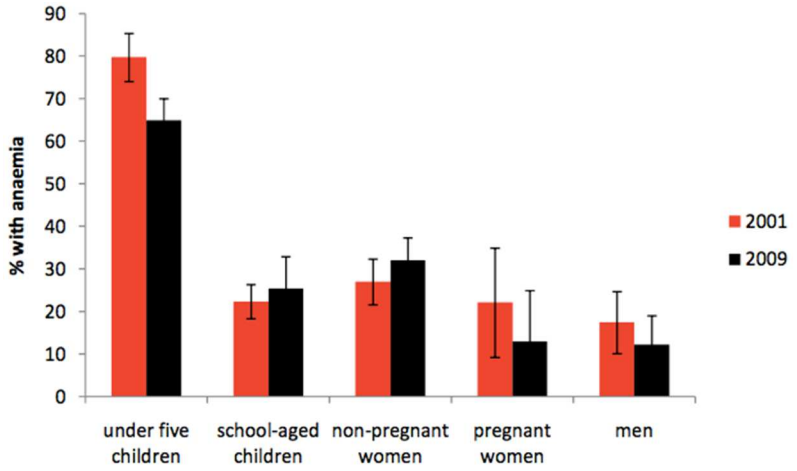

Figure 4. Prevalence of anemia in Malawi (Malawi government micronutrient survey 2009)

According to the National Micronutrient Survey report 2009 the prevalence of iron deficiency anemia in Malawian women of the reproductive age group was 26.1% from 32.4% in 2001, and in under-five children it was 49.1% from 61.5% in 2001 (Figure 4). Although anemia is decreasing there is still a significant number of school aged children, men, reproductive age, and pregnant women affected by iron deficiency anemia

(using transferrin as a biomarker). Iron is important for normal development of children not only when they are young after birth but beginning right in utero.

Therefore it is important to note that the micronutrient survey report 2009 shows iron deficiency in Malawi is common in women of reproductive age group (Figure 4) even before they conceive babies (Malawi Government, 2009). This is a cause of worry in that Malawian women go into pregnancy with reduced iron stores yet it is well known that pregnancy increases the nutritional demands of micronutrients (Berti et al., 2011; Black, 2001; Cao and O'Brien, 2013; Cetin et al., 2010; Pasricha et al., 2013).

Perhaps it is not surprising that anemia is common in pregnancy as well (see Figure 4) because these women cannot make up for the increased demand of nutrients in pregnancy when they could not meet their own need before pregnancy. Hence chances are high that they may transfer the iron deficiency to the unborn child risking poor physical and cognitive development of baby right from the uterus unless the placenta saves them by pooling nutrients towards the fetus at the expense of the malnourished mother (Black, 2001; Lager and Powell, 2012). This could also partly explain the occurrence chronic malnutrition in children (stunting).

Iodine

The recommendation of WHO is that only 50% of the population of school-aged-children and non-pregnant women should have urinary iodine levels of less than 50 ug/L in a country. According to Malawi government micronutrient survey report 2009, only 12.6% of school-aged children and 12% of non-pregnant women had urinary iodine status below 50 ug/L. Of the households surveyed over 90% had iodized salt in the house

and over 80% of salt samples from these households had the recommended iodine of 15 ppm (NSO-ICF, 2017).

However, half (50%) the population store their iodized salt in an open container and 45% of the at risk group, women of reproductive age, did not know that iodine deficiency results in goiter. Knowledge deficit on importance of iodine and its proper storage has implications on iodine intake the population in the long term (Glinoer et al., 1995). This is evidenced by the fact that only 17% of school-aged children had heard about iodized salt (NSO-ICF, 2017). This is critical in that the school-aged population, which is good at retaining information and passing it to others, is deficient of knowledge.

Another important issue to note is that some household (17%) had salt with higher iodine than the recommended levels, indicating problems with quality control in the industry where the salt is iodized. Salt in Malawi is imported already iodized or is bought in bulk, repackaged into smaller bags and iodized in the country. Salt is required to be repackaged in smaller bags so that when traders open it, it should not overstay in the shops before it reaches the consumer who will likely keep it in an open jar for sometime (NSO-ICF, 2017).

However, problems with illegal entry of salt have been reported and this is usually not iodized, because if passes through normal borders the port health officers will test it and if not iodized the consignment will be sent back or detained for destruction, hence a lot of it uses the informal routes and find its way to the markets. The it is surprising that only a small proportion of the population (12.6%) has iodine deficiency since most salt readily available to rural communities is un-iodized as it is cheap (unpackaged) compared to the industrially packaged one.

Policies and programs addressing nutrition problems in Malawi

Malawi is a member of the United Nations; therefore it subscribes to many international treaties and agreements on human rights and international development goals. The best examples are the Millennium Development Goals (MDGs) stated above and the Scaling Up Nutrition, (SUN) 1000 days. Malawi like any other country in the world is concerned with the nutrition status of her people and has put mechanisms in place to combat inadequacies to save the next generation from effects of nutrient deficiencies. The Malawi government oversees all the nutrition activities in the country through the ministry of health's department of nutrition and HIV. The department ensures that all government departments have nutrition activities; NGOs involved in nutrition are doing and aligning their programs to the nutrition policy, which will be launched soon in 2016.

Programs and policies targeting women and children in Malawi

The major approach of Malawi government for fighting malnutrition is an adaptation of the global movement of scaling up nutrition being implemented by many developing countries. The initiative was started after the Lancet published maternal and child undernutrition series in 2007 describing the importance of investing in nutrition (Black et al., 2013). Malawi was among the first countries to launch the initiative in 2011. The program is called "Scaling Up Nutrition (SUN) 1000 special days." It uses direct nutrition and health specific interventions as well as multi-sector approaches to fight malnutrition with a special focus on the most critical period of development in children (from 0 to 24 months). Thus SUN aims to increase activities that make women to be

nutritionally sufficient during pre-pregnancy period, during pregnancy and up to 2 y after birth of the child and beyond.

One of the programs that have been implemented in the SUN movement is supplementation of IFA to pregnant women in antenatal clinics. Knowing that iron inadequacy is a global problem and that during pregnancy iron is very crucial, it is being supplemented in all pregnant women universally in Malawi. Since it is recommended worldwide that supplementation of iron and folate more efficient folic acid is also given although its effect is much better before and very early in pregnancy. This is according to WHO which recommends that in countries that have anemia prevalence of 40% or more universal iron and folic acid supplementation be done with antimalarial prophylaxis in malaria endemic areas (WHO, 2012). Pregnant women receive the iron and folic acid supplements regardless of hematologic status from the day of their first antenatal visit throughout pregnancy. The IFA is given in tablet form, which comprises of 60 mg iron and 0.25 mg combined. The women are expected to take the tables daily for a period of at least 91 days as recommended by WHO. Every monthly visit the pregnant woman gets a supply that lasts approximately just over one month. Therefore the number of antenatal visits achieved by a pregnant woman may potentially determine how many pills of iron and folic acid are consumed. However, there is no program in place to check whether or not the woman actually takes all the iron and folic acid supplements. The program runs on trust that the women understands the importance of taking the supplements and will not miss the doses and will finish the whole monthly supply.

During the first visit the pregnant woman gets a diagnostic wake up where different primary diseases are screened. The woman will give samples of blood, urine and

stool for screening HIV, malaria, worms, and for checking hemoglobin levels among others. After screening the women are given anti-helminthes treatment as well as anti-malaria prophylaxis in the first and last trimester of pregnancy. The prophylaxis treatment of malaria is given as a single dose taken at once in the first trimester, repeated in the third trimester.

During the same first antenatal period the pregnant women get free mosquito nets treated with insecticide to protect themselves and under-five children from mosquitoes that transmit malaria and antimalarial as recommended by WHO (WHO, 2012). Malaria in pregnancy is a priority area just like other nutrition programs because malaria causes anemia, morbidity and also mortality. The other important health measure taken in early pregnancy is the vaccination that pregnant women receive against tetanus. This is to boost the antibodies, which they will pass to the fetus so that the child will be protected from tetanus infection.

The other activities in the SUN program include agronomic bio-fortification of crops for example the bio-fortification of sweet potatoes (orange-fleshed sweet potatoes) with vitamin A, and promotion of its consumption so that the general population especially women have sufficient stores of vitamin A. The government distributes the seed (potato vines) to farmers to plant for their own consumption.

Every six months there is a massive campaign in Malawi to mobilize health workers in ministry of health and all concerned sectors like ministry of education and ministry of agriculture to distribute vitamin A supplements and deworming pills as well as health talks. The officers will go to schools and clinics to give supplements to all

children aged 6 months to 12 years. Each child will get one drop of vitamin A in an oil capsule ranging from 100,000 IU to 200,000 IU every six months.

At the same time school going children (above 6 y old) and infants also receive de-worming tablets to clear parasites and reduce the risk of anemia. The Malawi government through the ministry of education has introduced a school-feeding program which is rolling out all over country, ensuring school going children are not hungry while at school.

Once every year the ministry of health with other concerned ministries, conduct breastfeeding week. This is conducted with massive campaign for breastfeeding and promoting exclusive breastfeeding for six months as well as proper complementary feeding after six month. The ministry of health officers go to schools, villages and streets to spread messages about hygienic feeding practices and the benefits of breastfeeding. There are also posters and billboards on town streets and jingoes/adverts on radio and television promoting breastfeeding especially the first six months (exclusive breast feeding).

When the worst comes to the worst severely malnourished children are treated in nutrition rehabilitation centers located in hospitals. Malawi is implementing another program called active community management of acute malnutrition (CMAM) where children at risk of malnutrition or with mild malnutrition are identified actively by community workers as early as possible and treated or referred to treatment centers before they worsen.

Children with diarrhea receive zinc supplements. The children receive 20mg of zinc supplements as tablets to take for at least 10 days. This is also a program, which has

been adopted to reduce the risks of micronutrient deficiencies in children. Another reason zinc is given to children during diarrhea illness is that zinc supplementation has been known to reduce both the occurrence and frequency of diarrhea. Zinc boost immunity by increasing cell growth. It also stabilizes the metabolic rate, balances the blood sugar and improves sense of smell, which may help control diarrhea and help improve appetite with appropriate diet. Zinc supplementation is now in the national guidelines in the management of diarrhea in children in Malawi.

Another strategy being used to increase micronutrients intake in both women and children and the general population at large is food fortification. Industrial fortification of vitamins A and B- complex in cooking oil, sugar and maize flour is being implemented by the manufacturing industry and enforced by Malawi government. Unfortunately the fortified commodities are consumed by those living in cities and towns where supermarkets are, which are not accessible by rural people who comprise 85% of the population. This is the major weakness to the approach as it targets wrong people. Due to rural and urban disparity people in urban areas have lower risk of micronutrient malnutrition and are few, majority (85% of Malawians) reside in rural areas with no access to fortified industrial products and increased risk of deficiencies.

Universal iodization of salt has been adopted by Malawi, although it benefits the whole population, it targets women and children more. In 1995 salt iodization Act was established by parliament and salt iodization regulations were formed in 1998. This act makes mandatory for the industry to iodize salt. This enables agencies to prosecute any one selling salt without iodine. This is critical because Malawi does not manufacture salt it is all imported either already iodized or it is iodized within Malawi. Food has very low

iodine content in Malawi, therefore iodized salt is the major and reliable source (Watts et al., 2015). However, some traders are still not adhering to the regulations, this is evidenced by the presence of salt without iodine in households during the MDHS survey despite the enforcement of the regulations (NSO-ICF, 2017).

The regulation states that only iodized salt should be imported into the country or some licensed traders will be allowed to bring salt without iodine in bulk, which will be iodized in the country. The certified industry is allowed to repackage iodized salt into smaller bags no more than 40 kg to put on the retail markets for consumers. Two steps that are prone to violations are firstly some unlicensed traders may import and smuggle salt without iodine and successfully put it on the market. Secondly, the licensed traders may decide not to iodize after importing salt and just repackage and sell to consumers. The government has two mechanisms to combat these malpractices, first it has employed port health officers who work at ports and ensure that imported salt entering the country is iodized. The second mechanism the government uses the Malawi Bureau of Standards officials who go out in retail shops with test kits and sample salt from shelves and discard salt not meeting the standards. Now with the iodization act, the bureau will be able to prosecute those selling un-iodized salt.

Compliance to prenatal supplements

According to Young et al. 2000, in Malawi compliance of pregnant mother to iron and folic acid supplementation is poor in the currently adopted method of daily dose from first antenatal visit to delivery day. A randomized controlled trial in northern Malawi recruited 413 pregnant women. Of them, 211 got iron and folic acid (60 mg iron and 0.25 mg folic acid) every day and 202 women got the supplements once a week at increased

dose (120 mg iron and 0.50 mg folic acid). The results showed that compliance was poor in the daily dose group than the weekly supplemented group (Young et al., 2000).

The reasons reported most for non-compliance to iron supplementation were side effects and forgetting to take the pills, s among others. It appears the daily supplementation becomes a burden for pregnant women to take constantly and consistently. In another study, which utilized the national data collected using a pre-tested questionnaire revealed that a large proportion of women know anemia (96.6%) but nausea from the metallic taste of iron was a drawback to compliance. Most women tried to take iron tablets with food until they finally stopped after a month only (Kalimbira et al., 2009). Non-compliance has been reported in Ghana West Africa as well, compared to Malawi, however knowledge about supplements was low in Ghana (Hill et al., 2007). Misconceptions about iron supplements were high as taking medicine while healthy was a new concept to them. Others had thoughts that supplements were for family planning, or for the childless. When the purpose of the supplementation was explained, the participants accepted the intervention of prenatal supplementation (Hill et al., 2007).

It has also been reported that access to iron supplements by pregnant women is low, although prenatal supplementation is considered universal in most developing countries. In Ethiopia only 35% of pregnant women were reached and received iron tablets. Of those who received supplements, only 3.5% complied to taking the iron supplements and the rest did not (Gebremedhin et al., 2014). This means that only 12% of the target population receives the benefit of prenatal iron supplementation. These studies show that although micronutrients supplementation shows the potential increasing serum levels the targeted population is not being met and those reached are not utilizing the

supplements. Therefore it may be necessary to re-focus the intervention and plan on how best the goal can be achieved, as the program is reaching very small numbers of pregnant women risking anemia and poor development of children.

Role of placenta

Normal placental development relies on an adequate supply of such micronutrients as iron, iodine and selenium in placental biogenesis and angiogenesis (Khera et al., 2015; Meher et al., 2015). Efficient placental vascular function relies on multiple micronutrients supply too (Owens et al., 2015). Placental size and functions are important for transferring nutrients for fetal growth and better birth outcomes. Therefore with the evidence that micronutrient deficiencies exist in Malawi and that compliance to supplementation programs is poor, the risk of malnutrition increases, there is need for more evidence on the efficacy of micronutrient supplementation in Malawi in order to develop working programs.

Secondly, micronutrients are important for placental health, fetal growth and newborn health. Owens and others (2015) in their study in Gambia, west Africa demonstrated that multiple micronutrient supplementation have the potential to modify placental vascular function (Owens et al., 2015). Low selenium concentration in the placenta was associated with hypertensive diseases in pregnancy (pre-eclampsia) which leads to growth restriction in the newborn due to poor development and vascularization of placenta (Mistry et al., 2008). As early as 1930s selenium concentration in placental tissue was described as a proxy to concentration in cord blood or fetal selenium status. In animal studies (rats and sheep from 1938, 1965 to 1990s) 14% of selenium in the diet of well

nourished dam was found in the off-spring against a concentration gradient (Hadjimarkos et al., 1959; Westfall et al., 1938).

Nutrient transport and kinetics in the placenta

Fetal development comprises of entire intrauterine process of differentiation, growth and maturation between conception and birth. The conditions under which the fetus develops are recognized to have major impacts on the future health of the newborn child (Holme et al., 2015). The developmental environment of the fetus is dependent on maternal nutrition and metabolic state, and on the placental function, as most nutrients have to be transported from the maternal circulation across the placenta to the fetus.

Thus the major function of the placenta is transport, and serves as the only link of the fetus to the outside world unlike other mammals that give birth to an immature young and have to continue development independent of the placental connection. The placenta has another function; it secretes hormones and growth factors that change the maternal metabolism markedly. Hence the placenta controls to large extent the environment in which the fetus develops and grows (Holme et al., 2015). The main energy source of the fetus and the placenta is glucose. Glucose is transported via the facilitated carrier-mediated diffusion using glucose transporters of the GLUT family (Holme et al., 2015; Lager and Powell, 2012).

Apart from energy the fetus also needs amino acids, fatty acids and micronutrients for growth and development, which have to cross through the syncytiotrophoblast. The syncytiotrophoblast is the primary barrier limiting nutrient transport across the human placenta. As discussed above syncytiotrophoblast has two polarized plasma membranes, a maternal facing microvillus plasma membrane (MVM) and a basal plasma

membrane oriented towards the fetal circulation. These plasma membranes express numerous nutrient transporters which may be regulated by fetal, maternal and placental signals (Lager and Powell, 2012).

Amino acids are transported across cellular membranes through either mediated active transporter processes, which are accumulative thus only sending nutrients towards one side against a concentration gradient usually co-transporting with sodium, or the exchanger system that exchanges amino acids between intracellular and extracellular compartments. There are over 20 amino acid transporters in the human placenta responsible for facilitating trans membrane transport (Lager and Powell, 2012).

Fatty acids mainly triglycerides are carried by lipoproteins across the placenta, but first they are hydrolyzed by placental lipases (Lager and Powell, 2012). Most vitamins cross the placental membranes by simple diffusion especially the B vitamins.

Placental iron transport

Iron is moved from the placenta to fetal circulation against a concentration gradient. Here are many disagreements concerning the transport of iron in the placenta with some proposing that the mechanism is not different as that involved in absorption of iron from the duodenum. However, the absorption of nonheme iron by enterocytes in the duodenum is controlled by shading of cells after 3 days when there is excess iron thereby avoiding toxicity (da Silva et al., 2014). The placenta lacks the capacity of shading cells storing excess iron and the syncytiotrophoblast has to keep excess iron until its the fetus to avoid toxicity or it will be discarded during parturition.

The process recognized involves reduction of Ferric (Fe^{3+}) to ferrous (Fe^{2+}) which is the first step in the assimilation of nonheme iron and is mediated by the brush-border ferri-reductase activity (Cao and O'Brien, 2013).

An enzyme called Duodenal Cytochrome B (DcytB) has been implicated in the process. Although it does not appear to be an essential gene, DcytB is highly regulated in response to iron status in both animals and human populations it modifies ferritin levels through a promoter (da Silva et al., 2014). After reduction by ferri-reductase or other enzymes, uptake of the ferrous form of iron is mediated by divalent metal transporter-1 (DMT-1). Another model of iron transport across the placenta and is the one that fits very well with placenta physiology is the vesicular trafficking pathway. This mechanism uses iron exporter ferroportin to transfer diferric inorganic iron intracellularly by compartmentalizing it in a membranous vesicle and surrender this to iron free Apo-transferrin(da Silva et al., 2014).

According to Cao et al, the serum diferric-transferrin binds to placental transferrin which is abundantly expressed on the apical side of the syncytiotrophoblast, leading to endocytosis of the transferrin-transferrin complexes (Cao and O'Brien, 2013). Iron dissociation is achieved by acidification of the vesicle and iron is reduced by a new family of ferri-reductase, Steap 1,2,3, and 4, later transported out into the cytoplasm through a protein channel DMT-1 or a DMT-1 independent pathway which is said to exit in the placenta. Once in the cytoplasm iron is incorporated into its storage form ferritin or is delivered to transferrin in the fetal circulation (Cao and O'Brien, 2013).

Allen et al (2000) Serum transferrin carries iron from the maternal circulation to transferrin receptors located on the apical surface of the placental syncytiotrophoblast,

holotransferrin is endocytozed, iron is released, and Apo-transferrin is returned to the maternal circulation. The free iron then binds to ferritin in placental cells where it is transferred to Apo-transferrin, which enters from the fetal side of the placenta and exits as holotransferrin into the fetal circulation (Allen, 2000).

