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Addis Ababa University

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Acronyms

AAS - Atomic Absorption Spectroscopy

ANC - Antenatal Care

ASF - Animal Source Food

BMI - Body Mass Index

BP - Blood Pressure

DDS - Dietary Diversity Score

EDHS - Ethiopian Demographic Health Survey

EDTA - Ethylene Diamine Tetra Acetic Acid

EPHI - Ethiopian Public Health Institute

Hb - Hemoglobin

HIV - Human Immune Virus

IDA - Iron Deficiency Anemia

IQ - Intelligence Quotient

IUGR - Intra Uterine Growth Retardation

LBW - Low Birth Weight

MMR - Maternal Mortality Rate

NGO - Nongovernmental Organization

SPSS - Statistical Package for Social Science students

TIBC - Total Iron Binding Capacity

UNICEF - United Nations Children's Fund

USA - United States of America

WHO - World Health Organization

Abstract

Background: Micronutrients are life sustaining nutrients that are needed in small quantities for effective functioning of human metabolic activities and development. Deficiency of most micronutrients can result in adverse pregnancy and birth outcomes.

Objective: To determine the influence of third trimester pregnant women's dietary habit on anemia status, cord blood micronutrient concentration and neonatal birth weight.

Method: A prospective case series study design and convenient sampling technique were used. 123 third trimester pregnant women were interviewed to assess their socio-demographic characteristics, dietary pattern, and clinical conditions. The hemoglobin values of the women were determined using HemoCue. Among these subjects, neonatal birth weight and cord blood was collected from 57 subjects out of whom 49 delivered at term. The cord blood micronutrient concentrations were determined using atomic absorption spectroscopy. Descriptive analysis, Bivariate, and multivariable regression analysis were the statistical tools used in this study.

Results: About 35.8 % of the pregnant women were anemic. The mean age (years) of the pregnant women was 25.8 ± 5.4 (SD) and 55.3 % were multiparous. The most commonly consumed food by the subjects was cereals (98.4%) and coffee was found to be the most commonly consumed drink (93.5%). Majority of the pregnant women (57.8 %) had three meals a day. Maternal factors such as, dietary diversity score ($\beta = 0.413$, $P = 0.003$), level of education ($\beta = 0.541$, $P < 0.001$), body mass index ($\beta = 0.162$, $P = 0.013$), consumption of cereals ($r = 0.407$, $P = 0.001$) and carbonated drinks ($\beta = 0.954$, $P = 0.002$) had positive linear relationship with hemoglobin. Whereas, factors such as the amount of cups of tea taken per day ($\beta = -0.374$, $P = 0.021$), consumption of legumes ($\beta = -0.579$, $P = 0.014$), roots and tubers ($\beta = -0.700$, $P = 0.004$), and level of fasting ($r = -0.374$, $P = 0.038$) were negatively associated with hemoglobin. Preeclampsia ($r = 0.258$, $P=0.038$), and vitamin A-rich foods ($\beta = -0.228$, $P = 0.048$) were found to be predictors of anemia. The maternal age ($\beta = -1.867$, $P = 0.038$), body mass index ($r = -0.330$, $P = 0.010$) and preeclampsia ($\beta = -0.653$, $P = 0.001$) showed negative correlation with cord blood iron, calcium and zinc concentration respectively. The amount of coffee taken per day was also negatively associated with cord blood calcium concentration ($\beta = -24.997$, $P = 0.018$). Parity, consumption of roots and tubers and umbilical cord blood iron concentration were important predictors of neonatal birth weight ($\beta = -0.336$, $P = 0.007$, $\beta = -0.196$, $P = 0.037$and $\beta = -0.158$, $P = 0.005$) respectively.

Conclusion: The dietary pattern, nutritional status, and pre-eclempsia of third trimester pregnant women were associated with maternal anemia and cord blood micronutrient concentration. Root and tuber consumption, cord blood iron concentration, and parity were negatively associated with neonatal birth weight. Health education that includes reproductive health and optimal nutrition during pregnancy is needed.

Key words: anemia, third trimester pregnant women, umbilical cord blood micronutrient concentration, dietary habit

Chapter one

1. Background

Micronutrients are life sustaining nutrients that are needed only in small quantities for effective functioning of brain, the immune system and energy metabolism. Deficiency of micronutrients affects about 2 billion people worldwide. The groups most vulnerable to micronutrient deficiencies are pregnant women, lactating women and young children; mainly because they have a relatively greater need for vitamins and minerals and are more susceptible to the harmful consequences of deficiencies. For pregnant women, these include a greater risk of dying during child birth or of giving birth to an underweight or mentally impaired baby (Dairo *et al* 2009).

Micronutrient deficiencies occur not only in developing countries where inadequate access to micronutrient dense food is a frequent problem, but also in industrialized countries where food habits and preferences can favor foods of low micronutrient content (Abebe 2013). In developing countries monotonous diet predominantly cereal and legume based diet with little consumption of animal source food remain the major public health problems contributing to micronutrient deficiency related problems during pregnancy which may affect the neonatal birth weight (Taseer 2011).

If pregnancy occurs during adolescence, anemia not only increases maternal morbidity and mortality, but increases the incidence of poor birth outcomes in infant (i.e. low birth weight and prematurity) and also negatively impact iron status. Maternal under-nutrition (inadequate dietary intake) is significantly linked with infant low birth weight, due to preterm birth or intrauterine growth restriction, an underlying factor in 60-80% of neonatal deaths (Unicef/WFP/WHO 2010). Low maternal body mass index is associated with intrauterine growth restriction and low birth weight. Thus, ensuring adequate dietary intake during pregnancy is crucial.

According to data from EDHS 2011, maternal mortality ratio (MMR) in Ethiopia was very high (676 per 100,000 live births). Stunting prevalence is also high (44%) which part of it may be from intrauterine growth retardation (IUGR). In addition, inadequate dietary intake during pregnancy may be part of the problem (EDHS 2011).

Although pregnant women in the third trimester relied on a monotonous diet in Ethiopia, prevalence of inadequate iron intake was surprisingly low (4%) whereas, inadequate Zinc intake was very high (99%) and it was also indicated that such dietary deficits could be overcome by regular consumption of cellular animal protein (Abebe et al 2008).

Generally, the prevalence of micronutrient deficiency is becoming the major public health concern than before in developing countries like Ethiopia. Therefore, to prevent such nutritional deficiencies problem solving studies and implementation of different cost effective strategies are crucial.

1.1 Statement of the problem

Maternal factors such as socio-economic, dietary pattern body mass index (BMI) and umbilical cord blood micronutrient concentrations have an effect on the birth weight. Deficiency of micronutrients during intrauterine period is closely related to morbidity and mortality of the newborn (Sahu et al 2013, Sharma *et al* 2013 and Shao *et al* 2012).). Thus, pregnant women have increased demand of micronutrients and they are crucial for the health of the mother and the developing fetus (Black 2001).

Maternal malnutrition is caused by inadequate dietary intake (calories, proteins and micronutrients). A mother chronically undernourished will likely give birth to a reduced weight baby compared to others. During pregnancy, there is an increase in nutrients requirements to take care of fetal, placenta and increase in maternal weight. The impact of micronutrient deficiency on pregnancy and birth outcome is associated with increased maternal morbidity and mortality, abortions, stillbirth, low birth weight, premature birth, congenital abnormalities and mental deficiencies in early childhood (Hornstra *et al* 2004, Black 2001).

Iron deficiency is the most common nutritional deficiency in pregnancy and has an important impact on maternal and fetal morbidity and mortality. Although the sign of anemia is not manifested, pregnant women at any stage have iron deficiency anemia. Maternal iron deficiency during pregnancy induces anemia in developing fetus; however, the severity tends to be less than in the mother. This is because placental iron transporter proteins are up regulated in maternal iron deficiency resulting in an increased efficiency of iron influx and a consequent minimization of fetal anemia (Gambling *et al* 2001). During fetal life zinc is required to synthesize protein, promote fetal growth and responsible for embryogenesis. In addition, zinc regulates immune cells, aids in the development of fetal brain and the mother in the first and second stage of labor (Hurley *et al* 1966 and Rathore *et al* 2011). Zinc deficiency can lead to clinically relevant disturbances in tissue functions and particularly important for birth weight of neonates (Khadem *et al* 2012). Studies indicated that zinc deficiency is responsible for 4.4% of child death and 1.2% of the burden of diseases in Latin America, Asia and Africa (Fischer *et al* 2009). Furthermore, the calcium need of both mothers and fetus significantly increases during third trimester. The fetus puts most of its bone calcium accumulation during third trimester. The maternal calcium absorption increases at this stage to maintain the fetal demand with an increase in maternal calcium turnover (Ritchie *et al* 1998). There are biological limits to a pregnant women's capacity to increase calcium absorption and if she does not consume adequate amounts of dietary calcium, she may be at increased risk for gestational complications, such as preeclampsia and preterm delivery or long term morbidities, such as excessive bone loss (Hacker *et al* 2012).