During deficiency the expression of these transport proteins may be up regulated thus the placenta is able to adapt to maternal supply. As for heme iron the human placenta exhibits expression of different proteins involved in uptake and utilization; For example heme carrier protein [proton-coupled folate transporter (PCFT)], heme carrier protein-1 (HPC-1) and Feline Leukemia virus C receptor 1 (FLVCR 1). FLVCR is an iron exporter though (Cao and O'Brien, 2013).Net flux of roughly 270g of iron is transferred across the placenta to be accumulated by the developing fetus especially during the last 10 weeks of gestation. During late gestation approximately 5.6mg per day of iron is trafficked through the placenta to meet fetal demands from diet or endogenous maternal sources (Cao and O'Brien, 2013).

In summary iron transport involves three mechanisms, first DcytB enzyme reduces Fe^{3+} to Fe^{2+}, which is easily transported across the syncytiotrophoblast in a process mediated by divalent metal transporter-1 (DMT-1). The second mechanism, which is common to heme iron as well, involves serum transferrin bringing ferrous to transferrin receptors into the microvillus spaces and ultimately a transferrin-transferrin complex is made and iron is endocytozed into the syncytiotrophoblast later ferrous s exocytozed to the fetal circulation. The third mechanism of iron transport uses heme carrier proteins to export heme, for example proton-coupled folate transporter (PCFT), heme carrier protein-1 (HPC-1) and Feline Leukemia virus C receptor 1 (FLVCR 1).

Placental iodide transport

Sodium iodide symporter (NIS) is central in the transport of iodide in the body. It is an 85kDa membrane protein that couples the translocations of sodium (Na^+) and iodine (I^-) (da Silva et al., 2014). Two Sodium ions are translocated from an area of high concentration to an area of low concentration, while in the same direction iodide will be translocated against a concentration gradient, from low to high. The above two sodium ions will be translocated across the membrane along with one iodide anion into the cell. NIS is a transmembrane glycoprotein which couples the inward transport of two Na^+ ion, along with the transport of one I^- ion against its electrochemical gradient (Andersen, 2015). Apart from placental site NIS is also found in the basolateral, membrane of the follicular cells of the thyroid, the lactotrophs, the lactating mammary gland, in salivary glands, sweat glands, in the gut mucosae and choroid plexus of the cerebral lateral ventricle (da Silva et al., 2014). In the same mechanism (NIS) used by gut cells to absorb iodine from intestinal lumen is also used by placenta to transport iodine to the fetus.

CHAPTER THREE: METHODS

Research site and design

Data for this research was collected from a cross sectional study carried out from December 2016 to January 2017. This is a lean period in terms of the availability of food in Malawi. It is also a festive season when Christians celebrate Christmas and everyone else celebrates the New Year. Despite having insufficient food during this season Malawians will try to save a little money to buy some special food for December 25th and 1st January of each year. The study site is the Lilongwe District Hospital Maternity Wing (locally known as Bwaila Maternity Wing), located in the capital city and central region of Malawi. The Bwaila Maternity Wing is strategically positioned inside the city, and provides services to pregnant women from different ethnic groups, educational backgrounds and socio-economic levels. The Lilongwe District Hospital serves as a referral hospital for smaller rural hospitals in the district. Thus, it serves rural, peri-urban and urban populations. Furthermore, the hospital is located in central Malawi, a region that has a higher prevalence of LBW births than the national level. All these reasons combine to making this an appropriate research site.

The health system structure in Malawi is organized into five major levels. The lowest level is a health post (Health Surveillance Assistance, HSA). This is an individual with one year medical training who stays in the village. The major role of a HSA is to identify sick children and pregnant women, give them first aid treatment and advise them to go to the hospital. They have basic medications, such as painkillers, chlorine for the treatment of water, anthelminthic agents, antimalarial drugs and vaccines. The HSA also

runs growth-monitoring clinics for children under five, once or twice a month. The second level is a health center, which is a small simple rural building manned by a medical assistant (2 y medical training) and one or two midwives. They conduct normal pregnancy deliveries, treat simple illnesses like diarrhea and malaria and make referrals of complicated pregnancies and diseases. The third level is the community hospital, which has an operating theatre and can conduct minor surgeries especially caesarian sections of non-complicated cases. There are more medical personnel and midwives, the hospital is manned by an experienced physician assistant (a clinical officer, with 3 y of medical training and one year of residency), or one general practitioner. These individuals perform minor surgeries and prescribe for other diseases. They make referrals of all complicated cases to the next level.

The fourth level is a district hospital, which provides the highest level of care. There are 28 districts in Malawi and each has its own district hospital except one, which is an island. Here more services are available, including major surgeries and consultations for complicated diseases. The services provided however depend on the type and level of experience of the staff. Several physician assistants, general practitioners, nurses and midwives and one or two specialists provide services at the district level. The highest level of care is provided by central hospitals, of which there only four institutions in the whole country. At central hospitals, specialists such as Oncologists, Ophthalmologists, Nephrologists, Surgeons, Internal Medicine Specialists, Pediatricians and Obstetricians and Gynecologists who provide surgical and advanced obstetric care (e.g., hysterectomies, fistula and ruptured uterus repairs) are available. Subjects with diseases requiring a specialty not available in the country are treated outside of Malawi and the

Malawian government pays all the medical bills. Health care is paid for by taxes in Malawi and the Malawian government uses the tax money to provide citizens with hospitals, pays for training medical personnel and buys all necessary equipment and medical drugs. There are private hospitals (expensive and owned by individuals) and mission hospitals (run by missionaries and subsidized by donors). According to this health system structure, most district hospitals are located in townships and smaller hospitals treat the rural people in villages. Catchment areas determine how many patients seen at each hospital. Rural hospitals see tens of thousands of patients while district hospitals treat hundreds of thousands of patients. However depending on where they live, pregnant women may find it easier to go to the district hospital than the community hospital, hence, making a district hospitals into locations that provide care to both rural and urban populations. This is the situation for Lilongwe district hospital, although it is located in the city, it is close to some peri-urban and rural populations who seek obstetric and medical care here.

Ethical clearance / IRB approval

Ethical clearance or IRB approval was obtained from Michigan State University and the National Health Sciences Research Committee (NHSRC) in Malawi. At the hospital level, the Lilongwe District Health Officer (DHO), the overseer of all health services in the district, was contacted for approval to use the facility and their patients and a letter of support for this research was issued. The nurse/midwife in-charge of the maternity unit was contacted to provide her support. She communicated information to the members of staff in the unit about the study and urged them to give support every day of the data collection. We obtained consent (Appendix D) from the pregnant woman or

the mother/husband of the pregnant woman. If she could not write, we obtained a fingerprint of the thumb of her non-dominant hand was as proof of her consent to voluntarily participate in the research. Data and blood sample collection from human subjects only commenced after the pregnant woman gave voluntary consent to participate in the study.

Training of research team

The study team was comprised of the following individuals: the Principle Investigator (PI) and a Study Coordinator from Michigan State University. There were 4 midwives from the Lilongwe District Hospital's Bwaila Maternity Wing and a laboratory technologist from the Central Medical Diagnostics (CMED) Laboratory, the main branch of Lancet laboratories in Malawi. Lancet laboratories have other branches in Ghana, Tanzania and South Africa. Our samples for folate analysis were sent to the Lancet lab in South Africa.

The PI and study coordinator were in charge of and responsible for all research activities both at the hospital and in the laboratory, making sure all supplies and materials were available and correct procedures were followed. The research midwives were responsible for administering the questionnaires to the pregnant women, abstracting data from medical records, collection of blood specimens from pregnant women before delivery, and measuring newborn anthropometrics after birth and giving incentives ($5 worth of baby wrapping cloth) to women after all data and blood samples were collected. The midwives worked in teams of 2 per shift (two for the day shift and two for the night shift) to maximize research participant recruitment. The Laboratory Technologist was responsible for providing specimen bottles and guidance for proper collection of

specimens and handling of samples before analysis. He was also responsible for preparation and shipping of folate blood samples for analysis at Lancet laboratories in South Africa.

The research team received a one-day long training on study protocol in general and sample collection procedures specific for folate. Although the midwives collect blood samples daily in their work, the handling procedure is slightly different for folate samples.

The training emphasized respect and safety of human subjects as required by MSU IRB and the National Health Sciences Research Committee (NHSRC) in Malawi. Midwives were taught how to appropriately obtain consent respectfully during recruitment of human subjects into the study. The study coordinator went through the consent forms approved by the MSU IRB and the survey instrument covering each item on the questionnaire (Appendix F). This was to make sure that the midwives understood the meaning of each question as well as the purpose for asking the question. The training also covered demonstrations of how to take anthropometric measurements of pregnant women and newborns. The midwives were trained to do neonatal anthropometry in pairs; emphasis was on birth length and head circumference because these are not routinely done in the clinic. Weight is the routine measurement for neonates born in Malawi.

The Laboratory Technologist trained midwives on the use of proper specimen bottles for serum and RBC folate. He also demonstrated on how to draw venous blood and put specimens into containers from the syringe without excessive pressure to avoid breaking red blood cells. The Laboratory Technologist trained midwives on how to measure hemoglobin using a Hemocue Hb 201+ (this was a refresher, as midwives

already knew how to do this). Emphasis was made on safety of the midwives during the handling blood and bodily fluids as well as safety of the human subjects. The training session ended with hands on practice of the following experimental procedures: how to measure hemoglobin using Hemocue Hb 201+, how to administer the questionnaire to patients in the hospital and how to take anthropometric measurements on pregnant women and newborn babies.

Study subjects

All pregnant women coming for delivery of their babies at the Bwaila Maternity Wing of Lilongwe District Hospital were asked to participate in the study. Recruitment of subjects followed convenience sampling and included women of all age groups with viable singleton pregnancy or twin gestation delivering at 28 weeks or more. The women were residing in Lilongwe district whether rural peri-urban or urban, which is the catchment area for this hospital. We recruited women with pregnancies of various gestation ages (28 weeks and upwards).

The study excluded dyads where the mother had severe anemia (requiring a blood transfusion), the presence of placenta previa (or history of bleeding during pregnancy due to early partial separation of placenta), delivery involved instrumentation, and those where an infant had brain trauma. The study also excluded mothers of babies whose delivery involved instrumentation, and had brain trauma, mothers with severe anemia (receiving a blood transfusion) and placenta praevia (or history of bleeding during pregnancy due to early partial separation of placenta). Additionally participants must not have had a severe medical condition known to severely affect maternal nutrition status, placenta health or newborn health. In summary, we excluded all obstetrical and medical

emergencies. Babies and mothers that were in intensive care unit (ICU), high dependence unit (HDU) or required constant medical support and monitoring were excluded, so that their medical care was not interrupted.

Data collection

Demographics, antenatal care, and maternal anthropometrics

Using a short questionnaire (Appendix F) basic characteristics of the pregnant woman were collected: age of the mother, gestation age of the pregnancy, gravidity (number of pregnancies), parity (number of deliveries), gestation age, mother's weight, education level of the mother, socio-economic status (occupation of the mother, annual household income, and area of residence. Two types of maternal weight were recorded. The first maternal weight taken on her first prenatal visit (at 3 months gestation for most women, earlier for a few women). The maternal weight at the first prenatal clinic visit was abstracted from the health passport book (taken as proxy to pre-pregnancy weight, since most women don't know their pre-pregnancy weight). The second maternal weight was taken on the day of the survey just before delivery of the baby using Seca weighing scale (Seca, Chino, CA). Most of the maternal characteristics are routinely documented on the labor-monitoring chart, therefore only the parameters that are not available from the chart were asked from each the pregnant woman to avoid duplication.

The questionnaire also contained questions on antenatal care that the pregnant woman was able to get during pregnancy. This included information on iron and folic acid supplement use, which was obtained from self-reports and confirmed by checking medical records (health passport book, a little hand book that contains all medical information and previous treatments of the woman, she carries it everywhere with her).

We used the same questions as are used in the Malawi demographic and health survey. The questions ask if the participant received or bought iron and folic acid supplements in pregnancy; if she took any of the iron and folic acid pills; how many pills was she able to take during pregnancy. Our questionnaire also included questions on the number of antenatal visits the woman attained, at what gestation of the pregnancy (trimester) she started prenatal care, and reasons for starting prenatal care early or late (Kalimbira et al., 2009). Information about vaccinations, malaria prophylaxis and anti-helminthes use during pregnancy was abstracted from the medical record (health passport book). We also obtained medical history about sickness during the current, pregnancy, such anemia (blood transfusions) and if the pregnant woman was on any long-term treatment or chronic diseases such as diabetes, hypertension and asthma/allergies. History of contraceptive use prior to the current pregnancy was obtained. (Abioye et al., 2016; Ahmed et al., 2015)

The midwives performed the anthropometric measurements on pregnant women who participated in the study. Immediately after administering the questionnaire, the subject was asked to step on weighing scale while the midwife recorded her weight. We used a Seca weighing scale validated by UNICEF. This make and model scale is used during the Malawi demographic and health survey data collection. The weight was recorded to the nearest 0.1 g Then the woman's height was measured using a stadiometer. The woman stood on the foot board with her back straight as possible against the measuring surface of stadiometer, making sure the heels, buttocks and shoulders touched the measuring surface. When the midwife was convinced that the woman was in an appropriate position (according to the training provided), s/he would lower down the

headboard to touch the pregnant woman's head. The woman had to remove anything that she was wearing on her head and make braided hair as flat as possible by loosening her hair and pressing the headboard. The pregnant woman had to remove shoes and heavy clothes (subjects were dressed in light clothes) during the anthropometric measurement process. Height was recorded to the nearest 0.1 cm.

Blood specimen collection

We collected blood samples from women before they gave birth to their newborn. Upon obtaining consent and explaining the procedure the pregnant woman was made comfortable in a sitting position or lying on the bed, whichever was preferred by the subject. A tourniquet was applied around the upper left arm and a 70% alcohol swab was used to clean skin the on the site (cubital). After cleaning, a 10 ml syringe with 21 gauge needle was used to withdraw a venous whole blood sample from the cubital region.

Each blood specimen was put in two different sample tubes 5 mls each, one for serum and the other for red blood cell (RBC) folate analysis. The third specimen type was taken using a Microcuvette from the same 10 mls to measure iron status (hemoglobin) right away on the bedside using a Hemocue Hb 201+ (described in detail below). The blood sample for folate was allowed to flow freely into the specimen bottle by vacuum pressure, after piercing the rubber cover with the needle of the syringe.

Sample for RBC folate analysis was put in a 5 ml tube containing anticoagulant (ethylenediaminetetraacetic acid, EDTA). The EDTA kept sample fluid and not clotted so that there was adequate plasma for analysis of hematocrit (needed for calculation of RBC folate later). After putting blood inside the tube it was gently shaken to mix the blood with anticoagulant. The serum folate sample was put in the tube without anticoagulant to

allow cells to separate from serum after clotting. This tube had a gel to separate the red cells and from serum after clotting cells. Samples were kept at room temperature for 2 – 6 hours until they were transferred to laboratory outside the Bwaila maternity wing (research site hospital) at the African Bible College Clinic – Center for Medical diagnostics (CMED). At CMED the RBC folate blood sample were centrifuged and frozen, kept at – 80□. All samples for folate were further transferred to Lancet laboratories in South Africa (Lancet Johannesburg RSA) for analysis.

Hemoglobin and folate analyses

Hemoglobin measurements were done on the bedside using a Hemocue Hb 201+ (HemoCue America, Brea, CA). For women who gave a whole blood sample for folic acid, the same sample was used to prepare a specimen for hemoglobin measurement. After blood sample collection, as described above, we used a microcuvette (HemoCue America, Brea, CA) to get a drop (5 microliters) of whole blood. The microcuvette takes up blood by capillary pressure when it is put close to a drop of blood. To ensure no air bubbles went into the microcuvette, there must be sufficient blood, and when microcuvette touched the drop of blood, we ensured there was no break in the flow of blood going into microcuvette until it was adequately filled. If an obvious bubble was present in the microcuvette or not enough blood got into it, the specimen was discarded and the sample recollected to ensure credible hemoglobin measurements. A properly prepared microcuvette specimen was loaded into the Hemocue Hb 201+. After a minute the reading was noted and recorded to the nearest 0.1 g/dl. After the reading was recorded, the loader was opened and the used Microcuvette removed and disposed of accordingly.

Folic acid analysis was done in South Africa by Lancet laboratories (Lancet, Johannesburg, South Africa) using ARCHITECT assay kits on the ARCHITECT *i* system**.** The ARCHITECT Folate assay is a two-step assay for the quantitative determination of folate in human serum, plasma, and red blood cells (RBC) using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Two pre-treatment steps mediate the release of folate from the endogenous folate binding protein. In pre-treatment step 1, sample and pre-treatment reagent 2 (Dithiothreitol or DTT) are aspirated and dispensed into a reaction vessel (RV). In pre-treatment step 2, an aliquot of sample/pre-treatment reagent 2 mixture is aspirated and dispensed into a second RV. Pre-treatment reagent 1 (potassium hydroxide or KOH) is then added. An aliquot of the pre-treated sample is transferred into a third RV, followed by the addition of folate binding protein (FBP) coated paramagnetic microparticles and assay specific diluent. Folate present in the sample binds to the FBP coated microparticles. After washing, a pteroic acid-acridinium labeled conjugate is added which binds to unoccupied sites on the FBP-coated microparticles. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting Chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of folate in the sample and the RLUs detected by the ARCHITECT *i* optical system. In the Folate RBC assay, an initial manual pre-treatment step converts RBC-bound folate to measurable folate, after which these samples are processed as described above. The results are reported in Nano moles per liter and categorized according to the World Health Organization's protocol and cutoffs. See results section below.

Neonatal anthropometry, APGAR scores, and placenta weight

Any obstetrician or midwife on duty delivered the newborn of each research participant. The study midwives were only collecting data and would assist whenever there was an emergency that needed their service urgently. Delivery of the baby happened a few minutes after completing the questionnaire for most mothers, some even before completing the questionnaire but after giving a blood sample, yet others it took several hours after questionnaire administration to 2 days in extreme cases. The delayed delivery cases were mainly due to false labor. After delivery of the baby the midwife or obstetrician weighs and records the weight and APGAR scores of the neonate. The research midwife abstracted the weight and APGAR scores from the labor chart. (Haeussner et al., 2013). Using a Seca weighing scale the baby was weighed naked and weight was recorded to the nearest 0.1 g immediately after birth. APGAR scores and weight were taken from the labor charts because they are routine measurements done on every newborn in the hospital, unlike birth length and head circumference, which are not routinely done. We ensured minimal interruptions in the care and bonding of the newborn and mother. This was due to Malawi ministry of health guidelines and international standards, which require that babies be given to their moms within 30 minutes after birth.

APGAR is an acronym named after Dr. Virginia Apgar who started assessing neonatal prognosis at birth. Within the first 1 minute of life of the newborn at birth APGAR scores are taken and repeated after 5 minutes (Bondevik et al., 2001; Zhou et al., 1998). The baby is scored out of 10 with an aggregate score below 7 as worrisome and above 7 considered to be better score, as it is associated with greater survival. Newborns rarely score 10 out of 10 in the first minute because newborns almost always appear a

little blue at birth because circulation and respiration is not fully established or may not cry although they might be breathing normally. APGAR stands for the following:

A= Appearance (0-2); whether the baby was pink, pale, or blue at birth.

P=Pulse (0-2); the peripheral pulses whether regular and of good volume, however, in the first minute central pulses including the heart beat are assessed. A pulse between 100 and 180 was considered normal and tachycardia or bradycardia would be indicated by derangements.

G=Grimace (0-2); the muscular response on the face and other parts of the body are assessed as well as reflexes.

A= Activity (0-2); whether newborn is active, moving limbs spontaneously at birth or floppy and flabby.

R=Respirations (0-2); was there a spontaneous cry or not, even if there is no cry, effort is made to observe if newborn is breathing without difficulties, not grunting or showing labored respiration and the lower part of the chest is not severely sunken in.

Each parameter's score ranges from 0 to 2. However the research midwives made sure that all necessary steps were undertaken to score the newborn and that the scores were a true reflection of the overall health of the newborn at birth.

The length of the newborn at birth was measured using an infantometer (Seca, Chino, CA) by two midwives. The newborn was laid on their back against the measuring surface of infantometer. One midwife restrained and positioned the head against the headboard while the other straightened the limbs and took the reading. The birth length was recorded to the nearest 0.1 cm.

The head circumference was taken using a tape (Seca, Chino, CA) measure in centimeters. One midwife did this measurement with newborn on the mother's lap or two midwives when baby was put on examination couch or the neonatal resuscitative table. The tape was placed around the infant's head passing through the sinciput just above the eyebrows (1 cm) and behind the head on the occipital region. When the tape was placed around the head nicely (not too tight or too loose) the reading was taken to the nearest 0.1cm and verified by the assistant. The newborn head was quickly wrapped, to reduce heat loss as babies are prone to cold injury and the newborn was given back to the mother for continued care.

Placenta weight

The placenta was collected at the end of third stage of labor and the cord was cut at least 10cm, long enough for easy clamping to stop the bleeding which kept the surroundings clean and maintained fresh weight. The mother was reminded of the request at the beginning of the study that, apart from measurement of the baby, the placenta would be weighed as well. Using a digital laboratory weighing scale (Ohaus, NJ, USA), we took the weight of the placenta. The weighing scale was put on a flat surface and a kidney dish was put on it first. The weight of the kidney dish weight was tared and the placenta was put in the dish for reading of its weight. When the digits stopped flashing, the reading was recorded to the nearest 0.1 g. After taking the weight, the placenta was discarded according to the routine procedures of the hospital and later incinerated. The disposing of the placenta was done in the presence of the mother to avoid unnecessary rumors and beliefs associated with collecting placentas. This arrangement was well accepted by the participants.

Statistical analyses

All the data analyses were conducted using Microsoft Excel (2016) and SPSS version 24 (SPSS Inc. Armonk, NY). We generated z-scores for weight for height (WHZ), Height for age z-scores (HAZ), weight for age z-scores (WAZ), body mass index z-scores (BMIZ) and head circumference z-scores (HCZ) using the WHO anthro (3.2.2.) software. This software is recommended by the World Health Organization (WHO) for assessing and monitoring of child anthropometry (WHO anthro 3.2.2 January 2011). The software is used to calculate and determine anthropometric measurements on the spot and is also used to individualize data and keep a record for group of children with separate electronic record for each child.

We calculated summary statistics in SPSS using descriptive and frequencies. We also determined associations and relationships between maternal health and compliance to IFA factors, hemoglobin and folate status, placenta weight and birth weight, length, head circumference and APGAR scores using correlations. Finally, we determined the degree of influence of iron and folic acid supplements on hemoglobin and folate and birth outcomes using general linear model (GLM) controlling for covariates. The major dependent variables were WHZ, HAZ, WAZ, HCZ and placenta weight. Covariates were education level, maternal age, number of antenatal clinic visits, gravidity (number of pregnancies), residence, annual household income, and gestation age in weeks.

CHAPTER FOUR: RESULTS

Out of 220 pregnant women who gave consent to participate in the study, seven women delivered twins and were excluded from the final analysis.

Sociodemographics

Sociodemographic and other health information of the pregnant women in the present study are summarized in Table 2. The majority of the pregnant women were between 20-35 y of age (70.9%), whereas 18.8% and 10.5% were younger than 20 y of age and over 35 y of age, respectively. The majority of pregnant women had some education: 51.6% with primary school education, 40.8% completing secondary school (high school), and 1.4% had a college education. The participants were from rural (32.9%), peri-urban (37.6%), and urban (29.6%) areas. The majority (75%) reported living on less than a dollar a day with the lowest reporting an income of about $250 per year. Only 11% of the mothers reported that they engaged in skilled labor (teacher, tailor, mechanic, hotel attendant, etc.) whereas 53.6%, 0.9% and 17.4% were housewives, students and small-scale business women, respectively. Although 10.8% reported being subsistence farmers, most of these women were subsistence farmers producing foods during rainy season, as farming is not considered an occupation for women in Malawi.

Table 2. Sociodemographic information of pregnant women who participated in the study.

Variable	Category	n	Percent
Age (n=213)	< 20y	40	18.8
	20y – 34y	151	70.9
	≥ 35y	22	10.3
Education Level (n=213)	Never been to school	12	5.6
	Junior primary	28	13.1
	Senior primary	82	38.5
	Secondary	87	40.8
	College	4	1.9
Mother's residence (n=213)	Rural	70	32.9
	Peri-urban	80	37.6
	Urban	63	29.6
Annual HH income quartiles (n=154)	USD $249		
	USD $499		
	USD $832		
	USD $13,869		
Occupation (n=213)	None	26	12.2
	House wife	114	53.5
	Skilled labor*	11	5.2
	Small scale business	37	17.4
	Student	2	0.9
	Subsistence farmer	23	10.8

*Skilled labor = Teachers, tailors, mechanic, hotel attendant, hospital attendant, petrol pump attendant, and community worker. Housewives are subsistence farmers during the rainy season.

Prenatal care, iron and folic acid (IFA) supplement use and anemia status

Results of prenatal care, IFA use and anemia status are shown in Table 3. The participants' average number of pregnancies was three and the maximum was nine (Appendix C). Primgravida women (first pregnancy) comprised 30% of all pregnancies and grandmultigravida (more than 4) formed 14%. About 16% had less than 36 weeks of gestation, indicating premature delivery. The number of prenatal care clinic visits ranged from 0 to 6 times with an average of 3 visits during the pregnancy. The majority participants in this study started antenatal care in their second trimester of pregnancy (74.8%) and another 16.9% in their third trimester, with only 8.6% in their first trimester. Participants who reported receiving IFA pills comprised 87.7% of the total and 86.9% reported taking IFA pills during pregnancy. Intake of IFA pills started in the second trimester as well for most pregnant women (78.7%).

The anemia status of the participants is shown in Table 3. About 40% were anemic (below 11 g/dl) with 20% moderate anemia (7-9.9 g/dl) and 1% severe anemia (below 7 g/dl). Recognizing that any degree of anemia in pregnancy increases the risk of morbidity and mortality for both the pregnant woman and her expected newborn, the high rate of anemia is clearly a public health issue. The majority of the participants had folate deficiency with 66.3% having serum folate below 10 nmol/L and 53.5% having red blood cell folate below 400 nmol/L.

Medical history (Table 3) indicates that 21 of the 213 participants (10%) were on antiretrovirals (HIV treatment). However less than 5% had other common diseases such as diabetes, hypertension, asthma and tuberculosis.

Table 3. Maternal characteristics related to prenatal care, iron and folic acid supplement use, anemia status and medical history.

	Variable	Category	n	Percent
Prenatal care	Gestation age at delivery (n=189)	< 32 wk	9	4.8
		33 – 35 wk	20	10.6
		≥ 36 wk	160	84.7
	Maternal height categories (n=185)	≤ 150 cm	48	22.5
		> 150 cm	137	64.3
	Total number of pregnancy (n=213)	1	70	32.9
		2 – 4	113	53.1
		> 4	30	14.1
	1^{ST} prenatal visit (n=200)	First trimester	18	8.6
		Second trimester	157	74.8
		Third trimester	35	16.7
	Total prenatal visits # (n=207)	0	3	1.4
		1 – 2	39	18.8
		3 – 4	140	67.6
		> 4	25	12
	Received IFA supplements (n=212)	Yes	186	87.7
		No	26	12.3
	Took IFA supplements (n=206)	Yes	179	86.9
		No	27	13.1
	Trimester IFA supplements started (n=183)	First Trimester	20	10.9
		Second trimester	144	78.7
		Third trimester	19	10.4
	# IFA supplements taken (205)	0 - 30	77	37.6
		31 - 60	76	37.1
		61 - 120	42	20.5
		121 - 180	10	4.9
IFA intake and anemia status	Maternal Hb* (n=199)	< 7 g/dl	2	0.9
		7 - 9.9 g/dl	44	20.7
		10 - 10.9 g/dl	39	18.3
		≥11 g/dl	114	53.5

Table 3. (cont'd)

IFA and Anemia	Serum folate** (n=101)	< 10 nmol/L	67	66.3
		≥10 nmol/L	34	33.7
	RBC Folate** (n=101)	< 400 nmol/L	54	53.5
		≥ 400 nmol/L	47	46.5
Medical and drug history	Tuberculosis drugs (n=213)	Yes	2	0.9
	Antiretroviral*** (n=212)	Yes	21	9.9
	Steroids (n=210)	Yes	5	2.4
	Anti-diabetics (n=213)	Yes	1	0.5
	Anti-hypertensive (n=212)	Yes	1	0.5
	Other medical drugs (n=212)	Yes	1	0.5

* Maternal hemoglobin was measured prior to delivery; **Folate cutoffs are according to (WHO, 2015) guidelines for assessing folate status in Populations; *** Antiretrovirals are given to all HIV positive women throughout pregnancy.

Compliance to IFA supplements and timing of prenatal care

Tables 4 and 5 show reasons for timing of antenatal care clinic visits and reasons for taking iron and folic acid supplements for a particular duration or amount for those who received IFA pills. Participants who started prenatal care in the first trimester said it was because the nurse had advised them (5) or they got sick in the first trimester (8) and when sought treatment at the hospital they had to start prenatal care immediately. Most pregnant women started prenatal care in the second and third trimesters and the majority (55%) declined to give a reason for their delay. Another leading cause for delay was waiting for pregnancy to grow followed by long distance to the hospital and lack transport.

Compliance to IFA intake was a challenge (Table 11). In the category of those who took supplements for only one month (0-30 pills) the leading reason was a shortage of pills at the hospitals. For pregnant women who took IFA pills for at least 2 months (31 – 60 pills) negligence (65.7%) and nausea (25.4%) were the leading reasons for none compliance to take of IFA pills. Overall negligence is the leading reason (38.6%) followed by a shortage of pills at the hospitals (29.9%), nausea (20.7%) and starting late (7.6%). There was a 1.6% group of participants who reported discarding the pills after receiving without taking any pills.

Table 4. Timing and reasons of the first prenatal care visits**.**

	First trimester		Second trimester		Third trimester		Total	
Reasons	n	Percent	n	Percent	n	Percent	n	Percent
Advised by nurse	5	27.8	0	0	0	0	5	2.4
Didn't know when to start ANC	0	0	4	2.5	0	0	4	1.9
Distance, no transport money	1	5.6	11	7	3	8.6	15	7.1
Friends told her to wait	0	0	5	3.2	1	2.9	6	2.9
No doctor at the hospital	0	0	0	0	2	5.7	2	1
Not answered*	2	11.1	96	61.1	17	48.6	115	54.8
She did not know she was pregnant	0	0	7	4.5	2	5.7	9	4.3
Sick	8	44.4	4	2.5	2	5.7	14	6.7
Waited for husband to authorize	0	0	3	1.9	1	2.9	4	1.9
Waited for pregnancy to grow	0	0	24	15.3	7	20	31	14.8
Went to traditional birth attendant first	2	11.1	3	1.9	0	0	5	2.4
Total	18	100	157	100	35	100	210	100

*Not answered = women who opted out to not give a reason, mostly because they started late and did not want to be rebuked by medical personnel.

Table 5. Reasons for taking certain number of IFA supplement pills during pregnancy.

IFA pills taken		Reasons						
		Bought some	Nausea	Negligence	Started late	Stock outs	Discarded IFA	Total
0 -30	n	0	9	8	4	44	3	68
	Percent	0	13.2	11.8	5.9	64.7	4.4	100
31 - 60	n	0	17	44	3	3	0	67
	Percent	0	25.4	65.7	4.5	4.5	0	100
61 - 120	n	1	9	16	6	7	0	39
	Percent	2.6	23.1	41	15.4	17.9	0.0	100
121 - 180	n	2	3	3	1	1	0	10
	Percent	20	30	30	10	10	0	100
Total	n	3	38	71	14	55	3	184
	Percent	1.6	20.7	38.6	7.60	29.9	1.6	100

Note: IFA is taken one pill daily hence one pill is equivalent to one day of intake or 180 pills is equal to 6 months intake. (WHO recommends a minimum of 180 pills during pregnancy).

Newborn characteristics

Neonatal characteristics (Table 6) show that boy/girl newborn ratio was 1.09 or 52.1% and 47.9%, respectively. LBW newborns comprised 16% of the total deliveries in this study. The mean, median and range of birth length were 47.8 cm, 48.4 cm, and 32.5 -57.0 cm, respectively. The mean, median and range of head circumference at birth were 34 cm, 35 cm, and 25 - 40 cm, respectively. Over 50% of newborns had low APGAR scores (below 6) in the first minute of life but improved significantly after 5 minutes. After 5 minutes 90% had good APGAR scores (above 9). However, 10% had poor scores even after 5 minutes, which usually predicts a poor quality of life of newborns as they may have neurological complications.

Z-scores for newborn anthropometry (Table 6) indicate that 20.0% of neonates had height for age z-scores (HAZ) below -2, 14.55% had weight for age z-scores (WAZ) below -2, and 28.71% had a weight for height z-scores (WHZ) lower than -2. This shows that a significant number of newborns are stunted and underweight (low birth weight) at birth, indicating poor health during pregnancy.

Table 6. Newborn anthropometry and health characteristics at birth.

Variable	Category	n	Percentage
Sex (n=213)	Male	112	52.1
	Female	101	47.9
Birth weight (n=213)	<1500 g	2	0.9
	1500 g – 2499 g	32	15
	2500 g – 3499 g	144	67.6
	>3500 g	35	16.4
APGAR scores at 1 min (n=209)	< 6	9	4.4
	6 – 8	98	46.9
	9 – 10	102	48.8
APGAR scores at 5 min (n=209)	< 7	7	3.4
	7 – 9	14	6.6
	10	188	90
Z-scores			
Child's HAZ (n=213)	<-2	46	22.0
	≥-2	163	78.0
Child's WAZ (n=213)	<-2	31	14.6
	≥-2	182	85.5
Child's WHZ (n=213)	<-2	60	28.7
	≥-2	149	71.3
Child's HCZ (n=213)	<-2	16	8.1
	≥-2	197	91.9
Prevalence of LBW (n=213)		34	16
	Male	15	16.1
	Female	19	15.8

APGAR score is out of 10. HAZ=height-for-age z-scores, WAZ=weight-for-age z-scores, WHZ=weight-for-height z-scores and HCZ =head circumference-for-age z-scores. LBW = Low birth weight < 2500g.

Hemoglobin, serum and RBC folate status by IFA taken

IFA pills taken by the women during pregnancy and the mean maternal hemoglobin, serum folate and RBC folate concentration before delivery are shown in Figures 5, 6, and 7, whereas scatter plots of the same are shown in Figure 8.

Maternal hemoglobin levels are positively correlated with the number of IFA pills taken. Hemoglobin levels reached from 10.7 g/dl, 11.3 g/dl to 11.7 g/dl in response to the reported number of IFA pill taken from <45, 45-89, to ≥ 90, respectively. The correlation between IFA pills taken and maternal hemoglobin was positive with a Spearman's correlation coefficient of r=0.1846, P=0.0102 (Table 7).

The maternal serum folate concentrations of pregnant women before delivery was positively associated with the reported IFA pills taken during the pregnancy (Figure 6). The IFA supplement intake of <45 to ≥90 pills corresponded to a mean serum folate status of 7.9 nmol/L and 10.2 nmol/L, (P=0.175) respectively. This positive association between IFA pills taken and mean serum folate status was marginally significant, r = 0.1839, P = 0.0699 (Table 7). The scatter plot in Figure 8 shows that the majority of pregnant women took about 60 pills and only 4 women took between 121 – 180 pills.

The relationship between IFA pills taken and maternal RBC folate concentration before delivery are shown in Figure 7. The mean RBC folate status increases steadily from 463.1 nmol/L and to 498.8 nmol/L for the IFA pills between <45, and ≥90 (P = 0.949) respectively. A spearman's correlation coefficient is small (r = -0.0329) and was found statistically insignificant at P= 0.7481 (Table 7). The scatter plot in Figure 8 shows the same poor association between the IFA pills taken and RBC folate levels, however it is positive.

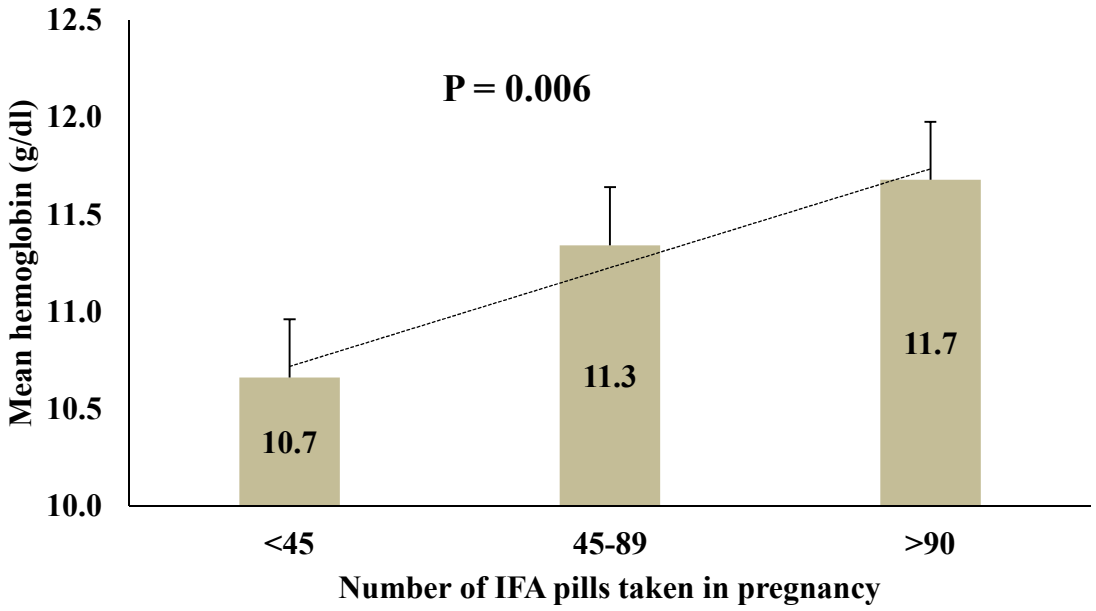

Figure 5. Mean maternal hemoglobin levels by number of IFA pills taken during pregnancy.

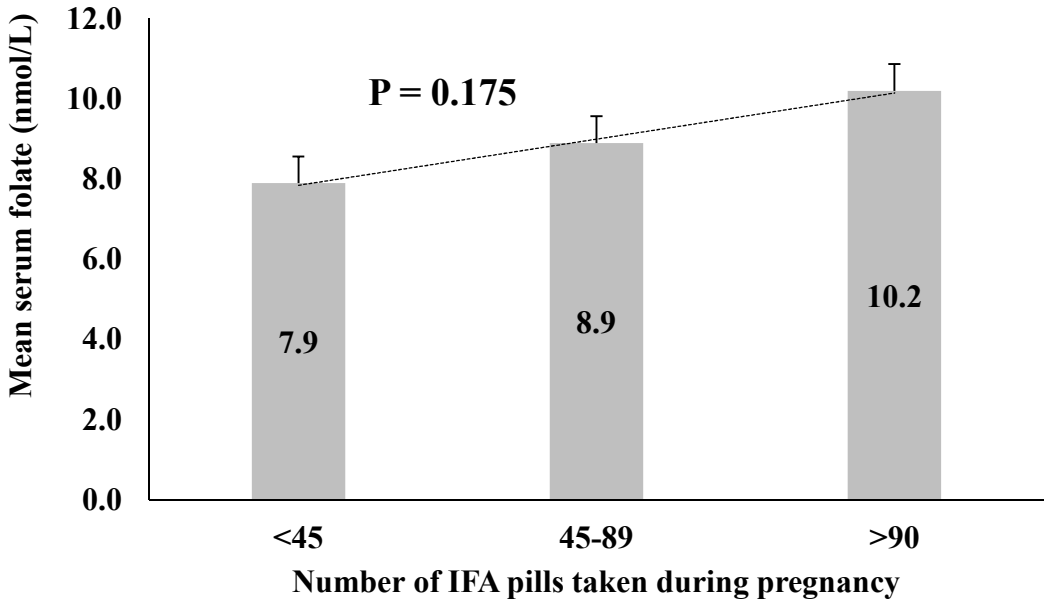

Figure 6. Mean maternal serum folate levels by number of IFA supplement pills taken during pregnancy.