In low and middle income countries, complications from pregnancy and child birth are leading cause of death despite 81 % of the pregnant women receive antenatal care services at least once over the period 2005-2012 (WHO 2013). Therefore, this study was designed to assess whether neonatal birth weight is affected by maternal dietary habit, anemia status and/or umbilical cord blood micronutrient concentration. Thus, the findings of this study are imperative to design recommendations for policy makers and health educators to promote maternal and child health.

1.2 Research question

Is there any significant association between third trimester pregnant women's dietary habit, anemia status, cord blood micronutrient concentration, and the neonatal birth weight?

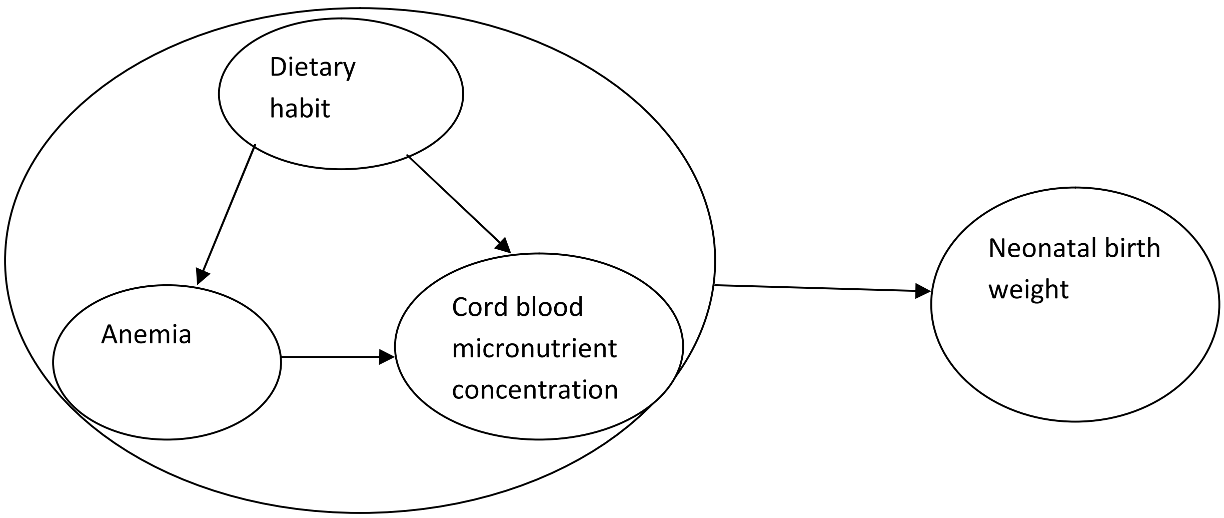


Fig. 1 Conceptual framework of the study

1.3. Objective

To determine the association of third trimester pregnant women dietary habit, anemia status and cord blood micronutrient concentration with neonatal birth weight.

1.4 Specific objectives

1. To assess the dietary habit of third trimester pregnant women
2. To determine the anemia status of pregnant women during their third trimester
3. To determine the neonatal birth weight
4. To analyze concentration of micronutrient in cord blood
5. To study the relationship between maternal micronutrient status and neonatal birth weight

Chapter two

2. Literature review

2.1 Nutrition and pregnancy

Nutritional deficiencies are preventable etiological and epigenetic factors causing congenital abnormalities, first cause of infant mortality. Micronutrient deficiencies, such as folate deficiency has a well established teratogenic effect (Safi et al 2012).

Maternal nutritional status has important implications for the health of both mothers and children. Women in poor nutritional health face a greater risk of an adverse pregnancy and are more likely to give birth to children who are not healthy (Macro international inc. 2007).

Nutritional requirements during pregnancy increase to support fetal growth and development as well as maternal metabolism and tissue accretion. Micronutrients are involved in both embryonal and fetal organ development and overall pregnancy outcomes. Several factors may affect directly or indirectly fetal nourishment and the overall pregnancy outcomes, such as the quality of diet including intakes and bioavailability of micronutrients, maternal age, and the overall environment. The bioavailability of micronutrients during pregnancy varies depending on specific metabolic mechanisms because pregnancy is an anabolic and dynamic state orchestrated via hormones acting for both redirection of nutrients to highly specialized maternal tissues and transfer of nutrients to the developing fetus. The timing of prenatal intakes or supplementations of specific micronutrients is also crucial as pregnancy is characterized by different stages that represent a continuum, up to lactation and beyond. Consequently, nutrition during pregnancy might have long-lasting effects on the well-being of the mother and the fetus, and may further influence the health of the baby at a later age (Berti et al 2010).

Maternal malnutrition is caused by inadequate dietary intake (calories, proteins and micronutrients). A mother chronically undernourished will likely give birth to an underweight baby. During pregnancy there is increase in nutrients requirements to take care of fetal, placenta and increase in maternal weight. The impact of micronutrient deficiency on pregnancy and birth outcome is associated with increased maternal morbidity and mortality, abortions, stillbirth, low birth weight, premature birth, congenital abnormalities and mental deficiencies in early childhood (Hornstra et al 2004, Black 2001).

American Journal of nutrition reported that pregnant women usually meet their increased energy needs but do not always meet their increased micronutrient requirements. Thus, the supply of micronutrients has been related to positive pregnancy and infant outcomes (Krauss-Etschmann et al 2007). Another similar journal also revealed that maternal zinc concentration was associated with neonatal development and its deficiency can result in prolonged labour pain, spontaneous abortion, maternal mortality and decreased birth weight (Tamura *et al* 1996).

2.2 Diet, micronutrients and neonatal birth weight

Micronutrients are life sustaining nutrients that are needed only in small quantities for effective functioning of human metabolic activities and development. Deficiency of micronutrients affects about 2 billion people all over the world. The groups most vulnerable to micronutrient deficiencies are pregnant women, lactating women and young children. (Dairo *et al* 2009).

Calcium concentration was found to be associated with pregnancy complications like gestational hypertension and preeclampsia (Ramakrishnan 2002). During fetal life zinc is required to synthesize protein, promote fetal growth and responsible for embryogenesis. It also plays major role in regulation of immune cells (Hurley *et al* 1966). In pregnancy, zinc also aids in the development of fetal brain and the mother in the first and second stage of labor (Rathore *et al* 2011). At least 17 million infants are born every year with LBW, representing about 16% of all newborns in developing countries. Nearly 80% of all intra uterine growth retarded newborns who are LBW and full term are born in Asia. About 15% and 11% are born full term with LBW and IUGR in middle and western Africa respectively and approximately 7% in the Latin America and Caribbean region (ACC/SCN 2000).

Infants born with low birth weight (less than 2500 grams) suffer from extremely high rates of morbidity and mortality from infectious disease, and are underweight, stunted or wasted beginning in the neonatal period through childhood. Infants weighing 2000−2499g at birth are 4 times more likely to die during their first 28 days of life than infants who weigh 2500−2999 g, and 10 times more likely to die than infants weighing 3000−3499 g (ACC/SCN 2000). Stunting and other forms of undernutrition are clearly a major contributing factor to child mortality, disease and disability. For example, a severely stunted child faces a four times higher risk of dying (Unicef 2013).

In developing countries, major determinants for LBW are poor maternal nutritional status at conception, low gestational weight gain due to inadequate dietary intake and low short maternal stature due to the mother's own childhood undernutrition and/or infection (ACC/SCN 2000).

A literature review report of 70 articles by Kristle Elzabth in USA indicated that there was no evidence to establish a relationship between maternal eating disorders and the occurrence of fetal birth defects or delay in developmental millstones. Evidence did support a relationship between the occurrences of adverse pregnancy outcomes in women with eating disorders (Neuhalfen 2007).

According to UNICEF, in south Asia, each year between 15 to 30 million infants, i.e. up to 20% of all infants are born with a low birth weight. Hence, LBW infants are at risk of 40fold greater chance of dying in the neonatal period, a 50% greater chance of serious development problems, e.g. learning disabilities and mental retardation, IQ point decrease of 5 to 10 point, long term disabilities and premature death. The report also indicated that the causes of LBW baby is due to maternal nutrition, high maternal blood pressure, teenage pregnancy, multiple birth and intra uterine growth retardation (IUGR) (Unicef 2002).

Birth weight is affected to a greater extent by the mother's own foetal growth and here diet from birth to pregnancy, and thus, here body composition at conception. Mothers in deprived socio-economic conditions frequently have low birth weight infants (Wardlaw *et al* 2004).