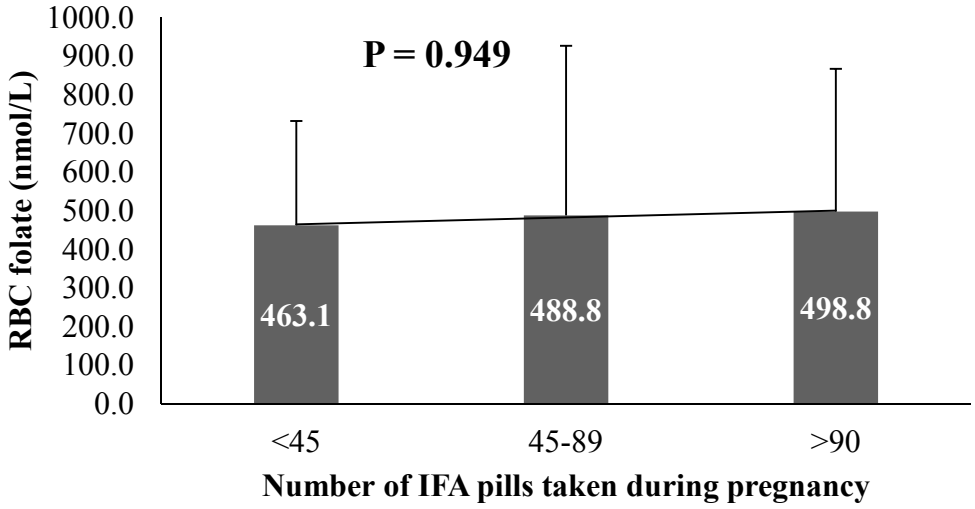

Figure 7. Mean maternal RBC folate levels by number of IFA supplement pills taken during pregnancy.

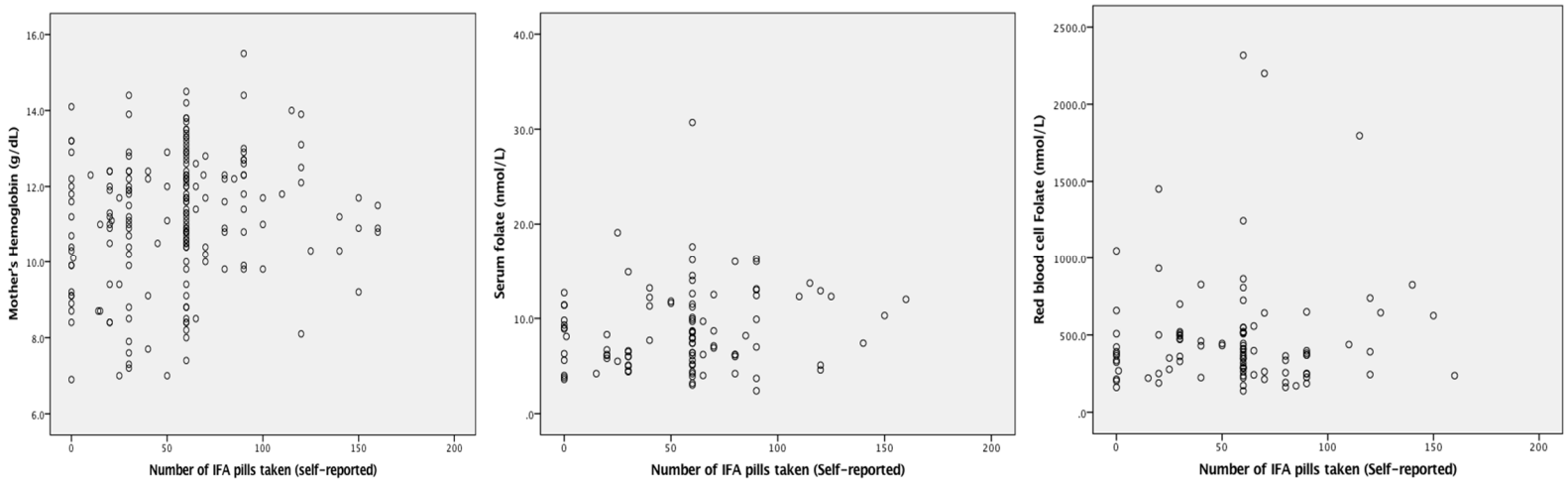

Figure 8. Scatter plots of hemoglobin, serum folate and RBC folate levels by IFA pills taken.

Table 7. Spearman's correlation matrix of maternal and newborn characteristics and biomarkers.

		# of IFA	Mother WT - 1	Mother WT - 2	Mother - HT	*Hb-2	Serum folate	RBC folate	Placenta WT	Birth WT	Birth Length	Head Circ
# of IFA tablets	*Corr coeff*	1.0000	0.0599	0.0042	0.1759	0.1846	0.1839	-0.0329	0.0087	0.1761	0.0247	0.0442
	P-value		0.4261	0.9610	**0.0189**	**0.0102**	**0.0699**	0.7481	0.9118	**0.0113**	0.7272	0.5308
	n	206	179	136	178	193	98	98	165	206	202	203
Mother WT – 1	*Corr coeff*			0.8910	0.1794	0.1025	0.0697	0.0266	0.3325	0.2758	0.2420	0.2521
	P-value			**<.0001**	**0.0215**	0.1798	0.5261	0.8089	**<.0001**	**0.0001**	**0.0010**	**0.0006**
	n			129	164	173	85	85	148	185	181	182
Mother WT – 2	*Corr coeff*				0.2121	0.1489	0.0537	-0.0287	0.3550	0.3204	0.2476	0.2194
	P-value				**0.0150**	0.0836	0.6542	0.8107	**<.0001**	**0.0001**	**0.0034**	**0.0094**
	n				131	136	72	72	116	141	138	139
Mother height	*Corr coeff*					0.1819	-0.1529	-0.0432	0.3389	0.3975	0.3511	0.2121
	P-value					**0.0163**	0.1413	0.6791	**<.0001**	**<.0001**	**<.0001**	**0.0039**
	n					174	94	94	150	185	182	183
Hemoglobin	*Corr coeff*						0.3905	-0.0167	0.0172	0.2346	0.2002	0.1582
	P-value						**<.0001**	0.8688	0.8258	**0.0009**	**0.0049**	**0.0268**
	n						100	100	166	199	196	196
Serum folate	*Corr coeff*							0.2502	-0.0467	0.0622	0.051	0.1522
	P-value							**0.0116**	0.6692	0.5366	0.6183	0.1346
	n							101	86	101	98	98

Table 7. (cont'd)

RBC folate	*Corr coeff*	-0.1493	-0.075	0.0146	-0.0231
	P-value	0.17	0.456	0.8865	0.8213
	n	86	101	98	98
Placenta WT	*Corr coeff*		0.5727	0.4161	0.3709
	P-value		**<.0001**	**<.0001**	**<.0001**
	n		171	170	171
Birth WT	*Corr coeff*			0.6428	0.613
	P-value			**<.0001**	**<.0001**
	n			209	210
Birth Length	*Corr coeff*				0.5457
	P-value				**<.0001**
	n				209
Head Circ	*Corr coeff*				1
	P-value				
	n				210

Circ, circumference; Hb, hemoglobin; HT, height; WT, weight.
*Hb -2 (Hemoglobin), serum and RBC folate were measured prior to delivery.
Mother WT-1 = Mother's weight at first prenatal visit, which varied among women
Mother WT-2 = Mother's weight on the day of survey before delivery.

Hemoglobin, folate status and newborn anthropometry by IFA pills taken

The number of IFA pills taken during pregnancy and its association with biomarkers (hemoglobin, serum and RBC folate and newborn anthropometry (birth weight, birth length, head circumference and z-scores of newborn anthropometric measurements) are shown in Tables 8-13 and in Figure 9.

Table 8 shows demographic information and prenatal care characteristics of mothers by number of IFA supplement intake. Supplement intake did not differ according to demographic and other prenatal care characteristics like age, residence, education level, number of pregnancies, timing of starting prenatal care of supplements. However, there were significant differences according to gestation age and total number of prenatal visits achieved by the pregnant woman.

Hemoglobin status (10.7 ± 1.6 g/dl, 11.3 ± 1.8 g/dl and 11.7 ± 1.6 g/dl, P= 0.006, Table 9) was positively associated with the number of IFA pills taken, 45, 45-89 and above 90 pills, respectively . The associations between IFA pills taken during pregnancy and mean serum folate (7.9 ± 3.6 nmol/L, 8.9 ± 4.9 nmol/L and 10.2 ± 4.1 nmol/L, respectively, P = 0.175) and RBC folate status immediately before delivery (463.1 ± 270 nmol/L, 488.8 ± 38.5 nmol/L and 488.8 ± 469.5 nmol/L, respectively, P = 0.949) were not statistically significant.

Table 10 shows demographics that mothers' age, residence (rural, peri-urban, and urban), gravidity (number of pregnancies), parity, number of prenatal care clinic visits, and trimester of starting intake of IFA pills did not differ between mothers who gave birth to normal weight versus LBW newborns. Factors that differentiated the two groups

were education level (P=0.016), trimester of first prenatal clinic visit (P=0.003) and gestation age (P= <0.001) Table 9.

Table 10 shows the comparison of mothers' and newborn variables between normal weight and LBW newborns. Mean hemoglobin status between mothers of normal and LBW newborns were 11.4 ± 1.6 g/dl and 9.4 ± 1.6 g/dl P = <0.001, respectively. However, the difference in serum and RBC folate between mothers of normal and LBW newborns were not significant, 9.1 ± 4.4 nmol/L 7.5 ± 3.6 nmol/L, respectively (P = 0.230). The same was observed with RBC folate status between normal and LBW 494.6 ± 413 nmol/L and 489.8 181.1 nmol/L respectively, (P = 0.942).

Table 8. Sociodemographic and prenatal care characteristics by number of IFA supplements taken during pregnancy.

	# of IFA supplements taken during pregnancy (tablets)						
	<45 (n=81)		45–89 (n=89)		≥90 (n=36)		
	n	%	n	%	n	%	P-value
Age							
<19y	13	16.1	10	11.2	1	2.8	0.210
19-29y	53	65.4	59	66.3	25	69.4	
30-35y	7	8.6	10	11.2	8	22.2	
≥36y	8	9.9	10	11.2	2	5.6	
Missing	*7*						
Residence							
Rural	31	38.3	27	30.3	10	27.8	0.631
Peri-urban	33	40.7	31	34.8	14	38.9	
Urban	17	21.0	31	34.8	12	33.3	
Missing	*7*						
Total number of pregnancies							
1	25	31.3	32	36.0	12	33.3	0.364
2	22	27.5	17	19.1	5	13.9	
3	11	13.8	18	20.2	5	13.9	
4	13	16.3	11	12.4	6	16.7	
≥5	9	11.3	11	12.4	8	22.2	
Missing	*8*						
Total number of births							
0	2 6	32.1	31	35.2	12	33.3	0.225
1	24	29.6	17	19.3	5	13.9	
2	11	13.6	19	21.6	5	13.9	
3	11	13.6	10	11.4	6	16.7	
≥4	9	11.1	11	12.5	8	22.2	
Missing	*8*						

Table 8. (cont'd)

Gestation weeks							
<37	32	46.4	18	22.0	7	21.9	**0.003**
≥37	37	53.6	64	78.1	25	78.1	
Missing	*30*						
Education							
Never Been to School	7	8.6	5	5.6	0	0.0	0.371
Junior Primary	13	16.1	10	11.2	4	11.1	
Secondary Primary	25	30.9	35	39.3	19	52.8	
Secondary or more	36	44.4	39	43.8	13	36.1	
Missing	*7*						
First prenatal visit							
First trimester	8	10.1	8	9.0	1	2.8	0.472
Second trimester	51	64.6	74	83.2	29	80.6	
Third trimester	20	25.3	7	7.9	6	16.7	
Missing	*9*						
# of prenatal visits							
0–2	28	35.4	7	8.0	6	17.1	**0.006**
3	23	29.1	36	40.9	15	42.9	
4	22	27.9	36	40.9	5	14.3	
5–6	6	7.6	9	10.2	9	25.7	
Missing	*11*						
IFA supplements started in							
First trimester	10	17.0	8	9.1	2	5.7	0.350
Second trimester	37	62.7	73	83.0	33	94.3	
Third trimester	12	20.3	7	8.0	0	0.0	
Missing	*31*						

Total number of births = parity, total number of pregnancies = gravidity

Table 9. Mother's and newborn's variables by number of IFA supplements taken during pregnancy.

	Number of IFA supplements taken during pregnancy (pills)									
	<45 (n=81)			45–89 (n=89)			≥90 (n=36)			
	n	Mean	SD	n	Mean	SD	n	Mean	SD	P-value
Mother's variables										
Age (years)	81	24.5	6.5	89	25.6	6.5	36	25.6	5.8	0.512
Body weight- 1(kg)*	70	58.1	9.3	77	60.1	10.6	32	59.1	8.3	0.459
Body weight- 2(kg)	44	65.3	9.6	63	65.3	11.4	29	66.0	12.3	0.961
Hb-1(g/dl)**	14	8.5	1.5	11	9.6	1.5	4	10.9	1.6	**0.029**
Hb-2 (g/dl)	72	10.7	1.8	87	11.3	1.6	34	11.7	1.6	**0.006**
Serum folate (nmol/L)	34	7.9	3.6	45	8.9	4.9	19	10.2	4.1	0.175
RBC folate (nmol/L)	34	463.1	270.0	45	488.8	438.5	19	488.8	369.5	0.949
Placenta weight (g)	63	560.7	147.1	72	573.6	126.5	30	580.8	147.7	0.774
Baby's variables										
Birth weight (g)	81	2844.2	524.3	89	2903.9	461.4	36	3188.9	452.2	**0.002**
Birth length (cm, lying down)	79	47.7	3.4	87	47.9	3.5	36	48.1	3.9	0.852
Head circumference (cm)	80	34.2	2.5	87	34.5	1.8	36	34.9	1.8	0.294
APGAR score of baby at 1 min	81	8.1	1.5	85	8.0	1.5	36	8.2	1.1	0.838
APGAR score of baby at 5 min	81	9.8	0.9	85	9.7	1.3	36	9.6	1.6	0.785
	n	%		n	%		n	%		
Prevalence of LBW										
LBW	17	20.1		12	13.5		2	5.6		**0.027**
Normal weight	64	79.0		77	86.5		34	94.4		
Missing	*7*									

*Body weight- 1 was measured at first prenatal clinic visit that vary in trimesters among women.
**Hb-1 (hemoglobin) was measured at the first prenatal clinic visit that varies in trimesters among women.
Hb-2 (hemoglobin), serum and RBC folate samples were taken prior to delivery.

Table 10. Differences in characteristics between mothers of normal weight and LBW newborns.

	Birth weight of newborn				
	Normal weight (n=179)		Low birth weight (n=34)		
	n	%	n	%	p-value
Age					
<19y	18	10.1	7	20.6	0.235
19-29y	120	67.0	20	58.8	
30-35y	23	12.9	5	14.7	
≥36y	18	10.1	2	5.9	
Residence					
Rural	58	32.4	12	35.3	0.722
Peri-urban	68	38.0	12	35.3	
Urban	53	29.6	10	29.4	
Gravidity					
1	54	30.3	16	47.1	0.081
2	40	22.5	7	20.6	
3	31	17.4	4	11.8	
4	27	15.2	4	11.8	
≥5	26	14.6	3	8.8	
Missing	*1*				
Parity					
0	55	30.7	15	45.5	0.146
1	42	23.5	7	21.2	
2	32	17.9	4	12.1	
3	25	14.0	4	12.1	
≥4	25	14.0	3	9.1	
Missing	*1*				
Gestation age (weeks)					
<37	38	24.1	22	71.0	**<0.001**
≥37	120	76.0	9	29.0	
Missing	*24*				
Education level					
Never been to school	8	4.5	4	11.8	**0.016**
Junior primary	21	11.7	7	20.6	
Senior primary	69	38.6	13	38.2	
≥ Secondary	81	45.3	10	29.4	

Table 10. (cont'd)

Trimester of first prenatal visit					
First	11	6.2	7	21.9	**0.003**
Second	134	75.3	23	71.9	
Third	33	18.5	2	6.3	
Missing	*3*				
# of prenatal clinic visits					
0–2	33	19.0	9	27.3	0.267
3	64	36.8	12	36.4	
4	55	31.6	9	27.3	
5–6	22	12.6	3	9.1	
Missing	*6*				
Trimester IFA pill intake started					
First	13	8.4	7	25.0	0.088
Second	126	81.3	18	64.3	
Third	16	10.3	3	10.7	
Missing	*30*				

Each variable was run after controlling the rest as covariates.

Table 11. Differences in characteristics of mothers who delivered normal vs. LBW babies.

	Birth weight						
	Normal weight (n=179)			Low birth weight (n=34)			
	n	Mean	SD	n	Mean	SD	P-value
Mother's variables							
Age (years)	179	25.6	6.2	34	23.4	6.8	0.064
# of IFA pills taken during pregnancy	175	56	37	31	40	28	**0.021**
Body weight-1 (kg)	159	60.1	9.9	26	54.6	8.4	**0.008**
Body weight-2 (kg)	125	66.4	11.2	16	59.5	8.6	**0.019**
Maternal Height	154	155.5	5.6	31	151.0	6.4	**<0.001**
Hb-1 (g/dl))	23	9.7	1.4	6	7.6	1.7	**0.005**
Hb-2 (g/dl)	172	11.4	1.6	27	9.4	1.6	**<0.001**
Serum folate (nmol/L)	87	9.1	4.4	14	7.5	3.6	0.230
RBC folate (nmol/L)	87	494.6	413.0	14	489.8	181.1	0.942
Placenta weight (g)	145	591.4	129.2	26	455.6	153.9	**<0.001**
Baby's variables							
Birth weight (g)	179	3071.8	388.0	34	2115.6	310.0	**<0.001**
Birth length (cm, recumbent)	175	48.6	3.1	34	43.6	3.3	**<0.001**
Head circumference (cm)	176	34.9	1.5	34	31.8	2.7	**<0.001**
APGAR score 1 min	175	8.1	1.4	34	8.1	1.3	0.887
APGAR score 5 min	175	9.7	1.3	34	9.8	0.9	0.653

Body weight-1: First prenatal visit weight, data were extracted from health passport book.
Body weight-2: Measured prior to delivery.
Hb-1: Hemoglobin status at first prenatal clinic visit, data was extracted from health passport book.
Hb-2: Hemoglobin level measured prior to delivery.

Risk of LBW by IFA pills taken during pregnancy

The results of multivariate odds ratio for LBW and IFA pills intake and other variables are shown in Table 12. The odds ratio was not significant for age categories, residence (rural, peri-urban, and urban), gravidity, parity, education levels, number of prenatal clinic visits, and trimester of starting IFA pill intake. IFA supplement intake during pregnancy reduces the risk of delivering a LBW newborn. As shown in Table 12, women who took IFA pills greater than 60 lower risk (OR = 0.11, CI: 0.02, 0.056, P = 0.008) compared with the reference group who took ≤ 30 pills (OR = 1.00). The risk of LBW was also lower for women who took ≥ 90 pills (OR = 0.19, CI: 0.04, 1.01, P = 0.051). Risk of LBW was lower for a gestation age ≥ 37 weeks (OR = 0.15, CI: 0.06, 0.40, P = <0.001) at their first prenatal care visit (OR = 0.06, CI: 0.01, 0.41, P = 0.005). This might suggest new cutoffs for prenatal IFA supplements in future. As demographic information seems to contribute very little to factors compelling women to adhere (compliance) to prenatal supplements intake. Gestation age lowered the risk of LBW, however, somewhat unusual was the starting of prenatal care, as those who started early seemed to have higher risk than those who were late.