A study conducted in Nepal indicated that birth weight is an important determinant of infants well being as LBW is known to increase the risk adult onset of disease like type 2 diabetes and ischemic heart disease. The study concluded that gestational weight gain has positive linear relationship (correlation) with the birth weight of infants (Shrestha *et al* 2010).

Another study in Brazil revealed that extremely preterm infants have low mineral reserves and, as a consequence, may have deficiencies in the postnatal period if they do not receive parenteral or enteral supplementation. There are controversies about the outcome and the influence of post-discharge nutrition on bone disease of prematurity (Trindae 2005). A research in Korea indicated that the intakes of iron and folate consumed by pregnant women from food sources alone were much lower than the recommended nutrient and this may have an impact on neonatal birth weight (Park *et al* 2012).

In China, a research done on dietary intake and food habits of pregnant women indicated that more than half (54.7%) of the women participated in the study increased their food consumption among which soup with varieties of meat and vegetables accounted for 85.5% of all food increases (Gao *et al* 2013). Whereas, another study conducted in Bangladesh among pregnant women revealed that betel nut consumption combined with tobacco is associated with lower serum foliate level and folate deficiency (Kader 2013).

A research conducted in Southern Ethiopia among pregnant women revealed that the prevalence of micronutrient intake inadequacy was very high. It was indicated that the prevalence of Zn and Ca intake inadequacy was 99% and 74% respectively. Whereas, iron intake inadequacy was surprisingly low (4%) (Abebe *et al* 2009). Human eating behavior depends on biological and cultural behaviors. Perceptions and food taboos are some factors that can affect the eating pattern of pregnant women.

2.3 Cord blood micronutrients

In United Arab Emirates, a study on maternal-infant micronutrient status and the effects on birth weight indicated that 25-hydroxy vitamin D concentrations were low in mothers and infants. Here, the maternal serum 25-hydroxy vitamin D correlated positively with birth weight while serum ferritin showed a negative correlation (Amirlak *et al* 2009).

Likewise, another study in India on umbilical cord blood nutrients in low birth weight babies in relation to birth weight and gestational age indicated nutrients like protein; cholesterol, triglycerides, calcium, magnesium, zinc, and iron were significantly lower in low birth weight babies compared to term control babies. These values were lowest in preterm low birth weight (70.25±24.59µg/dl, 75.73±22.65µ/dl and 5.67±0.89mg/dl for zinc, iron and calcium respectively) followed by term low birth weight (78.09±18.39µg/dl, 86.74±21.97µg/dl and 7.11±0.94mg/dl for zinc, iron and calcium respectively). Total iron binding capacity (TIBC) showed inverse association with iron. Some of the babies including control babies had protein, albumin, calcium, and iron below the normal range and mean albumin, calcium and iron levels were below the normal range in all the three sub-sets. In term control babies, the values of zinc, iron and calcium in cord blood were 92.24±19.4µg/dl, 96.25±21.08µg/dl and 8.08±0.96mg/dl respectively while the normal values were 64-118µg/dl, 100-250µg/dl and 9-11.5mg/dl respectively for each parameter (Elizabeth *et al* 2008).

Mothers with a reduced store of iron at term were able to provide sufficient iron for the fetus (Wong *et al* 1990). However, in Saudi Arabia, Zainab A. *et al* reported that there is no correlation between maternal hemoglobin value and cord blood iron concentration (Babay *et al* 2002). But, it is fact that physiologic regulation of iron transfer is determined at gut level and iron need of the fetus takes priority over the maternal requirement (O'Brien *et al* 2003).

In Peru, a study on changes on the concentration of biochemical indicators of diet and nutritional status of pregnant women across pregnancy trimesters showed that none of the women were vitamin A and E deficient at any stage of pregnancy and only 1/62 women (1.6%) was selenium deficient during the third trimester. However, 6.4%, 44% and 64% of women had ferritin levels indicative of iron deficiency during the first, second and third trimester respectively. Statistically significant changes throughout pregnancy were noted for nutritional indicators with little to no association with demographic characteristics. Levels of iron status indicators (ferritin, transfferin saturation and iron) were higher in cord serum than in maternal serum (Horton *et al* 2013).

A study involving effect of gestational age on cord blood plasma copper, zinc, magnesium, and albumin in USA indicated that while cord plasma zinc decreased from (116.3±45.2µg/dl to 94.1±18.7µg/dl to 89.4±14.9µg/dl to 87.0±9.5µg/dl) as gestational age at birth increases from 24-28 to 29-33 to 34-37 to 38-42 respectively the reverse is true for copper. There were no differences with gestational age in maternal plasma zinc or copper. However, maternal ceruloplasmin tended to decrease with gestational age. Maternal and cord blood plasma magnesium exhibited a strong correlation as well as between cord plasma magnesium and zinc ($P<0.01$) (Perveen *et al* 2002).

Correspondingly, a comparative study of trace elements and serum ceruloplasmin level in normal and preeclamptic pregnancies with their cord in India showed that in normal pregnancy, the copper and ceruloplasmin concentration were statistically significantly increased in maternal blood as compared to cord blood. Serum iron and zinc concentration were observed significantly raised in cord blood compared to maternal blood. The significant difference between was observed in all biochemical parameters between maternal and cord blood preeclamptic pregnancies. On comparison of the normal and preeclamptic pregnancy the serum iron significantly increased (153.4±40.3µg/dl) in normal pregnancy than preeclamptic (118.6±44.6µg/d). The concentration of zinc in cord blood in normal and preeclamptic pregnancy was 90.8±28.4 µg/dl and 79.9±24.7 µg/dl respectively. However, there was no statistically significant variation in serum zinc, copper and ceruloplasimin concentration (Rathore *et al* 2011). Indeed, depletion of maternal serum zinc concentration contributes to preeclampsia during third trimester (Al-Jameil *et al* 2014, Gupta *et al* 2014).

2.4 Anemia

Anemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children. It is a disease with multiple causes, both nutritional (vitamin and mineral deficiencies) and non-nutritional (infection) that frequently co-occur. It is assumed that one of the most common contributing factors is iron deficiency, and anemia resulting from iron deficiency is considered to be one of the top ten contributors to the global burden of disease (Badham *et al* 2007).

Pregnancy is associated with increased demand of all the nutrients like Iron, Copper, Zinc etc. and deficiency of any of these could affect pregnancy, delivery and outcome of pregnancy. With this consideration, a study report on 80 mothers from India showed that 34 had Iron deficiency anemia and their Hb levels were below 9.0gm/dl. Pregnant women had significantly lower Iron (72.7±7.3µg/dl) and Zinc (70.5±11.2µg/dl) levels while Copper was significantly higher (187.3±19.9µg/dl) (Upadhyaya *et al* 2004).

Cereal products, legumes and nuts contain iron and zinc inhabitants like 5 and 6 phospho inositol (Brune *et al* 1992). Hence, diets largely contain cereals are not good source of iron and eventually they can impair bioavailability of iron (Dallman *et al* 1980). On the other hand, consumption of foods rich in vitamin A is crucial because women with low serum vitamin A are 1.8 times at greater risk of acquiring anemia compared to women who are not vitamin A deficient (Ahmed *et al* 2003).

A cross-sectional study conducted in Pakistan by Abbasi A. *et al* on the causes of maternal anemia among pregnant women showed a linear but negative correlation between level of education and anemia i.e. as the level of education decreases anemia decreases. It was also showed that dietary habit was associated with hemoglobin values of the respondents. The intake had also association with hemoglobin concentration. The eating pattern of the respondents showed that about 33.9% take meal twice a day while 57% respondents practice it 3 times. It was also indicated that 6.9% respondents enjoy it 4 times a day while only 2.1% of the respondents take meal more frequently in a day. About 36.7% of respondents take tea once a day while

43.6%, 13.2% and 6.5% of the respondents take tea twice, trice and more than trice respectively. Among these study subjects about 52.9% have been taking iron supplement and 47% never took iron supplement (Abbasi *et al* 2013). Other studies also showed that maternal socio-demographic characteristics could be important indicators of maternal anemia (Sharma *et al* 2013).

A research in Ethiopia on prevalence and associated factors among pregnant women in an urban area of eastern Ethiopia indicated that the staple diet of the subjects was rice and Spaghetti (87.3%) and about 66.2% of them were used to eat three times a day. Majority (98.8%) of the respondents were drank tea and 77.9% drank tea before meal and 59.5% of them consumed tea more than twice a day. The prevalence of anemia was 56.8% with mean hemoglobin concentration of 10.79 ± 1.47. (Alene *et al* 2014).

In a research conducted in USA on anemia and pregnancy: a link to maternal diseases it was indicated that anemia is often worsened by chronic communicable and non-communicable diseases, the most important being malaria, HIV, tuberculosis and diabetes. When anemia occurs in pregnancy it not only results in poor pregnancy outcome in the short term but, in the long term it also leads to worsening of these chronic conditions, reduced work capacity and impaired cognitive development of the child (Gangopadhyay *et al* 2011).