The relationship of number of IFA pills consumed during pregnancy and mean birth weight of the newborn is shown in Figure 9. For pregnant women who took no pills the mean birth weight 2,887 g and dropped to 2,816.3 g for those who took 1–30 pills. From 1-30 pills of IFA any increase in IFA number results in increased mean birth weight of the newborn, 31-60, 61-120 and 121-180 pills has corresponding increase in mean birth weight of 2,909.9 g, 3,061.9, and 3,210 g respectively.

Table 12. Multivariate odds ratios and 95% confidence intervals of LBW by its risk factors

		Low birth weight			
		OR	95% CI		P-value
Age (n=204)					
	<19y	1.00			
	19-29y	0.98	0.25	3.92	0.981
	30-35y	3.00	0.35	25.68	0.316
	≥36y	1.38	0.09	20.27	0.814
Residence (n=204)					
	Rural	1.00			
	Peri-urban	1.17	0.43	3.16	0.760
	Urban	0.86	0.30	2.49	0.778
Gravidity (n=204)					
	1	1.00			
	2	0.58	0.18	1.88	0.360
	3	0.30	0.07	1.30	0.108
	4	0.34	0.06	2.08	0.244
	≥5	0.22	0.03	2.02	0.182
Parity (n=204)					
	0	1.00			
	1	0.55	0.17	1.84	0.333
	2	0.30	0.07	1.31	0.110
	3	0.41	0.07	2.56	0.341
	≥4	0.25	0.03	2.18	0.208
Gestation weeks (n=182)					
	<37	1.00			
	≥37	0.15	0.06	0.40	**<0.001**
Education (n=204)					
	Never Been to School	1.00			
	Junior Primary	1.04	0.17	6.37	0.965
	Secondary Primary	0.42	0.08	2.23	0.306
	Secondary or more	0.38	0.07	2.07	0.262
First ANC visit trimester (n=204)					
	First	1.00			
	Second	0.20	0.06	0.72	**0.013**
	Third	0.06	0.01	0.41	**0.005**
# of prenatal clinic visits (n=204)					
	0–2	1.00			
	3	0.60	0.19	1.92	0.388
	4	0.39	0.11	1.36	0.139
	5–6	0.32	0.07	1.53	0.153

Table 12. (cont'd)

Trimester IFA supplements started (n=177)				
First	1.00			
Second	0.29	0.06	1.35	0.115
Third	0.43	0.06	3.05	0.399
# of IFA pills taken in pregnancy (n=199)				
≤30	1.00			
31–60	0.55	0.22	1.42	0.220
>60	0.11	0.02	0.56	**0.008**
# of IFA pills taken in pregnancy (n=199)				
<45	1.00			
45–89	0.48	0.19	1.25	0.132
≥90	0.19	0.04	1.01	**0.051**

*****Adjusted for maternal age, education, total number of pregnancies, first prenatal visit trimester, and total number of prenatal visits.

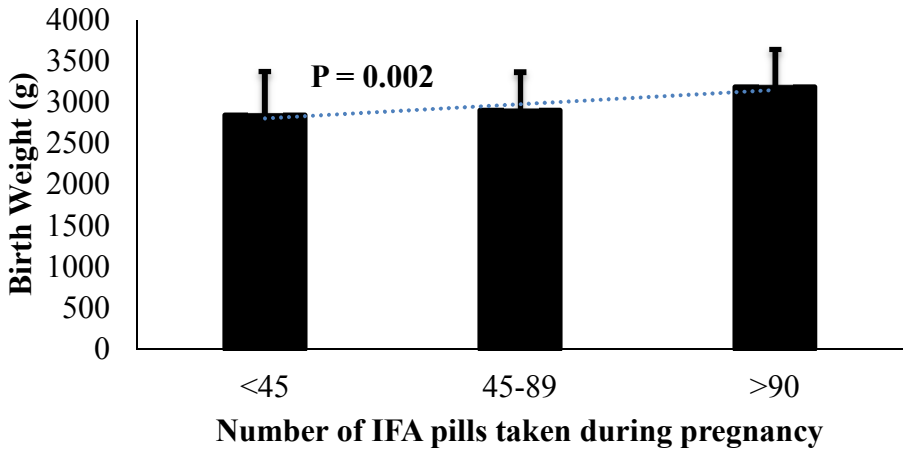

Figure 9. Mean birth weight by IFA pills taken during pregnancy.

Using mother's Hb, serum folate, and RBC as independent variables and the newborn anthropometric outcomes as dependent variables, the regression coefficients of number of IFA pills, mother's Hb, serum folate, and RBC folate for predicting newborn anthropometry vs. the number of IFA pills are shown in Table 13. After controlling for covariates (age, maternal height, gravidity, gestation, ANC visits, education, residence, and annual household income), mothers' hemoglobin levels were positively associated with WHZ and WAZ scores. An increase in mothers' Hb of 1 g/dl corresponded to an increase in the WHZ score of ($\beta = 0.232$, $P = 0.045$), when all covariates are constant. Also an increase in mothers' Hb of 1 g/dl corresponds to an increase in WAZ score of $\beta = 0.130$, $P = 0.021$. Higher serum folate levels were also significantly related to increased WAZ and HCZ scores of $\beta = 0.057$, $P = 0.043$ and $\beta = 0.119$, $P = 0.023$ respectively. However, mothers intake of IFA pills during pregnancy and RBC folate status of pregnant woman were not significantly associated with newborn anthropometric outcomes.

Table 13. Associations of mother's IFA pills taken and biomarkers with newborn anthropometry

Models	WHZ - scores		HAZ - scores		WAZ - scores		HCZ - scores	
	β	*P*-value	*β*	*P*-value	*β*	*P*-value	*β*	*P*-value
	(*n*=91)		(*n*=113)		(*n*=115)		(*n*=113)	
# of IFA pills	0.006	0.255	-0.001	0.889	0.003	0.219	-0.002	0.662
	(*n*=91)		(*n*=113)		(*n*=116)		(*n*=114)	
Mother's Hb	**0.232**	**0.045**	0.007	0.945	**0.130**	**0.021**	0.129	0.202
	(*n*=43)		(*n*=59)		(*n*=61)		(*n*=59)	
Serum folate	0.075	0.174	0.062	0.247	**0.057**	**0.043**	**0.119**	**0.023**
	(*n*=43)		(*n*=59)		(*n*=61)		(*n*=59)	
RBC folate	0.001	0.097	<0.001	0.910	<0.001	0.944	<0.001	0.747

All models were adjusted for maternal age, maternal height, gravidity, # of prenatal visits, education, residence, and annual household income. Each model also controlled for the other three variables considered in these four models.

CHAPTER FIVE: DISCUSSION

Prenatal supplements containing iron and folic acid are a short-term solution to the anemia problem in pregnant women in developing countries with high burden of anemia and food security challenges. The policy in Malawi supports that all pregnant women receive free prenatal IFA supplements at the prenatal clinics with every visit, throughout pregnancy. However, there have been challenges with compliance to these free supplements, risking anemia during pregnancy and poor birth out comes among this population. This research was conducted to evaluate the effect of non-compliance to taking prenatal supplements during pregnancy on prevalence of LBW in Malawi. Compliance to prenatal supplements itself depends on several other factors. Some of the factors explored in this study are; parity, availability of supplements at the hospital, timing of the first prenatal care clinic visit, level of education, age of pregnant woman, economic status, gestation age due to changes in early pregnancy, residence or distance to the hospital and number of pregnancies (gravidity).

We observed that the pregnant women involved in our study were mainly young women who were younger than 35 y (89.7%) with almost 20% being teenage mothers (under 20 y). This is in agreement with national data that show that Malawi is a young country compromised of a youthful population making over 50% of the national population, just like the rest of Sub-Saharan Africa. Another reason is that in Malawi life expectancy is low (below 60 y) because of health care and economic challenges (NSO-ICF, 2017).

Education level of the majority of the pregnant women fairly good, as only 5.6% had never been to school, with over 40% having attended primary school (middle school)

and another 40% attended secondary school (high school). This could be because a good number of pregnant women's resided in urban and peri-urban areas (over 60%) with the remainder coming reporting their residence as rural. Those living in the urban and peri-urban areas are likely to have access to education. Contrary to our expectation of such a literate population, we observed that their household income per annum was very low, with the majority living with less than a dollar a day. Although a good number was literate, stayed in urban and peri-urban areas they did not do any skilled labor. About 60% were housewives and those that could not mention their occupation, with only 11% and 17% reporting that they were doing skilled labor and small scale businesses respectively (Table 2).

Gestation length is highly associated with birth weight, i.e., the longer a woman gestates the higher the chances of allowing the fetus to grow and reducing the risk of low birth weight, assuming good nutrition and less infections of the pregnant woman. In our study we observed that prevalence of premature birth was about 15%, slightly lower than it was observed by other researchers recently in Malawi (Chagomerana et al., 2017), however, our findings are in line with national prevalence of premature births of 15% (NSO-ICF, 2017).

A significant number of women (22.5%) were shorter in height (≤150 cm). Short maternal height (≤150 cm) is associated with LBW (Raghunath et al., 2016). Average number of pregnancies (Appendix C) per woman was 3 with a minimum of 0 and maximum of 9. About 30% of the pregnant women were Primgravidae (first pregnancy), and 53% multigravidae (2-4 pregnancies) and 14% grandmultigravidae (more than 4 pregnancies). Although the fertility rate has dropped to 4.4 in 2016 from 6.3 the past

decade, the pregnant women in this study had more children than the national average (NSO-ICF, 2017). Most pregnant women started attending prenatal care in the second trimester of pregnancy (74.8%) and also start to take IFA in the same trimester. Number of visits ranged from 0 to 6 and the majority attended 3 to 4 visits (67%). Prenatal attendance is critical to health care of pregnant women in Malawi because on top of the general medical care, prenatal clinics serve as outlets for prenatal supplements to all pregnant women. The Malawian government through the Ministry of Health, distributes prenatal supplements (iron and folic acid) to be given for free to all pregnant women regardless of hematological status in all prenatal clinics. Therefore a woman can only have access to IFA supplements if she goes to the prenatal clinics, hence attendance is very important during pregnancy. The supplements are available in private pharmacies for those with financial power to access but the majority cannot afford among other priorities in the households. Besides it has been shown that those pregnant women who report buying their supplements and not receiving them from prenatal clinics have poor compliance, perhaps due to lack of counseling on side effects and other medical advice (Sununtnasuk et al., 2016).

Also to achieve WHO recommendation of taking a minimum of 180 pills of IFA, 6 months intake (WHO, 2012) throughout pregnancy requires starting of prenatal attendance early. Our research shows that compliance to prenatal supplements is likely to be a challenge as the majority of pregnant women, 74.5% (Table 3) started prenatal care in the second trimester of pregnancy and another 17% starting prenatal care in the third trimester. This renders a lot of pregnant women unable to take the required number of pills of supplements aside compliance factors.

In this study, 88% pregnant women reported receiving IFA pills at some point of their pregnancy and 87% reported taking at least IFA pill at some point in pregnancy which is in agreement with other published data. This seems to indicate that access to IFA pills may be improving but compliance to take them still depends on other factors (Sununtnasuk et al., 2016). Table 3 shows that although the majority reported receiving and taking IFA pills during pregnancy, only 4.9% (n=10) took above 121 IFA pills and none achieved the 180 pills minimum recommendation by WHO. This calls for a deep examination of the biggest anemia prevention program in the country, the prenatal supplementation program. In this study we found that 40% of pregnant women had some anemia (Hb <11g/dl), with 19% having mild anemia, 20% moderately severe anemia, and 1% very severe anemia requiring transfusion according to the guidelines of anemia treatment not only in Malawi but other countries in the world. Folate status showed that over 50% had serum folate <10 nmol/L and RBC folate lower than 400 nmol/L. The prevalence of anemia in this population is of great concern, considering that anemia has direct consequences on the fetus (Allen, 2000; Black et al., 2008b).

Based on the patients' medical and drug histories our study shows that the majority were free from major illnesses except 0.9% (n=2) were on anti-tuberculosis treatment, 2.4% (n=5) on steroids, on diabetic treatment 0.5% (n=1), on anti-hypertensive 0.5% (n=1), other drugs 0.5% (n=1) and on anti-retroviral (HIV treatment) 9.9% (n=21). Although HIV positive women comprised about 10% this did not affect the prevalence of LBW in our study, because Malawi practices "option-B plus" for all pregnant women. This regimen ensures all pregnant women start treatment as soon as they are diagnosed positive to reduce viral load regardless of immunological status. Malawi is one of the

leading countries in mother-to-child transmission of HIV but of the majority of babies are born HIV free from these positive mothers. Also Chagomerana and friends in their recent study found that those on HIV treatment did not significantly differ with their counterparts without HIV in terms of prevalence premature birth who are likely born LBW babies (Chagomerana et al., 2017).

Biomarker status by self-reported intake of IFA supplement

One of the major weaknesses of self-reported data is that it is very subjective. Because of this, the validity of results generated this way has always been questioned. On the other hand, the advantage of self-reported data is that it is easy to in large epidemiological studies it would be difficult and expensive for example to send a team to go door-to-door to collect data. Some studies have found contradicting results when they tried to validate self-reported data with verified data for compliance, even in clinical trials (Dieltjens et al., 2013; Kaunitz et al., 2015).

In Malawi compliance to prenatal supplements had been previously reported as poor, (Kalimbira et al., 2009; Young et al., 2000) but no one had validated self-reports of prenatal supplement intake nor actually examine the extent of this non-compliance in terms on biomarker status, let alone birth outcomes.

In our study we found that self-reported IFA pills taken during pregnancy are positively associated with biomarker status of the pregnant woman before delivery. Increased intake of IFA pills from 45, 45-89 and above 90 pills significantly increased mean hemoglobin status from 10.7 ± 1.6 g/dl, 11.3 ± 1.8 g/dl and 11.7 ± 1.6 g/dl (P= 0.006) respectively (Table 9). This is in agreement with what Alwan and friends (2014) found in their study which found that the intake of prenatal iron supplements was

associated with an increase in hemoglobin status of the mother more than dietary intake of iron (Alwan et al., 2014). In another clinical study it was found that self-reported intake of supplements was only confirmed in 53% of the women the other half lied about taking the supplements. They confirmed intake by performing stool analysis looking for iron, and intake was only confirmed in half the women involved in the study, and hematological changes were observed in those who actually took the supplements (Schultink et al., 1993). This shows that self-reporting data has some challenges but at the same time confirmed intake. In Ethiopia another study showed non-compliance to be over 90% mainly due to challenges of coverage of distribution of the supplements in the country and for the few that had access to the supplements, the majority did not comply due to side effects and forgetting to take the pills (Gebremedhin et al., 2014). However, in our study we found that IFA pills taken were positively associated with hemoglobin status in pregnant women, which provides evidence that the Malawi prenatal supplementation program has a chance to be successful. The major challenge in Malawi is that none of the subjects met the WHO recommendation of 180 pills during pregnancy - hence the need for policy changes to address the problem of not finishing the necessary pills.

IFA pills taken during pregnancy and self-reported by pregnant women was positively associated with increased mean serum folate and RBC folate status in the pregnant women before delivery, but this association was not statistically significant. The mean serum folate increase was 7.9 ± 3.6 nmol/L, 8.9 ± 4.9 nmol/L and 10.2 ± 4.1 nmol/L (P = 0.175) while that of RBC folate was 463.1 ± 270 nmol/L, 488.8 ± 38.5 nmol/L and 488.8 ± 469.5 nmol/L (P = 0.949) for IFA intake of 45 pills, 45-89 pills, and

≥ 90 pills respectively. Serum folate is a biomarker of short-term consumption of folate while RBC folate is a biomarker for long-term intake of folic acid. We are of the view that diet of the pregnant women might have impacted the folate data, as it is readily available in local vegetable and cereal diets than iron whose best source is meat and it is not readily available to the population at large. Partly the national fortification program of cereals with folic acid and vitamin B may also impact the results of the study. However, that would only affect a third of the pregnant women who came from rural areas, as fortified cereals are only found in cities accessible to urban dwellers and not rural and peri-urban residents.

Maternal hemoglobin and folate status and low birth weight

We wanted to investigate if pregnant women who reported taking prenatal supplements and showed improved hematological status, had any impact on the birth outcomes, particularly birth weight, of the newborn. It was observed that the mean birth weight was statistically different between the normal and LBW newborns, 3071.8 ± 388g and 2115.6 ± 310 g, P = < 0.001 (n=179) in Table 9. Mean hemoglobin status between mothers of normal and LBW newborns were 11.4 ± 1.6 g/dl and 9.4 ± 1.6 g/dl (P = <0.001), respectively. However, the difference in serum and RBC folate between mothers of normal and LBW newborns were not significant, 9.1 ± 4.4 nmol/L and 7.5 ± 3.6 nmol/L respectively (P = 0.230). The same was observed with RBC folate status between normal and LBW 494.6 ± 413 nmol/L and 489.8 181.1 nmol/L respectively (P = 0.942). These results indicate that overall maternal biomarker status is positively associated with birth weight. This is in agreement with many other studies which show that maternal hemoglobin has modifying effects on infant birth weight of women receiving prenatal

iron-containing supplements (Wang et al., 2016). In a randomized double blind multicenter clinical trial (n= 18,775 participants) in China it was found supplementation of iron and folic acid, folic acid alone or multiple micronutrient supplements (formulated by the United Nations) impacted birth weight depending on hemoglobin status of the pregnant woman. Folic acid did not show much impact on birth weight, perhaps this could explain our results above, that although there is positive association, it was not a statistically significant relationship. The supplement has to make significant change on maternal hemoglobin levels, and hemoglobin will modify the birth weight of the newborn (Wang et al., 2016), with more impact seen in those with better hemoglobin status at baseline. Those getting folic acid supplements alone did not have significant better birth outcomes but those with combined pills or multiple micronutrients. Another study showed that supplementation did not just improve newborn anthropometric measurements, but reduced the prevalence of anemia in mothers (Preziosi et al., 1997). Steer found that low hemoglobin is associated with LBW (Steer, 2000).

Prenatal IFA supplements are associated with low risk of low birth weight

We examined the association between the number of prenatal IFA pills taken during pregnancy and the risk of delivering a LBW newborn and found that pregnant women who self-reported taking supplements consistently (at least two months) lowered their risk of delivering a LBW newborn significantly. After establishing that IFA impacts biomarkers of hematological status of pregnant women and that biomarkers of supplement use (hemoglobin and folate) have modifying effects on birth weight, we wanted to examine if birth outcomes could be linked to "self-reported" IFA use during pregnancy. If self-reported use of IFA pills during pregnancy is validated to predict birth

outcomes in Malawi, then this could strengthen the messages taught in prenatal clinics for the anemia prevention program. It could also help reduce the cost of a routine hemoglobin check, which are rarely done, due to the lack of resources Malawi (only 30 out 213 women had their hemoglobin checked during prenatal care). We accepted the hypothesis that self-reported intake of IFA reduces the risk of low birth weight. The prevalence of LBW (Table 9) was 20.1%, 13.5% and 5.6% for IFA pill intake of < 45 pills, 45-89 pills, and ≥ 90 pills (P = 0.027) respectively. The more prenatal IFA pills were taken by pregnant women the lower the risk of low birth weights. According to Table 12 women who took 60 IFA pills or more had lower risk of delivering LBW (OR = 0.11, 95% CI: 0.02, 0.056, P = 0.008) compared with reference group who took ≤ 30 pills (OR = 1.00). The risk of LBW was also lower for women who took ≥ 90 pills (OR = 0.19, 95% CI: 0.04, 1.01, P=0.051). These observations were made on pill numbers of 60 – 90 or more. The question is whether or not pregnant women should take 180 pills (6 months pill intake) as recommended by WHO because we are seeing improvements at 90 pills (3 months).

Similarly Nisar and friends (2016) in their study which used nationally representative data of the Pakistan demographic and health survey (PDHS, 2002 – 2006) found that self-reported of IFA pills of any amount during pregnancy was positively associated with better perceived birth size and birth weight. Any amount of IFA pills taken was associated with reduced risk of having a smaller than average newborn by 18% (OR = 0.82, 95% CI: 0.71, 0.96) (Nisar and Dibley, 2016).