Furthermore, Indian journal of medicine reported that under nutrition of micronutrients have serious implications on the developing fetus. Nearly half the pregnant women suffer from varying degree of anemia, with the highest prevalence in India, which also has the highest number of maternal deaths in Asia region. The report also indicated that optimal weight gain during pregnancy and a desirable foetal outcome can be achieved through synergistic effects of improved food intake, food supplementation, improved micronutrient intake, education and the environment of the pregnant woman and her family (Muthayya 2009). In addition, a study conducted in southern Punjab among pregnant women showed that high frequency of anemia was related with poor dietary habits especially poor iron intake (Taseer et al 2011).

Similarly, in India, it was stated that prevalence of anemia is higher among pregnant women and preschool children. Even among higher income educated segments of population about 50% of children, adolescent girls and pregnant women were anemic. Inadequate dietary iron, foliate intake due to low vegetable consumption, perhaps low B12 intake and poor bioavailability of dietary iron from the fiber, phytate rich Indian diets were the major factors responsible for high prevalence of anemia. Increased requirement of iron during growth and pregnancy and chronic blood loss contribute to higher prevalence in specific groups. In India, anemia is directly or indirectly responsible for 40% of maternal deaths. There is 8 to 10 fold increase in MMR when the Hb falls below 5gm/dl (Kalaivani *et al* 2009).

In addition, another study in India revealed that prevalence of anemia was 96.3%, with mean Hb value of 8.84±1.12 and 7.85±1.58g/dl during the middle and last gestational ages respectively. This research also indicated that the food habit of the respondents was with two main meals a day. Thus, cereals intake suffice 40-50% of the total recommendations while the intake of tubers and dairy products were 30-40% of recommendations. Pulse and other vegetables intake was poor being only about 20% of the recommendation intake. Green leafy vegetables and fruits intake was even less than 10% and 20% of the desired levels respectively (Garg *et al* 2006).

Moreover, maternal anemia is associated with poor intrauterine growth and increased risk of preterm births and low birth weight rates. This in turn results in higher prenatal morbidity and mortality and higher infant mortality rate. A doubling of low birth weight rate and 2 to 3 fold increase in the prenatal mortality rate was seen when the Hb is <8g/dl. Parental height and maternal weight are determinants of intrauterine growth and birth weight. Thus, maternal anemia contributes to intergenerational cycle of poor growth and birth weight (Kalaivani 2009).

In Turkey, it was found that anemia prevalence among pregnant women was 27.1% (Hb<11.0g/dl). Having four or more living children and being at the third trimester were determinants of anemia in pregnancy. This report also indicated that tea intake reduces iron absorption (Karaoglu *et al* 2010). However, consuming tea between meals with vitamin C rich foods were recommended to prevent anemia (Zijp *et al* 2008).

In Jordan, it was revealed that the overall prevalence of anemia and mean Hb concentration among pregnant women were found to be 56.7% and 9.8±1.4g/dl respectively. The highest prevalence of severe anemia was found among third trimester pregnant women. It was also indicated that anemia prevalence advances greater as the gestation increases (Salahat *et al* 2012). On the contrary, a study conducted on the prevalence of IDA among pregnant women attending ANC at Al-Hada hospital of Saudi Arabia indicated that the prevalence of anemia was only 22.6% (Elzahrani *et al* 2012).

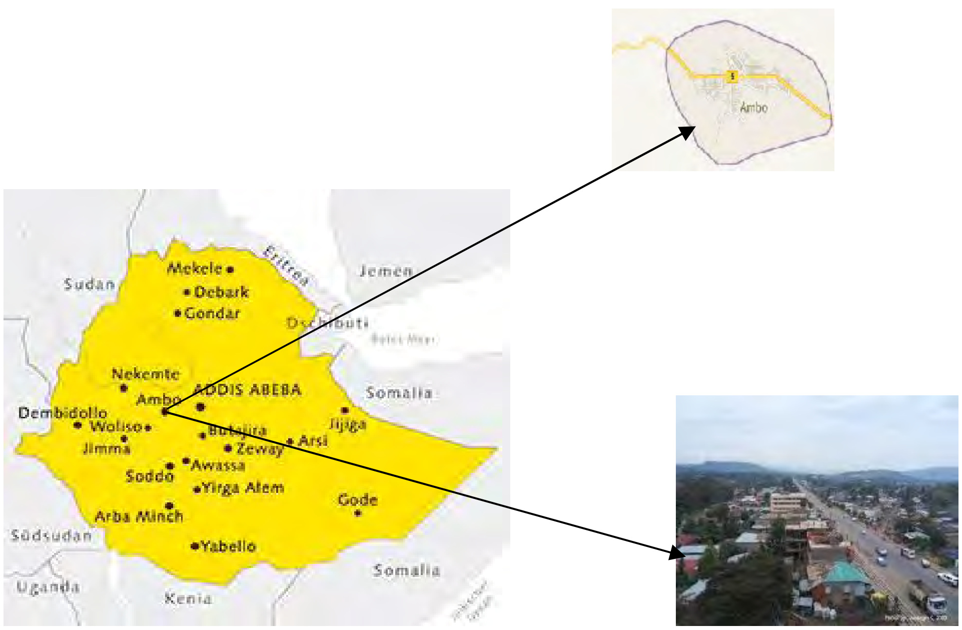
Likewise, another cross sectional study among pregnant women in Nigeria, Africa, revealed that there is only a moderate prevalence of anemia in pregnancy in this part of the world. This research revealed that anemia in pregnancy (Hb<11.0g/dl) and (Hb<10.0g/dl) were found to be 23.2% and 6.7% respectively. The etiology of anemia was found to be multifactorial; 40.2% had anemia of infection, 20.3% had plasmodium falciparum alone, 8.5% had HIV alone, 2.5% had HIV and malaria parasite co-infection, 8.9% undetermined infection and 0.6% had sickle cell anemia (Buseri *et al* 2008). Similar study in the same part of this world showed that the prevalence of anemia among antenatal clinic women was minimal compared to other parts of the world. The hemoglobin value among the study group was; Hb 10.14±1.45g/dl. Prevalence of anemia based on WHO criteria (hemoglobin value <11g/dl) and iron deficiency anemia based on ferritin <12ng/ml and transferin saturation <16% was found to be 21.3%, 13.5% and 7.69 respectively among pregnant women studied. The prevalence of IDA anemia was significantly higher in women in the third trimester of pregnancy compared to the second trimester (Erhabor *et al* 2013).

Similarly, a cross sectional study conducted in Kenya among third trimester pregnant women indicated the occurrence of mild iron deficiency and it was also revealed that the quantity of the food eaten has no significant correlation with the amount of iron in the food (Okongo et al 2012). The prevalence of anemia among pregnant women in south east Ethiopia was 27.9% among whom majority of the participants were rural residents with statistical significance (P=0.001) (Kefiyalew F *et al* 2014). Another similar study in Galgal gibe dam area indicated the prevalence of anemia is relatively high (53.9%). Majority, (62.2%) of the anemic women were rural residents and the place of the residence was statistically significantly associated with anemia (P=0.001) (Getachew *et al* 2012).

2.5 Micronutrients and the fetus

Iron transfer from mother to fetus is a regulated process involving iron uptake from the maternal circulation, its transport across the placenta and subsequent transfer into the fetal circulation (Srai *et al* 2002). The mechanisms behind iron transfer from mother to fetus are beginning to be clarified but much is still unknown. Iron metabolism during pregnancy maintains fetal iron levels at the expense of the mother. Most of the iron transferred to the developing fetus takes place during the last trimester of pregnancy. As pregnancy progresses, the amount of iron transferred from mother to fetus increases and at a term is directed against concentration gradient (Balesaria *et al* 2012). Iron nutrition tends to meet fetal needs except when the mother is very iron deficient (Shao *et al* 2012). In fetus, the concentration of iron and calcium increases with fetal size but zinc remains the same (Widdowson *et al* 1950).

Generally, people of all population groups in all regions of the world can be affected by Micronutrient malnutrition. Although the most severe problems of Micronutrient malnutrition are found in developing countries, people in developed countries also suffer from various forms of these nutritional problems.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Arsi
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Yirga Alem
Gode
Arba Minch
Südsudan
Yabello
Somalia
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Kenia

3.4 Inclusion and Exclusion criteria

All third trimester pregnant women attending antenatal clinic at Ambo hospital and Ambo health centers were included in this study. Neonatal birth weight and cord blood of the participants who gave birth at their home or referred to other health facilities were excluded. In addition, the birth weight and the cord blood of the twin babies were also not included.

3.5 Sample size and sampling technique

The sample size of the study was dependent on the number of women who attended the ANC during third trimester. Hence, 123 subjects were interviewed and followed up but only 57 participants cord blood was collected and the birth weight of 73 newborns was obtained due to inconveniences and work overload during labor. Besides, some women were referred to other health facilities other than the places included in the study, some gave birth at home and some encountered intra uterine fetal death.

3.6 Study variables

3.6.1 Dependent variables

Maternal Hb concentration, umbilical cord blood micronutrient concentration and neonatal birth weight.

3.6.2 Independent variables

Socio-demographic variables: marital status, ethnicity, religion, family size, education and occupation.

Woman characteristics: age, sex, number of children ever born, place of delivery, gestational age, gestational age at birth, breastfeeding status, antenatal care (ANC) visit, extra food during pregnancy, health status during pregnancy (hypertension, anemia, hemoglobin level), body mass index, medication, birth history and media access.

Environmental condition: water supply, sanitation and housing condition.

Dietary habit: consumption of fruits, dairy products, cereals and dark green leafy vegetables, drink intake.

3.7 Operational definition

Dietary habit: the dietary pattern of the study subjects.

Anemia: a woman whose hemoglobin concentration is less than 11g/dl or 110g/l of blood.

Third trimester pregnancy: a woman whose gestational age is greater than 24th week.

Birth weight: is a term used to describe the weight of the baby at birth.

Term: women with gestational age of 37-42 weeks.

Preterm: women with gestational age less than 37 weeks.

Post term: women with gestational age greater than 42 weeks.

Pre-eclampsia: a woman having three or more danger signs such as persistent swelling of feet, hands or face, increased breathlessness (especially on routine activity), headaches, blurring of vision, fever (temperature > $38^{O}C$) and high colored urine in the past two weeks accompanied with blood pressure of greater than 140/90mmHg.

Hypertension: blood pressure of greater than 140/90mmHg

3.8 Data collection process

The data were collected by the researcher, laboratory technicians', nurses, midwives and physicians.

Socio-demographic characteristics

The study subjects were interviewed using pre-structured English questionnaire translated in to local language, Afan Oromo; and all the personal details, environmental conditions and access to mass media were obtained by the researcher and nurses at ANC clinic.

Dietary habit

The study subjects were interviewed for their food habit using twenty four hour recall and two weeks food frequency questionnaire both by the researcher and nurses at ANC clinic.

Anthropometric parameters

The weight and height of third trimester pregnant women were obtained using balances and scales respectively. The data were calculated using the formula; weight/height square to obtain BMI of the subjects.

calibration standards were prepared by diluting the stock standards with deionized water as indicated below.

Table 1: Preparation of graded stock standard solution with deionized water

Element (standard)	Stock solution in ml					
Zn	0	1	2	3	4	5
Fe	0	1	2	3	4	5
Ca	0	1	2	3	4	5
Deionized water in ml	50	49	48	47	46	45
Total volume (ml)	50	50	50	50	50	50

The resultant concentration were 0, 0.1, 0.2, 0.3, 0.4 and 0.5ppm for zinc and iron; 0, 4, 8, 12, 16 and 20ppm for calcium.

Principle

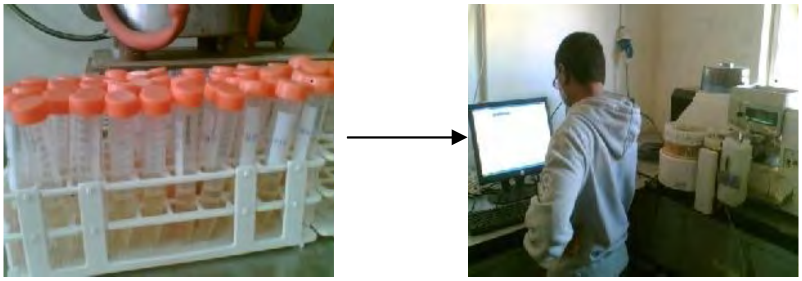
One milliliter of serum was diluted with 9 milliliters of deionized water and the concentration of elements were determined automatically using AAS; SHIMADZU AA6800.

Procedure

All the samples (n=57) were thawed and each of 1ml of serum from each tube was diluted with 9ml of deionized water. The contents were thoroughly mixed in the tubes using vortex mixer and measurement of the concentration of the elements were carried out using SHIMADZU AA6800.

Table 2: Instrument parameter for determination of micronutrients in cord blood

Element	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Burner height (mm)	Acetylene flow (L/min)	Air flow (L/min)	Recovery %
Zn	213.9	1.0	4	10	2.00	13.5	92-103
Fe	248.3	0.2	8	10	2.50	13.5	92-104
Ca	422.7	0.5	3	10	2.50	13.5	91-94



3.12 Ethical considerations

The proposal was presented to Addis Ababa University ethical review board for approval and the research was started after obtaining ethical clearance from Oromia regional health bureau. The benefit of the study was explained to health department of West Shoa zone, Ambo woreda health bureau, Ambo hospital and Ambo health center administrative officials. A written and signed consent was obtained from pregnant women during the study period.

Chapter four

4. Results

The socio-demographic characteristics of the third trimester pregnant women (n=123) is described in **table 1**. The mean age of the pregnant women was 25.8 ± 5.4 and the mean family size was 3.6 ± 1.6. Most of the study subjects (61%) were followers of the Ethiopian Orthodox Church, 35.8 % were Protestants, and 3.2 % were Muslim. Concerning their residence, most of the participants were from urban (59.3%) and peri-urban (20.3%). About one-third of the study participants were illiterate, 22% had primary, 26% secondary, and 22.7% tertiary level education. Most of the participants (75.6 %) were unemployed. Over half of the study subjects (56.1%) had information about nutrition during pregnancy, 40% of which had this information from health extension workers.

Table 3: Socio-demographic characteristics of third trimester pregnant women at antenatal clinics of Ambo governmental health institutions, West Shoa, Ethiopia, Apr-Aug 2014.

Variable	Frequency (%), Mean (SD)
Age of the study subjects	25.8(5.4)
Family size per household	3.6(1.8)
Religion	
Orthodox	75 (61.0)
Protestant	44(35.8)
Others	4(3.2)
Urban/rural livelihood	
Urban	73(59.3)
Peri-urban	25(20.3)
Rural	25(20.3)
Marital status	
Married	121(98.4)
Unmarried	2(1.6)
Ethnicity	
Oromo	107(87.0)
Amhara	15(12.2)
Gurage	1(0.8)
Level of education of the women	
Illiterate	36(29.3)
Primary school	27(22.0)
Secondary school	32(26.0)
Tertiary	28(22.7)
Employment status of the women	
Employed	30(24.4)
Unemployed	93(75.6)
Household's source of drinking water	
Wells	3(2.4)
River water	16(13.0)
Tap water	96(78.1)
Spring water	8(6.5)
Households with latrine facility	111(90.2)
Housing condition	
Roofing	
Iron corrugated sheet	114(92.7)
Hatched roof	9(7.3)
Flooring	
Soil	71(57.7)
Cement	46(37.4)
Other	6(4.9)
Households with access to mass media	92(74.8)
Women having information about nutrition in pregnancy	70(56.1)
Sources of information for women who had information in pregnancy	
Radio and television	9(12.9)
Community advocacy	6(8.6)
Health extension workers	29(40.0)
Other	27(38.5)

Information regarding the Body Mass Index (BMI), birth history and current birth outcomes of the pregnant women are described in **table 4**. The average BMI of the participants was 24.0 ± 2.4, and 65.9 % of the women were in the normal BMI range of 18-24. Very few had a BMI of < 18.5 (1.6%), 31.7% were in the BMI range of 25.0 to 29.9, and only 1 (0.8%) women had a BMI> 30. Majority of the women 65 (89%) gave birth at term while there were only 6 (8.2%) and 2 (2.8%) post term and preterm deliveries respectively.

Table 4: Maternal characteristics and pregnancy outcomes of the study subjects at Ambo governmental health institutions, West Shoa, Ethiopia, Apr-Aug 2014.