In another study in India, which also used national data set to examine the relationship between self-reported intake of IFA pills in pregnancy and LBW risk, found

inverse association. They found that at population level in a context where the burden of anemia is severe (prevalence ≥ 40%), IFA taken during pregnancy was significantly associated with decreased LBW (OR = 0.77, 95% CI: 0.68, 0.86, P = < 0.001. They concluded that measures to improve the implementation of prenatal supplementation program, would likely help to address India's burden of LBW (Balarajan et al., 2013). The situation in India applies to Malawi because they are both developing countries with existing food security challenges in its communities, have a high prevalence of anemia and prenatal IFA supplements have proved to be effective.

The findings in Table 13 summarize the influences of IFA and status of maternal biomarkers on newborn anthropometric z-scores. We used mother's hemoglobin, serum folate, and RBC as independent variables and the newborn anthropometric outcomes as dependent variables, the regression coefficients of number of IFA pills, mother's Hb, serum folate, and RBC folate for predicting newborn anthropometry vs. the number of IFA pills. Covariates were controlled and included age, residence, education, and house hold income among others. An increase in mothers' Hb of 1 g/dl corresponded to an increase in the WHZ score of ($\beta = 0.232$, $P = 0.045$), when all covariates are constant. Also an increase in mothers' Hb of 1 g/dl corresponds to an increase in WAZ score of $\beta = 0.130$, $P = 0.021$. Higher serum folate levels were also significantly related to increased WAZ and HCZ scores of $\beta = 0.057$, $P = 0.043$ and $\beta = 0.119$, $P = 0.023$ respectively. However, mothers intake of IFA pills during pregnancy and RBC folate status of pregnant woman were not significantly associated with newborn anthropometric outcomes. This shows that hemoglobin and folate status of the mother is associated with size of newborn at birth. Since it is known that prenatal supplements can correct

biomarker status, therefore compliance to prenatal supplements can impact newborn size at birth. This shows that compliance to prenatal supplements can actually reduce risk of LBW, infant mortality and stunting prevalence in Malawi.

Malawi has other nutrition programs that are aimed at improving the nutrition status of the population in general. These include improving food security by supporting production (farm inputs subsidy), industrial and bio-fortification of commonly consumed foods and encouraging locals to explore other foods to diversify diets rich in iron, vitamin A and other essential nutrients. However, the above approaches are long term as food security cannot be completely achieved without improvement of the economic status of the people. This leaves prenatal supplementation as the best short-term solution to the high prevalence of anemia. The biggest challenge that we were able to identify in this study was sustained intake of the IFA pills for longer than 3 months. Most pregnant women reported negligence, reduced supply of IFA supplements and late start of prenatal clinic attendance as major reasons for poor compliance (table 11 and 12). Now that we have demonstrated that self-reported intake of prenatal supplements is a valid tool to predict hematological status of biomarkers and birth out comes of a pregnant woman, it is high time a monitoring mechanism was put in place to evaluate the pregnant women about their compliance to IFA every time they go for prenatal clinics. Perhaps the monitoring mechanism could combine with pill counting (Young et al., 2000) to ensure compliance is being achieved and pregnant women adhere to IFA pills supplements. Pill counting has been employed successfully to improve adherence to ant-retroviral drugs in the treatment of HIV/AIDS. Another method being used in Malawi is directly observed treatment (DOT), where a patient takes drugs under the observation of medical

practitioner or a trusted guardian. DOT is being used in the treatment of tuberculosis (the first two weeks) and malaria prophylaxis in pregnant women at the prenatal clinic. An effective monitoring and evaluation mechanism would make Malawi realize more benefits from IFA supplementation program and make it more successful in lowering prevalence of low birth weight. Education of pregnant women on the benefits of IFA on their newborns would yield positive results because negligence was one of the major reasons for poor compliance. Lowering the risk of LBW has long term benefits such as reducing infant and child mortality, reducing stunting prevalence, which in turn has economic returns and improve national development (Win, 2016).

Summary and Conclusion

This research examined compliance to prenatal IFA in relation to LBW in Malawi. The results show that self-reported compliance to prenatal supplement use by pregnant women is associated with reduced risk of low birth weight. The mechanism being that prenatal supplements improve hematological status of hemoglobin and folate, which have direct impact on the developing fetus.

The total number of pregnant women involved in the study was n = 213, 70% were young (below 35 y), the majority were fairly literate (went to primary and secondary school) and they came from all corners of the district (i.e., rural, peri-urban and urban). The majority had very low household income, living with less than a dollar a day, only 11% were doing skilled labor and the rest were housewives and subsistence farmers. The average number of prenatal care clinic visits were 3.3, with most attendance in second trimester, received and took IFA supplements, however about 40% had anemia (hemoglobin < 11 g/dl).

We had hypothesized that women who self-reported taking prenatal supplements (complying) would have better biomarker status (improved hemoglobin and folate). This hypothesis is hereby being accepted based on results in Figure 5, 6, 7, and Table 8 and 9 which indicate a positive association between self-reported IFA supplements pills taken during pregnancy and hemoglobin and folate status before delivery. There was a statistically significant association between IFA intake and hemoglobin, although there was not a statistically significant association between both serum and RBC folate and IFA taken during pregnancy, mean folate status improved tremendously from those that <45 pills to those that took >90 pills. The implication of this finding is that it confirms that IFA supplementation still has the potential to fight anemia in pregnant women in Malawi and to reduce the prevalence of anemia (currently at 33%).

We also hypothesized that pregnant women with better biomarker status (less anemic) would have better birth outcomes (lower risk of delivering a LBW newborn). This hypothesis is also accepted based on results illustrated in Table 11. We observed that IFA biomarker status was much higher in women who delivered a normal weight newborn compared with those who had a LBW new born. The association was statistically significant for hemoglobin but not folate (Hemoglobin: 11.4 g/dl versus 9.4 g/dl P= < 0.001, serum folate: 9.1 nmol/L versus 7.5 nmol/L P=0.230, RBC folate: 494.6 nmol/L versus 489.8 nmol/L P=0.942). Again, these findings have the implication that improving maternal IFA biomarker status impacts newborn health, and reduces the risk of low birth weight. Routine checking of hemoglobin during prenatal clinics would facilitate prompt correction of anemic women and reduce the risk of delivering a LBW newborn. As seen in this study, 30 out 213 women only had hemoglobin checked in

prenatal clinic during pregnancy. The rest of the women just hoped that they were not anemic until they were enrolled in the study and had hemoglobin checked. In the absence of such a service then emphasis would be put on ensuring that all pregnant women are compliant to taking IFA supplements.

Finally we had hypothesized that self-reported compliance to prenatal iron and folic acid intake would be associated with lower risk of giving birth to a LBW newborn. Results in Table 7, 9, and Figure 9 indicate that the more IFA pills pregnant women took the better the birth outcome. The average birth weight increased from 2884.2 g, 2903.9 g and 3188.9 g ($P = 0.002$) with IFA pill intake of < 45, 45-89, ≥ 90 pills respectively. A multivariate analysis of odds ratio showed that women who took more than 60 pills reduced the risk of LBW significantly (OR = 0.11, 95% CI: 0.02, 0.056, $P = 0.008$). On this basis again the hypothesis is accepted. The implication of these findings is that self-reported intake of prenatal IFA can be used to predict birth outcomes. In other words, self-reported intake of IFA can be used as a monitoring tool for the prenatal supplementation program in Malawi, to enforce compliance in the interest of reducing LBW prevalence. However, the question still stands is whether we should stick to the WHO guidelines of a minimum of 180 pills (6 months intake) of IFA during pregnancy considering that >90 pills already shows significant difference.

Therefore, self-reported IFA intake (compliance) in pregnancy is a predictor of birth weight. Compliance can be improved if the Malawian government improves the supply of IFA pills in clinics, through education make every pregnant woman attend prenatal clinics early in pregnancy, and possibly adding pill count to the questions asked at follow up before supplying the next consignment of IFA pills.

Recommendations and future studies

In this study, we have demonstrated that self-reported compliance to prenatal IFA intake is associated with improved biomarker status before delivery (hemoglobin) and reduced risk of LBW. We also showed that taking ≥ 60 IFA pills or more is significantly associated with reduced risk of LBW. Therefore, we recommend that self-reported compliance be used as a monitoring and evaluation tool in prenatal clinics to ensure adherence to IFA intake as it will improve birth outcomes. Self-reported compliance to IFA intake is a valid tool to assess and predict hematological status in the pregnant women before delivery and newborn health at birth. We hereby also recommend that extensive education to be carried out in communities and prenatal clinics to pregnant women and women of child bearing age, so that they understand the importance of taking prenatal IFA supplements. Given the challenging levels of compliance to WHO recommendation of taking prenatal IFA through out pregnancy or at least a minimum of 180 pills, we suggest that prenatal clinics ensure that women take at least 60 – 90 pills of IFA since this has the potential to reduce risk of LBW.

However, more studies need to be done to find optimum minimum level of IFA intake, which gives similar beneficial results in Malawi women. Since ours was a cross sectional study, we recommend a longitudinal study which will take into account the changes in availability of food due to seasonality of food security in Malawi. A longitudinal population study may also show what happens to the infants who were born small, from mothers with low hemoglobin and folate status in terms of infant mortality and linear growth in general. We also recommend that such studies should include dietary recalls to account for differences in dietary exposure among pregnant women.

APPENDICES

Appendix A: Literature Review Articles

Table 14. Summarized literature review of selected articles.

Author	Objective	Design of the Study	Subject and Parameter	Major Findings
(Hjertholm et al., 2017)Hjertholm et. al 2017	To examine the association between maternal dietary during pregnancy intake and infant birth size	Cross sectional study in rural Malawi	Dietary intake (macronutrients and 11 micronutrients of 203 pregnant at 28 and 35 weeks gestation and neonatal anthropometry measured at birth (n=132). Used 3 day interactive 24HR recall	Milk, vitamin C and fat were positively associated with birth weight while carbohydrate had negative association
(Nisar and Dibley, 2016)	To examine the impact of antenatal IFA supplementation on perceived birth size and birth weight in Pakistan over a 5-year	Cross sectional study using the Pakistan demographic and health survey (PDHS) 2006-2007, n=5962	Any use of Iron and folic acid use and maternal perception of birth size and birth weight. They used multivariate logistic regression analysis and **adjusted** for 13 confounders	The risk of LBW was reduced by **18%** for IFA users and starting IFA in first trimester significantly reduced the risk of LBW in 19% of babies and 11% attributable risk of smaller birth weight for non-use of IFA

Table 14. (cont'd).

Author	Objective	Design of the Study	Subject and Parameter	Major Findings
(Owens *et al.* 2015)	Multiple micronutrient supplementation and placental function	Randomized, double blind, placebo controlled clinical trial in Gambia, (n =1137, and 415 pregnancies).	Subjects supplemented with UNICEF/WHO/United Nations University multiple micronutrient preparation (UNIMMAP) or placebo. Placental transport capacity at birth scanned.	Placental vascular function was modifiable by preconception multiple micronutrient supplementation (UNIMMAP)
(Abass et al., 2014)	Determine association between low plasma zinc and low birth weight	Case control in a hospital in Sudan	50 cases with birth weight of less then 2500g and 50 controls with birth weight above 2500g, checked serum zinc levels	Low maternal and cord blood serum zinc was associated with LBW in babies
(Rwebembera et al., 2006)	Relationship between infant weight and maternal zinc status	Case control study in a Hospital in Tanzania	84 cases with birth weight of less then 2000g and 84 controls with birth weight above 2000g, checked serum zinc levels	Low maternal and cord blood serum zinc was associated with LBW OR 2.6 (CI 1.36 – 5.73)

Table 14. (cont'd).

Author	Objective	Design of the Study	Subject and Parameter	Major Findings
(Kishosha et al., 2011)	To determine association of low selenium and goiter	Case control study in Uganda	46 cases and 46 controls. Blood samples were taken to determine selenium concentration	Mean serum selenium level in goiter patients (77.25ug/l (SD 16.78) lower than in the non-goiter controls (95.50 ug/l (SD 24.47), $p<0.005$.
(Gebremedhin et al., 2014)	Coverage and compliance for using prenatal iron supplements in eight rural districts of Ethiopia	Cross-sectional study in 8 random districts in Ethiopia	414 pregnant women and 1,573 women one post delivery. Data; multi-stage sampling and using a structured questionnaire	Coverage was only 35% of women. Only 3.5% took iron for 91 days as recommended by WHO. The odds of receiving iron supplements increased with increased antenatal attendance; 1, 2, 3, 4, had 0.04, 0.33, 0.50 and 0.60 respectively.
(Preziosi et al., 1997)	Iron supplementation on the iron status of mothers and infant health.	Randomized placebo controlled clinical trial in Niger	197 pregnant women received 100mg iron from 28 week gestation until delivery. Four blood samples taken from entry day, during labor, cord and heel of baby, and anthropometric	Neonatal length and Apgar scores improved by iron supplements. Iron decreased anemia.

Table 14. (cont'd).

Author	Objective	Design of the Study	Subject and Parameter	Major Findings
(Makrides et al., 2003)	Assessing effect of prenatal IDA and iron deficiency from low dosage (20 mg/d) of iron, and side effects	Randomized, double blind, placebo controlled clinical trial using iron supplementation in Australia	430 women followed up to 6 months postpartum after a low dose iron supplement of 20 mg given from 20 weeks gestation	No significant differences in gestational age at birth, birth weight, birth length, birth head circumference, Apgar scores, or the level of neonatal care required between the iron- supplemented and placebo groups.
(Hill et al., 2007)	Factors that affect adoption and adherence to weekly vitamin A supplementation in Ghana, (individual, cultural)	Field trial/community survey in Ghana	Semi-structured questionnaire administered during interviews and focus groups. 4-month capsule given to 60 women.	Knowledge low, taking medicines when healthy was new concept. Supplements accepted when motive explained. Factors affecting adoption: perceived side effects, vitamins are family planning; supplements are for the childless or pregnant, forgetfulness, herbal medicines and IEC

Table 14. (cont'd).

Author	Objective	Design of the Study	Subject and Parameter	Major Findings
(Thirukkanesh and Zahara, 2010)	To determine the compliance of vitamin and mineral supplementation of women in prenatal clinics in urban and rural areas	Cross-sectional study in Malaysia	118 mostly third trimester pregnant women aged >28, 62 urban and 56 rural. Used self-reported questionnaire and hemoglobin from prenatal records	49.2% complied with mineral and vitamin supplements, not different between rural and urban in compliance. Lower hemoglobin in non-compliant groups
(Steer, 2000)	Association of maternal Hemoglobin concentration and birth outcomes	Systematic review article	A presentation at a symposium Maternal nutrition in 1998 in Paris; new developments and implications	Low hemoglobin (<80g/L) is associated with low birth weight, SGA, and preterm birth >84g/L is considered optimal

Table 14. (cont'd).

Author	Objective	Design of the Study	Subject and Parameter	Major Findings
(Kalimbira et al., 2009)	Assess maternal knowledge and practices related to anemia and iron supplementation in rural areas	A cross-sectional study in northern, central and southern Malawi	629 women were randomly selected in all the 3 regions of the country and interviewed using a pre-tested questionnaire	22%, 28% and 33% in central, northern and southern respectively reported taking iron supplements for one month only. 43% non-compliance due to nausea
(Young et al., 2000)	Comparing the effectiveness of weekly and daily iron supplement and compliance	A simply randomized clinical trial at a prenatal unit in a hospital in northern Malawi	413 pregnant women, first group got 60mg iron/0.25 folate daily (n=211) and second got 120mg iron/0.50 folate weekly (n=202). Initial and later final Hemoglobin and compliance checked at 10th week	Initial hemoglobin for daily and weekly supplemented groups 105.7g/L and 104.4g/L. Final was 107.5g/L and 105.6 respectively, no significant difference. Self-reported and pill count compliance was higher in weekly supplemented group, also had less side effects

Appendix B: Architect i System for Folate Analysis

ARCHITECT i Systems reagents:

ARCHITECT Folate Reagent Kit (1P74-25, 1P74-35) 100 tests.

- Microparticle -1 Bottle (6.6 mL per 100□test bottle) Anti-Folate Binding Protein (mouse, monoclonal) coupled to microparticles affinity-bound with Folate Binding Protein (bovine), in TRIS buffer with protein stabilizers (human serum albumin and caprine). Minimum concentration: 0.08% solids. Preservatives: Sodium azide and antimicrobial agents.
- Conjugate - 1 Bottle (29.0 mL per 100-test bottle) Pteroic Acid (PTA) - acridinium labeled conjugate in MES buffer with protein stabilizer (porcine). Minimum concentration: 4 ng/mL. Preservative: antimicrobial agents.
- Assay specifc diluent -1 Bottle (5.7 mL per 100-test bottle/25.3 mL per 500-test bottle) Folate Assay Specific Diluent containing borate buffer. Preservatives: sodium azide and antimicrobial agents.
- Pretreatment 1 - Bottle (50.2 mL per 100-test bottle) Folate Pre-Treatment Reagent 1 containing potassium hydroxide.
- Pretreatment 2 - 1 Bottle (6.6 mL per 100-test bottle) Folate Pre-Treatment Reagent 2 containing dithiothreitol (DTT) in acetic acid buffer with EDTA.
- Specimen diluent - 1 Bottle (5.5 mL per 100-test bottle) Folate Specimen Diluent containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

ARCHITECT Folate (1P74-50)

- Manual Diluent -1 Bottle (4 mL) containing TRIS buffer with protein stabilizer

(human serum albumin). Preservative: sodium azide.

• Folate RBC Lysis Diluent (L2) - 1 Bottle (12.5 mL) containing citric acid and guanidine hydrochloride. Preservative: antimicrobial agent.

• Folate Lysis Reagent (L1) - 4 Bottles (285-385 mg each) containing ascorbic acid and guanidine hydrochloride.

• Pre-Trigger Solution - containing 1.32% (w/v) hydrogen peroxide.

• Trigger Solution - containing 0.35 N sodium hydroxide.

• Wash Buffer - containing phosphate buffered saline solution. Preservative: antimicrobial agents.

The specimens were mixed thoroughly by low speed vortexing for 10 times. To ensure consistency in results, specimens were transferred to a centrifuge tube and centrifuged before testing because they were frozen and thawed, also if they contained fibrin, red blood cells in serum, or other particulate matter.

Procedure for the Determination of Folate in Red Blood Cells (RBC)

NOTE: Hematocrit of EDTA specimen is determined first and used later to calculate the corrected value of RBC folate if the results fall out of range.

Part 1: Reconstitution of Folate Lysis Reagent (L1)

Reconstitution was done using one bottle of Folate Lysis Reagent (L1) by adding 30 mL distilled or deionized water.

• The reagent bottle was capped and mixed by inverting 10 times and let stand for 15 minutes.

Part 2: Preparation of Red Blood Cell Hemolysate

NOTE: The assay was initiated on the final hemolysate within 2 hours as recommended for

ARCHITECT *i* system.

• The reconstituted Folate Lysis Reagent (L1) was inverted an additional 10 times. Pipetted 1.0 mL into a suitable sample tube with a cap (example: 2 mL tube).

• Mixed whole blood tube by inverting 10 times to ensure a homogeneous sample.

•100 µL of whole blood sample was added to the sample tube containing the 1.0 mL of the reconstituted Folate Lysis Reagent (L1).

• Capped the tube and mixed by inverting 10 times or vortexing and allow to stand at room temperature (15-30 °C) for 90 minutes ± 5 minutes). Protect from light.

• Pipetted 100 µL ARCHITECT Folate RBC Lysis Diluent (L2) into a new sample tube (or ARCHITECT sample cup). Then add 100 µL of hemolyzed sample.

• Mix by swirling or vortexing and initiate assay on this sample within 2 hours.

Running the assay on ARCHITECT *i* system (Folate and Folate RBC Assays)

After preparing hemolysate and serum samples above, analysis preceded on the ARCHITECT *i* system.

Inverted the microparticle bottle 30 times until microparticle re-suspend. Once the microparticles have been re-suspended, the cap was removed and discarded. Wearing clean gloves, removed a septum from the bag and placed it septum on the bottle. The ARCHITECT Folate Reagent Kit was Loaded on the ARCHITECT *i* System. When running a serum or plasma specimen/control, Folate II (assay 685 "UNDILUTED") was selected on the ARCHITECT *i* system. When running a whole blood specimen or whole blood control, Folate RBC (assay number 686, "RBC DIL") was selected on the ARCHITECT *i* system.

Appendix C: Descriptive Statistics

Table 15. Summary descriptive statistics.

Category	Variable	n	Mean	Median	Std. Dev.	Minimum	Maximum
Newborn characteristics	Birth weight (g)	213	2919.2	3000.0	514.3	1300.0	4100
	Birth length (cm)	209	47.8	48.4	3.6	32.5	57
	Head Circumference (cm)	210	34.4	35.0	2.1	25.0	40
	APGAR Score 1minute	209	8.1	8.0	1.4	0.0	10
	APGAR score 5 minute	209	9.7	10.0	1.2	0.0	10
	Weight for height Z-scores	176	-0.671	-0.67	1.5846	-6.5	3.8
	Height for age Z-scores	208	-0.8	-0.6	1.7	-5.7	4.22
	Weight for age Z-scores	213	-0.9	-0.7	1.2	-5.0	1.57
	BMI for age Z-scores	207	-0.7	-0.6	1.5	-6.4	3.95
	Head circ Z-scores	210	0.2	0.4	1.7	-7.5	5.17
Pregnancy & antenatal care	Age	213	25.2	24.0	6.4	14	43
	Maternal WT- 1 (kg)	185	59.3	58.0	9.9	38.0	100
	Maternal WT- 2 (kg)	141	65.6	64.0	11.1	48.6	110
	Maternal height (cm)	185	154.7	154.0	5.9	141.0	170.5
	Gravidity	212	2.7	2.0	1.7	1.0	9
	Parity	212	1.6	1.0	1.6	0.0	8
	Gestation age	189	36.8	37.0	1.8	28.0	41
	Number of IFA	205	53.6	60.0	36.6	0	160
	Number of ANC visits	207	3.3	3.0	1.1	0	6

Table 15. (Cont'd)

Maternal IFA status	Hb- 1 (g/dl)	29	9.3	9.3	1.7	4.3	12.9
	Hb- 2 (g/dl)	199	11.1	11.2	1.7	6.9	15.5
	Serum folate (nmol/L)	101	8.8	8.0	4.4	2.0	31
	RBC Folate (nmol/L)	101	493.9	391.0	388.6	138.0	2317
Placenta weight	Placenta weight (g)	171	570.7	565.0	141.5	222.0	1029
Economic status	Annual HH income ($)*	154	884.8	499.3	1677.5	69.3	13,869.3

*USD $1= MK721 (Malawi kwacha), roughly at the time of the survey

Appendix D: Consent Form (English)

Research participant information and consent form

You are being asked to participate in a research study. Researchers are required to provide a consent form to inform you about the research study, to convey that participation is voluntary, to explain risks and benefits of participation, and to empower you to make an informed decision. You should feel free to ask the researchers any questions you may have.

Study Title: Compliance to prenatal supplements as a determinant of LBW in Malawi

Researcher and Title: Won Song, Professor of Human Nutrition Department and Institution: Food Science and Human Nutrition, Michigan State University

Address and Contact Information: Phone; +1 517 6143636 song@anr.msu.edu

1. PURPOSE OF RESEARCH

• You are being asked to participate in a research study of Compliance to prenatal supplements as a determinant of LBW in Malawi

- You have been selected as a possible participant in this study because you are pregnant and coming for delivery of your baby in this hospital.

- From this study, the researchers hope to learn how compliance to prenatal supplements affects the health of the newborn baby at birth. The results shall also be used to write a thesis to meet requirements of Masters study at Michigan State University.

- Your participation in this study will take about 15 minutes.

2. WHAT YOU WILL DO

- You will be asked a few questions on prenatal supplements use and your medical record on antenatal care will be abstracted. We will use part of the blood sample routinely drawn by midwives and doctors during admission to maternity ward (for grouping and

cross matching to keep blood in the bank for you in case you need a transfusion during labor).

- Upon delivery of your baby the midwife will collect a blood sample from the umbilical cord after it is cut and the placenta will be weighed. This will happen after making sure you are fine and will not be done by the same midwife responsible for your care and newborn.

- After your baby has received the initial care the research midwife will also abstract the medical record of your newborn specifically APGAR scores, weight, length and head circumference. If you choose not to participate in the study your care will not change, you will still have access to midwives and doctors.

3. POTENTIAL BENEFITS

The potential benefits to you for taking part in this study are that you will know the health status of your child if you choose to know the results and at the end we would like to give your newborn a gift (incentive) of a wrapper.

However, your participation in this study may contribute to the understanding if how the Malawi government's prenatal micronutrient supplementation to all pregnant women is working in terms of compliance. And it will add knowledge on how early malnutrition may start showing symptoms in newborns.

4. POTENTIAL RISKS

There are no risks to you or your baby for participating in the study, as it does not involve new procedures apart from those already routinely done in clinical practice. We shall collect the placenta for weighing and cord blood at the point when the nurse would throw away the placenta and cord. However, if some measurements were not done thoroughly on newborn, the researchers may want to repeat and that may lead to a short separation of you and your newborn. But we shall try our best to keep you or your guardian close while measurements are done.

□5. PRIVACY AND CONFIDENTIALITY □

• The data for this study are being collected confidentially. Information about you will be kept confidential to the maximum extent allowable by law. Only the research team, and the Michigan State University Human Research Protection program shall have access to the data. The data shall be stored at the Food and Nutrition Database Research Center in the department of Food Science and Human Nutrition at Michigan State University for a minimum of 3 y .

The results of this study may be published or presented at professional meetings, but the identities of all research participants will remain confidential.

6. YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW

• Participation is voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled. □

• You have the right to say no. □

• You may change your mind at any time and withdraw. □

• You may choose not to answer specific questions or to stop participating at any time. □

• Choosing not to participate in this study will not make any difference in the quality of any treatment you may □receive during labor and delivery of your baby.

□7. COSTS AND COMPENSATION FOR BEING IN THE STUDY

□There are no costs on your side for taking part in the study and therefore there is no compensation that you will get for being in this study.

□8. CONTACT INFORMATION

□If you have concerns or any questions about this study, such as scientific issues, how to do any part of it, or to report an injury, please contact the researcher: Dr. Won Song. 469

Wilson RD Trout Building RM 135, Michigan State University, MI 48824, or email song@anr.msu.edu or Phone; +15173533332

If you have questions or concerns about your role and rights as a research participant, would like to obtain information or offer input, or would like to register a complaint about this study, you may contact, anonymously if you wish, the Michigan State University's Human Research Protection Program at 517-355-2180, Fax 517-432- 4503, or e-mail irb@msu.edu or regular mail at 408 West Circle Drive, Olds Hall Room 207, MSU, East Lansing, MI 48824.

9. DOCUMENTATION OF INFORMED CONSENT

Your signature line below means that you voluntarily agree for you and your child to participate in this research study. Signature_____ Date _____/_____/_____

Your signature below means that you voluntarily agree for your child to participate in this research.

Signature_____Date _____/_____/_____ Signature of Assenting Child (15-17)_____Date_____/_____/____

You will be given a copy of this form to keep.

This consent form was approved by a Michigan State University Institutional Review Board. Approved 07/13/16 – valid through 07/12/17. This version supersedes all previous versions. IRB # 16-837.

Appendix E: Consent Form (Chichewa Version)

Kalata wa chivomelezo cholowa kafukufuku

Mukupemphedwa kutengapo gawo pa kafukufuku. Ochita kafukufuku amayenera kupereka chikalata ichi chofotokoza za ubwino ndi kuipa kotangapo gawo pa kafukufuku kuonenetsetsa kuti kutengapo gawo kwanu ndi chiganizo chomwe mwapanga mosakakamizidwa koma mwaufulu wanu. Choncho ndinu omasuka kufunsa funso lililonse lomwe mungakhale nalo kwa ochita kafukufuku.

Mutu wa kafukufuku: Kulondoloza kwa mankhwala ochulukitsa magazi operekedwa ku sikelo, ndi zotsatira zake pa ana operewera sikelo□

Mwini kafukufuku ndi udindo wake: Won Song, Professor wa zamadyedwe□athanzi Komwe akuchokera: Food Science and Human Nutrition, Michigan State University

Keyala ndi nambala ya lamnya: +1 517 614 3636, song@anr.msu.edu

1. CHOLINGA CHA KAFUKUFUKU

• Mukufunsidwa kutengapo gawo pa kafukufuku wa Kulondoloza kwa mankhwala ochuluktsa magazi operekedwa ku sikelo, ndi nthanzi la nsengwa/matenda posiyanitsa ndi kulemera kwa nsengwa, malilidwe, kulemera ndi msinkhu wa mwana pobadwa ku Malawi.

• Mwasankhidwa chifukwa chakuti ndinu oyembekezera ndipo mwabwera kudzachilira mu chipatala chino. □

• Ochita kafukufuku uyu akufuna kudziwa Kulondoloza kwa mankhwala ochulukitsa magazi operekedwa ku sikelo, ndi nthanzi la nsengwa/matenda posiyanitsa ndi kulemera kwa nsengwa, malilidwe, kulemera ndi kwa mwana pobadwa ku Malawi kuno. Zomwe zimayimira thanzi la mwana pamapeto pake. Zotsatira za kafukufuku zigwiritsidwa ntchito pokwaniritsa zofunikira pa maphunziro a ukadaulo ku sukulu ya □ukachenjede ya Michigan State University ku America. □

• Kafukufuku adzatenga pafupifupi theka la ola. □

2. ZOMWE TIDZACHITE □

Mudzafunsidwa mafunso ochepa za mankhwala amagazi aku sikelo ndiponso tidzafuna tilembeko zina zomwe zili m'buku lanu la kuchipatala. Tidzafuna tiyezeso kudziwa kuchuluka kwa magazi ndi mchere mmagazi anu omwe a nurse adzatenge kutumiza kwa oyeyeza.

Pamene mwana wanu wabadwa Namwino/mzamba adzatenga magazi pang'ono ku nsengwa komanso kuyeza pa sikelo nsengwayo. Mwananso akadzamaliza kulandira thandizo loyambilira tidzamuyesa sikelo, msinkhu, kukula kwa mutu ndi kulemba momwe analilira pobadwa.

Dziwani kuti ngati mutasankha kusatengapo gawo pakafukufuku uyu, thandizo lomwe a mzamba/Namwino kapena a dotolo adzapereke kwa inu silidzasintha chifukwa simunalowe mkafukufuku.

3. PHINDU LOLOWA MU KAFUKUFUKU

Phindu lotengapo gawo pa kafukufuku uyu ndi lakuti mudzadziwa thanzi la mwana wanu potengera momwe mumalondolozera mankhwala ochulukitsa magazi ku sikelo ngati mungasankhe kumva zotsatirazo. Komanso tidzafuna kupereka ka mphatso kangachepe kwa mwana wanu imene ili chitenje.

Komabe dziwani kuti kutengapo gawo kwanu mukafukufuku uyu kudzathandiza ngakhale Boma kudziwa momwe azimayi oyembekezera akulondolozera mankhwala amagazi operekedwa ku sikelo ndiponso kuwonjezera nzeru ndi ukadaulo kuti tidziwe ngati ana akudadwa a thanzi kapena onyetchera kale poyelekeza ndi momwe amayi anamwera mankhwala a magazi asachile.

4. KUIPA KOLOWA MUKAFUKUFUKU

Palibe vuto lilonse lomwe lingaoneke kwa inu kapena kwa mwana wanu chifukwa mwalowa mukafukufuku ameneyu chifukwa sitidzatenga magazi owonjezera pamwamba pa omwe amatengedwa kale kwa inu.

Mwana sitidzamubaya kapena kumutenga magazi koma kungomuyeza chabe sikelo ndi msinkhu. Ndipo tidzatenga magazi mu nsengwa pamene azamba akufuna kutawa atamaliza kukuthandizani.

Komabe pa nthawi yomwe tikuyesa mwana sikelo ndi msinkhu mudzasiyana naye mwana mwakanthawi tiphindi pang'ono. Ife tidzyesetsa kuti inu kapena okuyang'anirani akhale pafupi kuonetsetsa ndi kuwonerana zonse zomwe zikuchitika pa mwanayo.

5. KUKUSUNGIRANI CHINSINSI PA KAFUKUFUKU

Kafukufuku ndi wa chinsinsi. Wochita kafukufuku sadzapanga zoti zomwe inu mwanena ziululike monga mwamalamulo a dziko. Amene adzadziwa za zomwe inu mwatiuza ndi ochita kafukufuku okha ndi omwe anapereka chilolezo kuti kafukufufku achitike.

Zotsatira zidzasungidwa ku Food and Nutrition Database Research Center gawo la sayansi ya zakudya ndi madyedwe athanzi ku sukulu ya ukachenede ya Michigan State University ku America.

Dziwaninso kuti zotsatira zitha kudzaulutsidwa mmisonkhano ya akatswiri aza manyedwe athanzi ndi kusindikizidwa, koma dzina lanu silidzatchulidwa kapena kulumizidwa ndi zotsatira zanu kuti mudziwike ayi.

6. UFULU OLOWA, KUKANA NDI KUTULUKA MKAFUKUFUKU

• Kulowa mkafukufuku uyu ndu ufulu wanu. Simudzalandira chilango chifukwa chokana kutengapo gawo pakafukufuku, kapena kutaya mwayi uliwonse. Mutha kusiya popnda kukukanizani thandizo lilonse liene mukuyenera kulandira ngati chilango chifukwa chosalowa mukafukufuku ayi.

• Dziwani kuti muli ndi ufulu okana.
• Dziwani kuti muli ndi ufulu otuluka.
• Mutha kusankha kusayankha mafunso ena kapenanso kusiya kuyankha mafunso nthawi

iliyonse □
• Thandizo lanu silidzatengera kulowa kapena kukana kulowa kafukufuku ayi.

□7. MALIPIRO KAPENA CHIPEPESO MKAFUKUFUKU □

Inu simudzataya kapena kuwononga/kugwiritsa ntchito ndalama yanu kapena kanthu kalikonse (ngati malipiro) chifukwa mwalowa mkafukufuku ayi. Ndiponso zindikilani kuti simudzalandira malipiro kapena chipepeso chilichonse chifukwa cholowa mu kafukufuku ameneyu.

□8. KEYALA NDI MLAMNYA ZA MPHUNZITSI WA KAFUKUFUKU

Ngati mutakhala ndi mafunso kapena nkhawa zili zonse pa kafukufuku ameneyu, ngakhale panthawi yomwe mwatuluka mchipatala chonde lumikizanani ndi mkulu wakafukufuku yemwe ali Mphunzitsi wanga pa keyala ndi lamnya izi: Dr. Won Song. 469 Wilson RD Trout Building RM 139, Michigan State University, MI 48824, kapena email song@anr.msu.edu or Phone; +1517 353 3636. □

Ngati mutakhala ndimafunso kapena nkhawa zina zokhudzana ndi gawo kapena ufulu wanu mukafukufukuyu ndipo mukufuna kumva zambiri kapena kuthandizapo, ngakhale kupereka dandaulo pa kafukufuku ameneyu, mukhoza kulemba kalata kapena kuchita lamnya pa keyala ndi nambala ya lamnya zili mmusimu; Michigan State University's Human Research Protection Program pa 517-355-2180, Fax 517-432-4503, kapena e-mail irb@msu.edu or regular mail at 408 West Circle Drive, Olds Hall Room 207, MSU, East Lansing, MI 48824. □

9. UMBONI WA CHIVOMELEZO

kusainila kwanu mmusimu zitanthauza kuti mwavomereza mwaufulu popanda kukakamizidwa kuti inu ndi mwana wanu mulowe mkafukufuku.□

Tikitilani_____ Tsiku_____/____/_____ □

kusainila kwanu mmusimu zitanthauza kuti mwavomereza mwaufulu popanda kukakamizidwa kuti mwana wanu alowe □mkafukufuku.

Tikitilani (zaka zosakwanira 18)_____ Tsiku_____/_____/_____

Mudzalandira kalata iyi kuti inunso musunge.

This consent form was approved by a Michigan State University Institutional Review Board. Approved 07/13/16 – valid through 07/12/17. This version supersedes all previous versions. IRB # 16-837.

Appendix F. Survey Instrument

Name of interviewer …….

Date of interview …….

Official use
Code:.....

SECTION A: PERSONAL IDENTIFICATION / BASIC CHARACTERISTICS (From the chart)

ID	*Question*	*Response*	*Chart*	*Ask*	*Test*
A1	Name of patient		√		
A2	Age of patient		√		
A3	Height		√		
A4	Booking visit weight		√		
A5	Number of prenatal visits		√		
A6	Area of residence/village		√		
A7	Gravidity		√		
A8	Parity		√		
A9	Education; highest level of school Attained	(1) Junior primary (grade 1-4) (2) Senior primary (5-8) (3) Secondary (4) College (5) Never been to school	√		
A10	Occupation		√		
A11	Annual income of household head	**MK**……..		√	

SECTION B: ANTENATAL AND NUTRITION CARE

B1	Gestation age at first visit	(1) First trimester (Why?) …… …… (2) Second trimester	√		

		(3) Third trimester (why?) ……			
B2	Use of contraceptives prior to this pregnancy	(1) Hormonal (2) Physical (3) None	√		
B3	History of major illness in this pregnancy	(1) Yes required hospital admission (2) Yes, but just received prescription (3) Yes it resolved on itself (4) None		√	
B4	History of transfusion in this pregnancy	(1) Yes (2) No		√	
B5	Booking visit Hemoglobin (from health passport)	Hemoglobin……. **g/dl**	√		
B6	Screened for the following diseases:	1. Malaria……. (Yes/No) 2. HIV……. (Yes/No) 3. Syphilis……. (Yes/No) 4. UTI…….. (Yes/No) 5. Helminthes……. (Yes/No)	√		
B7	Vaccinated against tetanus or other	(1) Yes (2) No (3) Other ……	√		
SECTION C: MICRONUTRIENT SUPPLEMENTATION AND COMPLIANCE FACTORS					
C1	Did you receive iron and folate supplements	(1) Yes (2) None (why?) …….		√	
C2	How long did you take supplements?	(1) Less than a week (Why?) …….. (2) Less than a month (Why?) …… … (3) Two months or more		√	
C3	Which type of salt do you commonly use?	(1) Closed container, packet or bottle from shops (Why) …… …… (2) Sold by vendors at the market opened and exposed to the sun		√	
C4	Have you ever	Yes / No		√	

	heard about iodine?				
C5	Mention any importance of iodine?	(1) Correct response (2) Wrong response (3) Don't know		√	
C6	Did you receive anti-malaria prophylaxis (SP)?		√		
C7	Did you receive anti-helminthes prophylaxis (albendazole)?		√		
C8	Are you on long-term drug treatment for any chronic disease?	(1) Tuberculosis treatment (2) Anti-retroviral (3) Steroids (4) Other (specify)……		√	
SECTION D: PLACENTAL WEIGHT, NEWBORN APGAR SCORES & ANTHROPOMETRICS					
D1	Placental weight	…….. gs			√
D2	APGAR scores	At 1 minute …….. /**10** At 5 minutes …….. / **10**			√
D3	Birth weight	…….. **g**			√
D4	Birth length	…… **cm**			√
D5	Head Circumference	…… **cm**			√
D6	Chest circumference	…… **cm**			√

SECTION E: LABORATORY ANALYSIS OF MATERNAL

Maternal blood					
E1	Iodine	(1) TSH……. (2) T3……			√
E2	Iron	(1) Hemoglobin……. **g/dl** (2) Ferritin …… **ug/L**			√

END OF QUESTIONNAIRE

Appendix G: Survey Tool (Chichewa Version)

Dzina la Ofunsa Mafunso …….
Tsiku …….

Official use
Code:……..