Variable	Frequency (%), Mean (SD)
Body mass Index	24.0 (2.4)
<18.5	2 (1.6)
18.6 - 24.9	81 (65.9)
25.0 - 29.9	39 (31.7)
≥30	1 (0.8)
Gestational age at birth	
Pre-term (<37 weeks)	2 (1.6)
Term (≥37+ weeks to 42 weeks)	65 (52.8)
Post-term (≥42+ weeks)	6 (4.9)
Pregnancy category	
Primiparous	55 (44.7)
Multiparous	68 (55.3)
Number of children ever born	2.34 (1.5)
1-3	54 (79.4)
4-6	3 (19.1)
>6	1 (1.5)
Place of previous delivery	
Home	21 (30.4)
Health institution	48 (69.6)
Gap between previous delivery and current pregnancy in years	5.03 (3.8)
1-2	12 (17.6)
3-4	24 (35.3)
5-6	15 (22.1)
7 and above	17 (25)
Women who had history of abortion	23 (18.7)
Intentional abortion	9 (39.1)
Unintentional abortion	14 (60.9)
Neonatal birth weight	3.086 (0.5)
Preterm	2 (1.6)
Term	65 (52.8)
Post term	6 (4.9)

More than half of the study subjects were multiparous and had on average 2.3 ± 1.5 children. Close to 80% of the subjects had 1-3 children and the remaining 20% had 4-6 children. Out of the 73 (59%) that delivered in the health settings, and for whom birth weight of the children were available, the mean ± SD neonatal birth weight was 3.09 ± 0.47. There were only 2 (2.8%) preterm babies and were low birth-weight (weighed between 2-2.5 Kg).

The dietary pattern of the women in the 24 hours preceding the interview showed that cereals, followed by legumes and root and tubers are the most commonly consumed food groups. Considerable percent of study populations consumed Animal Source Food (ASF). On the other hand, a low consumption of vitamin A- rich vegetables and fruits, and a very low consumption of fruits and vegetables other than those that are vitamin A rich foods were observed (fig. 8).

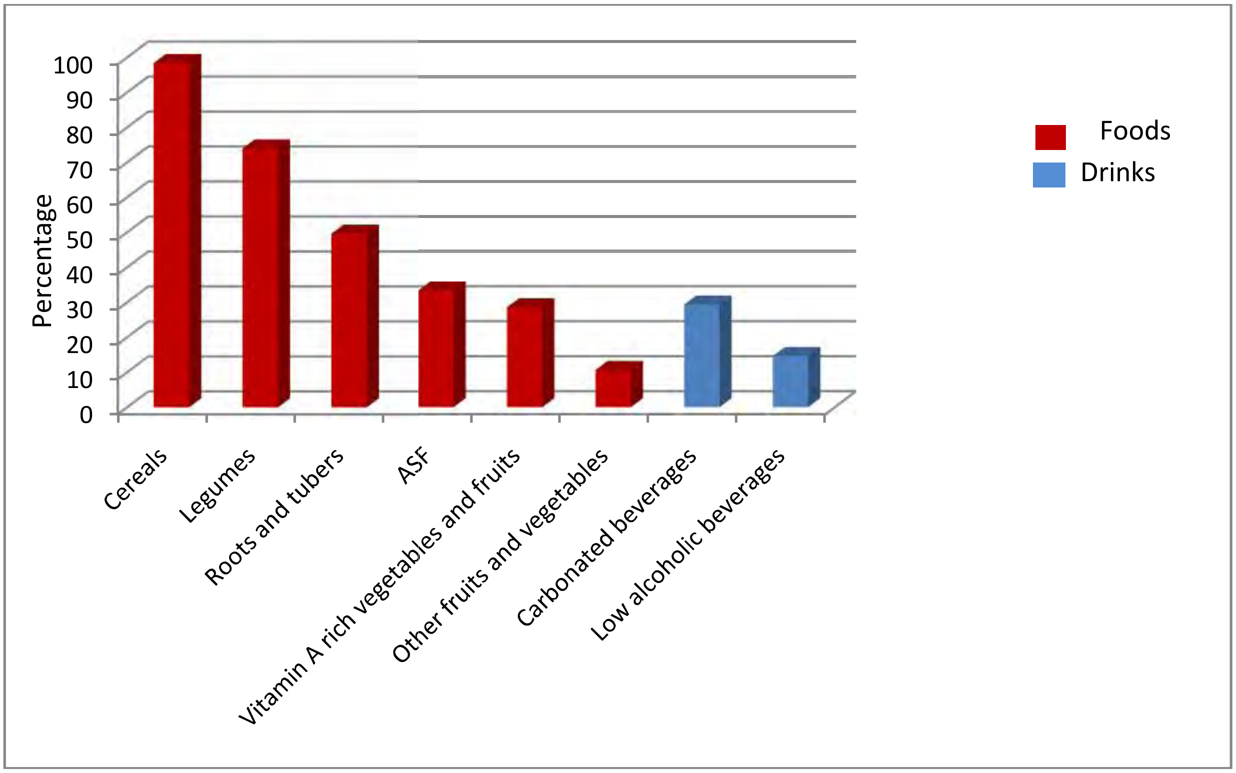


Fig 8: Dietary pattern (24hr recall) of third trimester pregnant women attending antenatal clinics at Ambo governmental health institutions, West Shoa, Ethiopia, Apr-Aug 2014.

In the past two weeks, the intake of some specific foods and drinks are presented in **table 5.** Coffee, carbonated beverages and tea were the most consumed drinks by 93.5%, 88.6% and 63.4% of the subjects in the past two weeks respectively. Regarding food intakes 78% of the participants consumed iron rich foods such as green leafy vegetables, meat, egg, bread and pulses which is higher than consumption of vitamin A rich foods such as papaya, mango, tomato, carrot and green leafy vegetables (62.6%). Calcium rich foods such as dairy products, cabbage and egg were consumed by 59.3% of the subjects. Zinc rich foods such as beans, fish and maize were consumed less frequently by the participants compared to other food groups. However, it is a little bit frequently consumed food than animal source foods.

Table 5: Consumption of some specific foods/drinks by third trimester pregnant women within the past two weeks, antenatal clinics of Ambo governmental health institutions, West Shoa, Ethiopia, Apr-Aug 2014.

Sr. no	Food/Drink type	Frequency (%)
1	Iron rich foods	96 (78)
2	Vitamin A rich foods	77 (62.6)
3	Calcium rich foods	73 (59.3)
4	Zinc rich foods	51 (41.5)
5	ASF	49 (39.8)
6	Carbonated beverages	109 (88.6)
7	Low alcoholic beverages	69 (56.1)
8	Coffee	115 (93.5)
9	Tea	78 (63.4)

The following table presents the top ten mixed dishes consumed by the study subjects. Teff injera was the most commonly consumed food in the form of firfir or accompanied with shiro or dinich wot (potato-based stew). The consumption of dairy products by the subjects was very low **(table 6).**

Table 6: Top ten mixed dishes consumed by the study subjects over the past 24hours. Ambo governmental health institutions, West Shoa, Ethiopia, Apr-Aug 2014

Sr. no	Mixed dish	Frequency (%)
1	Teff injera firfir	117 (95.1)
2	Teff injera firfir with shiro	79 (64.2)
3	Teff injera with dinich wot	58 (47.2)
4	Whole grain bread	33 (26.8)
5	Teff injera with misir	22 (17.9)
6	White bread	19 (15.4)
7	Carbonated drink (Mirinda)	16 (13.1)
8	Fruits and packed juices	15 (12.2)
9	Dairy products	14 (11.4)
10	Teff injera with scrambled egg	14 (11.4)

A surprisingly high number, 31(25.2 %) of pregnant women were fasting the Ethiopian orthodox fasting during pregnancy **(Table 7)**. The majority of the study subjects, 71(57.8%) were used to have three meals a day, and about 18 % ate only twice a day. Although coffee is a potential inhibitor of iron absorption, it was consumed by 93.5 % of the subjects among whom 39% were used to drink 1 to 2 cups per day, and >10 % of the subjects had coffee consumption exceeding 5 cups a day and coffee was mostly served immediately after a meal (77.2 %). Similarly, the consumption of tea was experienced by 63.4 % among whom 39.8 % consumed it with or right after a meal.

Table 7: Lifestyle characteristics of third trimester pregnant women attending antenatal clinics of Ambo governmental health institutions, West Shoa, Ethiopia, Apr-Aug 2014.

Variables	Frequency	Percentage
Women who had been fasting during pregnancy	31	25.2
Level of fasting		
Deprivation of animal source food except fish	14	45.2
Deprivation of all animal source foods	12	38.7
Deprivation of animal source food and fasting until 3:00pm	5	16.1
Number of meals per day		
Once a day	3	2.4
Twice a day	22	17.9
Three times a day	71	57.8
Four times a day	24	19.5
Five times a day	3	2.4
Breakfast eating habit	122	99.2
Always	102	82.9
Sometimes	20	16.3
Snack taking habit	74	60.2
Always	22	17.9
Sometimes	52	42.3
Coffee/tea consumption over the past two weeks		
Coffee	115	93.5
Sometimes	12	10.4
1 to 2 cups/day	48	41.7
3 to 4 cups/day	41	35.7
5 and above cups/day	14	12.2
Timing of coffee intake		
Before meal	3	3.5
With meal	5	4.3
Immediately after meal	95	82.6
Approximately 1hour after meal	10	8.7
Do not know	1	0.9
Tea	78	63.4
Sometimes	50	64.1
1 to 2 cups/day	28	35.9
Timing of tea intake		
Before meal	1	1.3
With meal	49	62.8
Immediately after meal	28	35.9

The health and clinical status of the women are presented in **table 8**. About half of the women reported that they had changes in appetite for food during their pregnancy, to be more specific, 72.6 % had a decrease in appetite whereas 27.4 % had experienced an increase in their appetite.

Table 8: Health and clinical status of third trimester pregnant women attending antenatal clinics at Ambo governmental health institutions, West Shoa, Ethiopia, Apr-Aug 2014.

Variables	Frequency (%)	Mean (SD)
Appetite changes during pregnancy	62 (50.4)	
Decrease in appetite	45 (72.6)	
Increase in appetite	17 (27.4)	
Pre-eclampsia[¥] during pregnancy	13 (10.6)	
Anemia during pregnancy	44 (35.8)	
Hemoglobin values of the women		11.3 (1.6)
Hemoglobin value of the anemic women		9.7(0.8)
Urban	26 (59)	
Peri-urban	12 (27.3)	
Rural	13 (29.5)	
Hemoglobin value of the non-anemic women	79 (64.2)	12.2 (1.0)
Urban	47 (59.5)	
Peri-urban	13 (16.5)	
Rural	12 (15)	
Women's blood pressure value		
less than 100/60mmHg (hypotensive)	18 (14.6)	
110/65 to 130/85mmHg (normal BP)	97 (78.9)	
greater than 140/90mmHg (hypertensive)	8 (6.5)	
Women who had been taking medication	18 (14.6)	
Iron supplement	3 (2.4)	
Iron folic acid supplement	10 (8.2)	
Anti-acid	2 (1.6)	
Other	3 (2.4)	
Timing of pregnancy at which iron or iron foliate supplement was started		
first trimester	1 (0.8)	
second trimester	8 (6.5)	
third trimester	4 (3.3)	
The dosage of the supplements being taken[£]		
One tablet daily (150 mg)	10 (8.2)	
Two tablets daily (300 mg)	1 (0.8)	
Three tablets daily (450 mg)	2 (1.6)	

¥: Persistent swelling of feet, hands or face, increased breathlessness (especially on routine activity), headaches, blurring of vision, fever (temperature > 38^{O}C) and high colored urine in the past two weeks
£: iron or iron foliate

Preeclampsia was observed in 10.6% (n=13) women and 65.5% (n=8) of these were term pregnancies. Among the studied subjects, 35.8% of the participants were found to be anemic when considering the WHO recommended cut-off of <11g/dl for anemia and the mean hemoglobin concentration of the anemic women was 9.7±0.8. Only 10.6% of the women have been taking iron or iron foliate supplements out of whom 23.1% took iron and 76.9% took iron foliate supplements. Even more surprising was that very few (0.8%) of the women had started to take the supplements during their first trimester.

The average concentration of cord blood micronutrients among term women are described in table 9. The average zinc, iron, and calcium concentration of cord blood was 1.58±0.48, 4.18±3.58 and 108±21.98µg/ml, respectively. The mean cord blood iron concentration for rural residents was 2.79±1.15µg/ml which is very low compared to those from peri-urban and urban residents. Although the mean cord blood iron concentration in anemic women was 4.93 ± 5.09µg/ml slightly higher than non-anemic ones (3.85 ± 2.69µg/ml), this was not statistically significant.

Table 9: Descriptive statistics of Zinc, Iron and Calcium concentration (µg/ml) in umbilical cord blood by maternal residential area, and anemia status among the study subjects (term births only) at Ambo governmental health institutions, West Shoa, Ethiopia. Apr-Aug 2014.

	Zinc		Iron		Calcium	
Variables	Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
Overall (49)	1.6±0.5		4.2±3.6		108.9±21.9	
Address						
Urban (24)	1.5±0.3		3.9±2.4		113.6±23.4	
Peri-urban (9)	1.7±0.6	0.48	7.2±6.6	0.34	92.7±22.8	0.55
Rural (16)	1.6±0.6		2.8±1.2		110.8±15.2	
Anemia status						
Anemic mothers (15)	1.7±0.6		4.9±5.1		116.5±19.4	
Non-anemic mothers (34)	1.5±0.4	0.51	3.9±2.7	0.50	105.5±22.5	0.25

The degree of association of hemoglobin with maternal factors is presented in **table 10**. A positive association between level of education and hemoglobin value was detected (r=0.352, P<0.001); similarly the association remains strong and significant in the multivariate analysis (β=541, P<0.001). BMI was positively correlated (r=0.242, P=0.007) with Hb concentration, and the association was retained in the multiple regression model (β=0.162, P=0.013). The amount of cups of tea taken per day was negatively associated with Hb concentrations (r=-0.267, P=0.018), even in the multivariate analysis (β=-0.374, P=0.021). For every additional cup of tea consumed per day, a 0.4 unit decrease in Hb was predicted. The consumption of roots and tubers was also negatively associated with Hb concentrations (r=-0.256, P=0.040), and its effect was even more pronounced in the multivariate model (β=-0.700, P=0.004). In contrast, the consumption of carbonated drinks was positively associated with Hb concentration, both in the Univariate (r=0.268, P=0.003) and multivariate models (β=0.954, P=0.002). In Pearson's correlation the overall dietary diversity score of the women showed positive correlation (r=0.180, P=0.046) with Hb. Additional strong statistical significance was also observed in multiple regression analysis (P=0.003).

Table 10: Factors affecting maternal hemoglobin values among study subjects at Ambo governmental health institutions, West Shoa, Ethiopia. Apr-Aug 2014.

	Bivariate analysis			Multivariate analysis§		
Variables	N	Pearson's r	P-value	β	Std. Error	P-value
Maternal characteristics and birth history						
Age	123	.004	.964	.020	.026	.430
Address	123	-.159	.080	-.012	.189	.086
Employment status	123	-.112	.219	.232	.353	.052
Level of education	123	.352	.001<**	.541	.148	.001<**
Family size	123	-.063	.091	-.130	.124	.298
Parity	123	.102	.419	.503	.536	.355
Birth spacing	68	.071	.666	-.129	.259	.621
Body mass index	123	.242	.007**	.162	.064	.013*
Maternal dietary pattern						
Average number of cups of tea taken/day	78	-.267	.018*	-.374	.311	.021*
Average number of cups of coffee taken/day	115	-.141	.132	-.262	.173	.132
Consumption of zinc rich foods	51	-.422	.001**	-1.341	.302	.001<**
Cereals	121	.407	.001**	.294	.229	.203
Legumes	91	-.106	.401	-.579	.233	.014*
ASF	41	.119	.347	-.592	.350	.096
Roots and tubers	61	-.256	.040*	-.700	.241	.004**
Carbonated drink	36	.268	.003**	.954	.301	.002**
DDS	123	.180	.046*	.413	.138	.003**
Level of fasting	31	-.374	.038*	-.234	.266	.386
Number of meals per day	123	.294	.001**	.685	.397	.096

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
§=multiple regression analysis

The level of fasting was negatively correlated with hemoglobin in Person's correlation (r=-0.374, P=0.038) but no additional evidence was observed in multiple regression analysis. On the contrary, the number of meals taken per day by the women showed statistically significant positive association with Hb (r=0.294, P=0.001). However, no statistical significance was observed in multiple regression analysis. Consumption of legumes was found to be associated with maternal Hb value (β = -0.759, P = 0.014).

The predictors of maternal anemia are presented in **table 11**. Preeclamptic women had a significantly higher likelihood of becoming anemic (r = 0.258, P=0.038), but the association was attenuated in multivariable analysis (P = 0.090). On the other hand, the consumption of vitamin A-rich foods was negatively associated with anemia (r=-0.295, P=0.017), even in multivariate analysis (β=-0.228, P=0.048). Tea consumption which was found to be associated with hemoglobin concentration was not strong predictor of anemia.

Table 11: Factors associated with anemia among third trimester pregnant women attending antenatal clinics of Ambo governmental health institutions, West Shoa, Ethiopia. Apr-Aug 2014.