GAWO A: ZOKHUDZA AMAYI

ID	*Funso*	*Yankho*	*Onani m'buku*	*Funsani*	*Yezani*
A1	Dzina la Amayi		√		
A2	Zaka za Amayi		√		
A3	Mudzi/kochokera		√		
A4	Sikelo yoyambilira		√		
A5	Ku sikelo munapita kangati?		√		
A6	Msinkhu wa amayi		√		
A7	Pakati ndi miyezi ingati?		√		
A8	Anaberekapo kangati?		√		
A9	Maphunziro	(6) Kufika sitandade 4 (grade 1-4) (7) Kufika sitandade 8 (5-8) (8) Sekondale (9) Kufika sukulu ya ukachenjede (10) Sanapiteko ku sukulu	√		
A10	Amagwira Ntchito yanji?		√		
A11	Pa chaka banja lanu mumapeza ndalama zingati?	**MK**……..		√	

GAWO B: ZA KU SIKELO NDI MADYEDWE ATHANZI					
B1	Sikelo munayamba muli ndi pakati miyezi ingati?	(4) Isanapitilire miyezi itatu (Chifukwa chiani?) …… …… (5) Miyezi yosapitilira isanu ndi umodzi (6) (chifukwa?) …… ……	√		
B2	Mumagwiritsa kulera uti musanatenge mimba iyi?	(4) Obaya/mapilitsi/noropulanti (hormonal) (5) Lupu (physical methods) (6) Palibe sindinatenge kulera	√		
B3	Munadwala matenda anji mimba iyi?	(5) Eya anatigoneka (6) Eya tinangolandira mankhwala (7) Eya zinasiya zokha (8) palibe		√	
B4	Munalandira magazi mimba iyi?	(3) Eya (4) Ayi		√	
B5	Magazi poyamba sikelo (onani buku)	Mulingo……. **g/dl**	√		
B6	Ku sikelo anakuyezani matenda awa:	6. malungo……. (eya/ayi) 7. HIV……. (eya/ayi) 8. Chindoko ……. (eya/ayi) 9. Likodzo…… (eya/ayi) 10. Njoka za mmimba……. (eya/ayi)	√		
B7	Katemera wa kafumbata	(4) Eya (5) Ayi (6) Wina ……	√		
GAWO C: KULONDOLOZA MANKHWALA OCHULUKITSA MAGAZI NDI MICHERE YOFUNIKA M'THUPI					
C1	Munalandira mankhwala ochulukitsa magazi	(3) Eya (4) Ayi (chifukwa?) …… …….		√	

C2	Munamwa mankhwala amagazi nthawi yayitali bwanji?	(4) Osapitilira sabata (chifukwa?) …… …….. (5) Osapitilira mwenzi(chifukwa?) …… …… (6) Miyezi iwiri kapena kupitilira		√	
C3	Kodi kawirikawiri mchere wanu mumagula kuti, ndipo wosungidwa motani?	(3) Ku golosala wa m'botolo/mpaketi (chifukwa) …… …… (4) Kwa mavenda/woyeza		√	
C4	Munabvapo za mchere wa ayodini?	(1) Eya (2) Ayi		√	
C5	Tchulani ubwino wa ayodine?	(4) Yankho lokhoza (5) Yankho lolakwa (6) Sadziwa		√	
C6	Munalandira mankhwala a malungo (SP)?		√		
C7	Munalandira mankhwala a njoka za mmimba (albendazole)?		√		
C8	Muli pa mankhwala aliwonse omwa nthayi yaitali?	(5) Eya a TB (6) Eya ma ARV (7) Eya a asima (steroids) (8) Ena (tchulani)…… ……		√	
GAWO D: NSENGWA/MATENDA NDI MWANA					
D1	Sikelo ya nsengwa (placenta)	…… ….. Magalamu			√
D2	Malilidwe a mwana (APGAR scores)	Mphindi imodzi …….. /**10** Mphindi zisanu …….. / **10**			√

D3	Sikelo ya mwana	…….. **g**			√
D4	Msinkhu	…… **cm**			√
D5	Kukula kwa mutu	…… **cm**			√
D6	Kukula kwa pachifuwa	…… **cm**			√

GAWO E: ZOTSATIRA ZA MMAGAZI (TO BE COMPLETED IN THE LAB)

ZOTSATIRA ZA MAGAZI A MAYI					
E1	Mchere wochulukitsa magazi	(1) Hemoglobin…….. **g/dl** **(2)** Serum folate ……... **ug/L** (3) RBC folate			√

MAFUNSO ATHERA APA

REFERENCES

REFERENCES

Abass RM, Hamdan HZ, Elhassan EM, Hamdan SZ, Ali NI and Adam I (2014) Zinc and copper levels in low birth weight deliveries in Medani Hospital, Sudan. *BMC Research Notes* **7**:386.

Abioye AI, Aboud S, Premji Z, Etheredge AJ, Gunaratna NS, Sudfeld CR, Mongi R, Meloney L, Darling AM and Noor RA (2016) Iron Supplementation Affects Hematologic Biomarker Concentrations and Pregnancy Outcomes among Iron-Deficient Tanzanian Women. *The Journal Of Nutrition*:JN225482.

Abubaker MS (2016) Anaemia and low birth weight in Medani, Hospital Sudan.

Ahmed EB, Ali EA, Mohamed EH, Saleh EA, Elbaset AKA, Mahmmed EM, Elaal ASA, Elsayed AM, Quora AF and Hashem ZM (2015) Assessment of iron and calcium supplements compliance among pregnant women attending antenatal care unit of Al-Sabah Banat primary health care unit in Ismailia, Egypt. *Journal of Medical and Biological Science Research* **1**:24-29.

Ahmed T, Hossain M and Sanin KI (2013) Global burden of maternal and child undernutrition and micronutrient deficiencies. *Annals of Nutrition and Metabolism* **61**:8-17.

Allen LH (2000) Anemia and iron deficiency: effects on pregnancy outcome. *The American Journal Of Clinical Nutrition* **71**:1280s-1284s.

Alwan NA, Cade JE, Greenwood DC, Deanfield J and Lawlor DA (2014) Associations of maternal iron intake and hemoglobin in pregnancy with offspring vascular phenotypes and adiposity at age 10: findings from the Avon Longitudinal Study of Parents and Children. *Plos One* **9**:e84684.

Andersen SL (2015) Iodine status in pregnant & breastfeeding women. *Danish Medical Journal* **62**.

Arnold L, Hoy W and Wang Z (2015) Low birthweight increases risk for cardiovascular disease hospitalisations in a remote Indigenous Australian community–a prospective cohort study. *Australian And New Zealand Journal Of Public Health*.

Balarajan Y, Subramanian S and Fawzi WW (2013) Maternal iron and folic acid supplementation is associated with lower risk of low birth weight in India. *The Journal Of Nutrition* **143**:1309-1315.

Berti C, Biesalski H, Gärtner R, Lapillonne A, Pietrzik K, Poston L, Redman C, Koletzko B and Cetin I (2011) Micronutrients in pregnancy: current knowledge and unresolved questions. *Clinical Nutrition* **30**:689-701.

Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, Haider BA, Kirkwood B, Morris SS and Sachdev H (2008) What works? Interventions for maternal and child undernutrition and survival. *The Lancet* **371**:417-440.

Black MM, Walker SP, Wachs TD, Ulkuer N, Gardner JM, Grantham-McGregor S, Lozoff B, Engle PL and de Mello MC (2008a) Policies to reduce undernutrition include child development. *The Lancet* **371**:454-455.

Black RE (2001) Micronutrients in pregnancy. *British Journal of Nutrition* **85**:S193-S197.

Black RE, Allen LH, Bhutta ZA, Caulfield LE, De Onis M, Ezzati M, Mathers C, Rivera J, Maternal and Group CUS (2008b) Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet* **371**:243-260.

Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, De Onis M, Ezzati M, Grantham-McGregor S, Katz J and Martorell R (2013) Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet* **382**:427-451.

Bondevik GT, Lie RT, Ulstein M and Kvåle G (2001) Maternal hematological status and risk of low birth weight and preterm delivery in Nepal. *Acta Obstetricia Et Gynecologica Scandinavica* **80**:402-408.

Boutayeb A (2006) The double burden of communicable and non-communicable diseases in developing countries. *Transactions of the Royal society of Tropical Medicine and Hygiene* **100**:191-199.

Bradley SEK and Mishra V (2008) HIV and nutrition among women in sub-Saharan Africa, in *DHS Analytical Studies No 16*, Macro International, Calverton, Maryland, USA.

Broadley MR, Chilimba AD, Joy EJ, Young SD, Black CR, Ander EL, Watts MJ, Hurst R, Fairweather-Tait SJ and White PJ (2012) Dietary requirements for magnesium, but not calcium, are likely to be met in Malawi based on national food supply data. *International Journal For Vitamin And Nutrition Research* **82**:191.

Bryce J, Coitinho D, Darnton-Hill I, Pelletier D, Pinstrup-Andersen P, Maternal and Group CUS (2008) Maternal and child undernutrition: effective action at national level. *The Lancet* **371**:510-526.

Cao C and O'Brien KO (2013) Pregnancy and iron homeostasis: an update. *Nutrition Reviews* **71**:35-51.

Cetin I, Berti C and Calabrese S (2010) Role of micronutrients in the periconceptional period. *Human Reproduction Update* **16**:80-95.

Chagomerana MB, Miller WC, Pence BW, Hosseinipour MC, Hoffman IF, Flick RJ, Tweya H, Mumba S, Chimbwandira F and Powers KA (2017) PMTCT Option B+ Does Not Increase Preterm Birth Risk and May Prevent Extreme Prematurity: A Retrospective Cohort Study in Malawi. *JAIDS Journal of Acquired Immune Deficiency Syndromes* **74**:367-374.

Chirwa E and Dorward A (2013) Agricultural input subsidies: The recent Malawi experience, *Oxford University Press*.

Coleman-Jensen A, Gregory C and Singh A (2014) Household food security in the United States in 2013.

da Silva V, Mackey A, Davis S, Gregory J, Ross A, Caballero B, Cousins R, Tucker K and Ziegler T (2014) *Modern Nutrition in Health and Disease*.

Dalal S, Beunza JJ, Volmink J, Adebamowo C, Bajunirwe F, Njelekela M, Mozaffarian D, Fawzi W, Willett W and Adami H-O (2011) Non-communicable diseases in sub-Saharan Africa: what we know now. *International Journal Of Epidemiology* **40**:885-901.

Dieltjens M, Braem MJ, Vroegop AV, Wouters K, Verbraecken JA, De Backer WA, Van de Heyning PH and Vanderveken OM (2013) Objectively measured vs self-reported compliance during oral appliance therapy for sleep-disordered breathing. *Chest* **144**:1495-1502.

Ellis F and Manda E (2012) Seasonal food crises and policy responses: A narrative account of three food security crises in Malawi. *World Development* **40**:1407-1417.

Gebremedhin S, Samuel A, Mamo G, Moges T and Assefa T (2014) Coverage, compliance and factors associated with utilization of iron supplementation during pregnancy in eight rural districts of Ethiopia: a cross-sectional study. *BMC Public Health* **14**:607.

Glinoer D, De Nayer P, Delange F, Lemone M, Toppet V, Spehl M, Grün J-P, Kinthaert J and Lejeune B (1995) A randomized trial for the treatment of mild iodine deficiency during pregnancy: maternal and neonatal effects. *The Journal of Clinical Endocrinology & Metabolism* **80**:258-269.

Hadjimarkos D, Bonhorst CW and Mattice JJ (1959) The selenium concentration in placental tissue and fetal cord blood. *The Journal Of Pediatrics* **54**:296-298.

Haeussner E, Schmitz C, von Koch F and Frank H-G (2013) Birth weight correlates with size but not shape of the normal human placenta. *Placenta* **34**:574-582.

Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M and Fawzi WW (2013) Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ* **346**:f3443.

Hill Z, Kirkwood B, Kendall C, Adjei E, Arthur P and Agyemang CT (2007) Factors that affect the adoption and maintenance of weekly vitamin A supplementation among women in Ghana. *Public Health Nutrition* **10**:827-833.

Hjertholm KG, Iversen PO, Holmboe□Ottesen G, Mdala I, Munthali A, Maleta K, Shi Z, Ferguson E and Kamudoni P (2017) Maternal dietary intake during pregnancy and its association to birth size in rural Malawi: A cross□sectional study. *Maternal & Child Nutrition*.

Holme AM, Roland MCP, Lorentzen B, Michelsen TM and Henriksen T (2015) Placental Glucose Transfer: A Human In Vivo Study. *Plos One* **10**:e0117084.

Kalimbira A, Mtimuni B and Chilima D (2009) Maternal knowledge and practices related to anaemia and iron supplementation in rural Malawi: a cross-sectional study. *African Journal of Food, Agriculture, Nutrition and Development* **9**:550-564.

Kaunitz AM, Portman D, Westhoff CL, Archer DF, Mishell DR and Foegh M (2015) Self-reported and verified compliance in a phase 3 clinical trial of a novel low-dose contraceptive patch and pill. *Contraception* **91**:204-210.

Khera A, Dong L-f, Holland O, Vanderlelie J, Pasdar EA, Neuzil J and Perkins AV (2015) Selenium supplementation induces mitochondrial biogenesis in trophoblasts. *Placenta* **36**:863-869.

Kishosha P, Galukande M and Gakwaya A (2011) Selenium deficiency a factor in endemic goiter persistence in sub-Saharan Africa. *World Journal Of Surgery* **35**:1540-1545.

Lager S and Powell TL (2012) Regulation of nutrient transport across the placenta. *Journal Of Pregnancy* **2012**.

Lau C, Ambalavanan N, Chakraborty H, Wingate MS and Carlo WA (2013) Extremely low birth weight and infant mortality rates in the United States. *Pediatrics* **131**:855-860.

Makrides M, Crowther CA, Gibson RA, Gibson RS and Skeaff CM (2003) Efficacy and tolerability of low-dose iron supplements during pregnancy: a randomized controlled trial. *The American Journal Of Clinical Nutrition* **78**:145-153.

Malawi Government (2009) A Report for the National Micronutrient survey 2009, pp 1 - 111, Malawi Health Ministry, Lilongwe.

Meher A, Sundrani D and Joshi S (2015) Maternal nutrition influences angiogenesis in the placenta through peroxisome proliferator activated receptors: A novel hypothesis. *Molecular Reproduction And Development* **82**:726-734.

Mistry HD, Wilson V, Ramsay MM, Symonds ME and Pipkin FB (2008) Reduced selenium concentrations and glutathione peroxidase activity in preeclamptic pregnancies. *Hypertension* **52**:881-888.

Morris SS, Cogill B, Uauy R, Maternal and Group CUS (2008) Effective international action against undernutrition: why has it proven so difficult and what can be done to accelerate progress? *The Lancet* **371**:608-621.

Müller O and Krawinkel M (2005) Malnutrition and health in developing countries. *Canadian Medical Association Journal* **173**:279-286.

Muula A, Siziya S and Rudatsikira E (2011) Parity and maternal education are associated with low birth weight in Malawi. *African health sciences* **11**.

Nagothu US (2014) *Food Security and Development: Country Case Studies*, Routledge.

Ngwira A and Stanley CC (2015) Determinants of Low Birth Weight in Malawi: Bayesian Geo-Additive Modelling. *PloS One* **10**:e0130057.

Nisar YB and Dibley MJ (2016) Antenatal iron–folic acid supplementation reduces risk of low birthweight in Pakistan: secondary analysis of Demographic and Health Survey 2006–2007. *Maternal & child nutrition* **12**:85-98.

NSO-ICF (2017) Malawi Demographic and Health Survey 2015 - 2016, pp 1 - 221, National Statistical Office (NSO) and ICF, Zomba Malawi & Rockville Maryland.

Owens S, Gulati R, Fulford AJ, Sosseh F, Denison FC, Brabin BJ and Prentice AM (2015) Periconceptional multiple-micronutrient supplementation and placental function in rural Gambian women: a double-blind, randomized, placebo-controlled trial. *The American Journal Of Clinical Nutrition* **102**:1450-1459.

Pasricha S-R, Drakesmith H, Black J, Hipgrave D and Biggs B-A (2013) Control of iron deficiency anemia in low-and middle-income countries. *Blood* **121**:2607-2617.

Preziosi P, Prual A, Galan P, Daouda H, Boureima H and Hercberg S (1997) Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. *The American Journal Of Clinical Nutrition* **66**:1178-1182.

Raghunath D, Kujur A, Dixit S, Sabnani S, Yadav S and Saroshe S (2016) Multivariate Analysis of the Factors affecting Low Birth Weight-A Case-Control Study in a Tertiary hospital of Central India. *Annals of Community Health* **4**:18-24.

Rogerson SJ, Brown HC, Pollina E, Abrams ET, Tadesse E, Lema VM and Molyneux ME (2003) Placental tumor necrosis factor alpha but not gamma interferon is associated with placental malaria and low birth weight in Malawian women. *Infection And Immunity* **71**:267-270.

Rwebembera AA-B, Munubhi E, Manji K, Mpembeni R and Philip J (2006) Relationship between infant birth weight≤ 2000 g and maternal zinc levels at Muhimbili National Hospital, Dar Es Salaam, Tanzania. *Journal Of Tropical Pediatrics* **52**:118-125.

Schultink W, van der Ree M, Matulessi P and Gross R (1993) Low compliance with an iron-supplementation program: a study among pregnant women in Jakarta, Indonesia. *The American Journal Of Clinical Nutrition* **57**:135-139.

Smith C, Ryckman K, Barnabei V, Howard B, Isasi CR, Sarto G, Tom S, Van Horn L, Wallace R and Robinson J (2016) The impact of birth weight on cardiovascular disease risk in the Women's Health Initiative. *Nutrition, Metabolism and Cardiovascular Diseases* **26**:239-245.

Steer PJ (2000) Maternal hemoglobin concentration and birth weight. *The American Journal Of Clinical Nutrition* **71**:1285s-1287s.

Steketee RW, Wirima JJ, Hightower AW, Slutsker L, Heymann DL and Breman JG (1996) The effect of malaria and malaria prevention in pregnancy on offspring birthweight, prematurity, and intrauterine growth retardation in rural Malawi. *The American Journal Of Tropical Medicine And Hygiene* **55**:33-41.

Sununtnasuk C, D'Agostino A and Fiedler JL (2016) Iron+ folic acid distribution and consumption through antenatal care: identifying barriers across countries. *Public Health Nutrition* **19**:732-742.

Thirukkanesh S and Zahara A (2010) Compliance to vitamin and mineral supplementation among pregnant women in urban and rural areas in Malaysia. *Pakistan Journal of Nutrition* **9**:744-750.

Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP and Steegers EA (2009) Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *British Journal of Nutrition* **102**:777-785.

Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, Sachdev HS, Maternal and Group CUS (2008) Maternal and child undernutrition: consequences for adult health and human capital. *The Lancet* **371**:340-357.

Wang L, Mei Z, Li H, Zhang Y, Liu J and Serdula MK (2016) Modifying effects of maternal Hb concentration on infant birth weight in women receiving prenatal iron-containing supplements: a randomised controlled trial. *British Journal of Nutrition* **115**:644-649.

Wardlaw TM (2004) Low birthweight: country, regional and global estimates, *UNICEF*.

Watts M, Joy E, Young S, Broadley M, Chilimba A, Gibson R, Siyame E, Kalimbira A, Chilima B and Ander E (2015) Iodine source apportionment in the Malawian diet. *Scientific Reports* **5**.

West KP and Christian P (2008) Antenatal micronutrients in undernourished people. *The Lancet* **371**:452-454.

Westfall B, Stohlman E and Smith M (1938) The placental transmission of selenium. *Journal of Pharmacology and Experimental Therapeutics* **64**:55-57.

WHO (2012) Guideline: daily iron and folic acid supplementation in pregnant women. *World Health Organization*.

WHO (2015) Serum and red blood cell folate concentrations for assessing folate status in populations. *World Health Organization*.

Win AZ (2016) Micronutrient deficiencies in early childhood can lower a country's GDP: The Myanmar example. *Nutrition* **32**:138-140.

Young M, Lupafya E, Kapenda E and Bobrow E (2000) The effectiveness of weekly iron supplementation in pregnant women of rural northern Malawi. *Tropical Doctor* **30**:84-88.

Zhou L-M, Yang W-W, Hua J-Z, Deng C-Q, Tao X and Stoltzfus RJ (1998) Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. *American Journal Of Epidemiology* **148**:998-1006.

Zimmermann MB (2011) The role of iodine in human growth and development, in *Seminars In Cell & Developmental Biology* PP 645-652, Elsevier.