Variables	Bivariate analysis			Multivariate analysis§		
	N	Pearson's r	P-value	β	Std. Error	P-value
Maternal characteristics and birth history						
Family size	123	-.082	.370	-.076	.054	.166
Body mass index	123	.232	.062	.337	.172	.059
Parity	123	.062	.624	.138	.290	.637
Birth spacing	68	-.012	.941	-.058	.075	.439
Maternal clinical conditions						
Medication	18	.119	.346	.171	.162	.298
Blood pressure	123	-.144	.252	-.038	.142	.792
Preeclampsia	13	.258	.038*	.327	.190	.090
Maternal dietary pattern						
Cereals	121	.149	.099	.111	.067	.099
Legumes	91	-.126	.319	-.195	.088	.060
Roots and tubers	61	-.104	.254	-.083	.072	.254
ASF	41	.131	.299	-.069	.105	.512
Consumption of calcium rich foods	73	.005	.966	-.019	.111	.863
Consumption of Vitamin A-rich foods	77	-.295	.017*	-.228	.113	.048*
Consumption of zinc rich foods	51	-.303	.014*	-.198	.110	.076
DDS	123	.121	.338	.124	.058	.056
Habit of drinking coffee	115	.032	.800	-.004	.268	.987
Habit of drinking tea	78	-.132	.145	-.133	.091	.145
Number of meals per day	123	.149	.237	.041	.096	.672

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
§=multiple regression analysis

Factors affecting cord blood micronutrient concentrations are shown in **table 12.** The maternal age was negatively correlated with cord blood iron concentration in both Univariate ($r = -0.274$, $P=0.028$) and multivariate analyses ($\beta=-1.867$, $P=0.038$). The amount of cups of coffee taken per day was found to be positively associated with cord blood zinc concentration in both Univariate ($r=0.356$, $P=0.008$) and multivariate analyses ($\beta=0.176$, $P=0.031$). For every cup of coffee consumed a 0.176 unit increase in zinc concentration was predicted. In contrast, tea consumption was negatively correlated with calcium concentration in cord blood in both Univariate ($r=-0.349$, $P=0.032$) and multivariate analyses. The coefficient of regression (β) indicated a strong variation in calcium concentration ($\beta=-24.997$) with tea consumption ($P=0.018$). Consumption of roots and tubers was positively associated with iron concentration in the cord blood in both Univariate ($r=0.355$, $P=0.006$) and multivariate ($\beta=2.108$, $P=0.019$) analyses.

Table 12: Factors affecting umbilical cord blood zinc, iron and calcium concentration among term births at Ambo governmental health institutions, West Shoa, Ethiopia. Apr-Aug 2014.

	Bivariate analysis			Multivariate analysis §					
	Zinc	Iron	Calcium	Zinc		Iron		Calcium	
Variables (n)	r / P-value	r / P-value	r / P-value	β/SE	P-value	β/SE	P-value	β/SE	P-value
Maternal characteristics									
Age in year (49)	.059/.344	-.274/.028*	.096/.256	-.006/.121	.958	-1.867/.872	.0381*	.619/6.141	.793
Body mass index (49)	-.152/.149	-.091/.266	-.330/.010*	.006/.144	.968	1.575/1.032	.134	-10.056/7.322	.177
Maternal clinical condition									
Medication (49)	.051/.363	-.170/.122	-.031/.417	.094/.196	.632	-1.785/1.602	.271	-5.012/10.005	.619
Preeclampsia (8)	-.473/.001<**	-.174/.116	.147/.156	-.653/.183	.001**	-1.066/1.672	.527	8.867/9.899	.375
Blood pressure (49)	.032/.414	.138/.172	-.215/.069	.088/.139	.529	.829/1.275	.519	-9.273/7.570	.227
Anemic (15)	-.144/.161	-.139/.170	-.233/.053	-.391/.219	.081	-.634/1.163	.588	-5.687/10.906	.605
Hemoglobin (49)	-.042/.758	-.039/.775	-.177/.187	.072/.060	.254	-.407/.427	.226	-2.896/3.353	.392
Maternal dietary pattern									
Average number of cups of coffee taken /day (45)	.356/.008**	.171/.130	-.041/.394	.176/.079	.031*	1.324/.790	.109	-3.168/5.471	.569
Average number of cups of tea taken/day (29)	-.070/.359	.133/.246	-.349/.032*	-.054/.185	.772	2.003/1.393	.166	-24.997/9.663	.018*
Vitamin A rich vegetables and fruits (13)	-.045/.381	-.193/.092	-.004/.489	-.069/.169	.689	-.387/1.376	.781	-11.130/9.226	.242
Cereals (49)	.143/.163	.222/.063	.064/.332	-.003/.147	.983	1.599/.944	.097	-11.130/9.226	.242
Legumes (36)	-.024/.436	-.072/.311	.182/.106	-.042/.101	.679	-.338/.621	.5896	.544/5.103	.206
Roots and tubers (20)	-.028/.425	.355/.006**	-.127/.192	-.039/.133	.770	2.108/.863	.019*	-1.550/6.412	.810
Carbonated drink (16)	-.004/.488	-.038/.398	-.014/.462	.045/.162	.782	-.536/1.071	.6194	.789/8.375	.570
DDS (49)	-.078/.298	.084/.282	-.013/.466	-.007/.062	.914	-2.246/3.353	.506	-.118/2.379	.961
Interaction between umbilical cord blood micronutrients									
Zinc (49)	-	.212/.144	-.085/.562	-	-	.028/.020	.177	.001/.003	.810
Iron (49)	.212/.072	-	-.241/.048*	.028/.020	.177	-	-	-.037/.023	.118

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

§ =multiple regression analysis

The factors associated with birth weight are presented in **table 13**. Birth weight was negatively associated with parity (r=-0.295, P=0.017), consumption of root and tubers (r=-0.233, P=0.031), and cord iron concentrations (r=-410, P=0.003), and these associations remained significant predictors in the multivariate model. Maternal anemia and hemoglobin concentration were however not associated with birth weight.

Table 13: Factors affecting neonatal birth weight among study subjects (term births only) at Ambo governmental health institutions, West Shoa, Ethiopia. Apr-Aug 2014.

	Bivariate analysis			Multivariate analysis§		
Variables	N	r	P-value	β	Std. Error	P- value
Maternal characteristics and birth history						
Age in year	65	-.060	.635	-.015	.011	.181
Address	65	-.028	.826	-.038	.069	.587
Employment status	65	.059	.640	.058	.152	.705
Parity	65	-.295	.017*	-.336	.121	.007**
Birth spacing	39	.136	.410	-.007	.077	.925
Body mass index	65	.100	.427	.067	.189	.725
Maternal clinical condition						
Anemia	15	.104	.408	.088	.175	.616
Blood pressure	65	.084	.508	.162	.195	.412
Hemoglobin	65	.053	.654	.038	.039	.327
Medication	9	.103	.416	.157	.171	.362
Maternal dietary pattern						
Cereals	64	-.112	.374	-.134	.100	.184
Legumes	46	.075	.555	.073	.071	.307
Roots and tubers	29	-.233	.031*	-.196	.092	.037*
ASF	24	-.075	.552	-.014	.098	.890
DDS	65	-.104	.412	-.012	.046	.800
Vitamin A rich vegetables and fruits	16	.144	.251	.127	.141	.372
Other vegetables and fruits	5	.107	.396	.263	.219	.235
Carbonated drink	24	-.095	.451	-.099	.121	.415
Habit of drinking coffee	61	-.170	.177	-.339	.236	.155
Concentration of micronutrients in cord blood						
Zinc	49	-.008	.955	.085	.141	.552
Iron	49	-.410	.003**	-.158	.020	.005**
Calcium	49	.114	.434	.000	.003	.890

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
§=multiple regression analysis

Chapter Five

5. 1 Discussion

This study is the first to provide information on the link between maternal dietary pattern, maternal anemia, and cord blood micronutrient concentration and neonatal birth weight. Pregnancy is a period of rapid fetal growth and cell differentiation for both the mother and the developing fetus (Black 2001). Consequently, it is a period at which the mother is at an increased risk of nutritional deficiencies that can have serious consequences to the development of the fetus. Micronutrient deficiencies, especially iron deficiency and anemia, are common during pregnancy. In this study, maternal dietary pattern was associated with hemoglobin concentrations, anemia, cord blood micronutrient concentrations and neonatal birth weight. The cord blood micronutrient concentrations were also found associated with the birth weight of the newborns.

Nutritional requirements during pregnancy are increased to support fetal growth and development as well as maternal metabolism and tissue accretion (Berti et al 2010). Micronutrients are involved in both the development of the embryo and the fetal organ development and overall pregnancy outcomes (Berti et al 2010). Several factors can affect directly or indirectly the development of the fetus and the overall pregnancy outcomes, such as the quality of the diet including intakes and bioavailability of micronutrients, maternal age, and the overall environment (Berti et al 2010). The bioavailability of micronutrients during pregnancy varies depending on specific metabolic mechanisms because pregnancy is an anabolic and dynamic state orchestrated via hormones acting for both redirection of nutrients to highly specialized maternal tissues and transfer of nutrients to the developing fetus (Berti et al 2010).

About one-third of the pregnant women in this study were anemic. Several reasons can explain this finding. Most studies indicated that maternal malnutrition contributes to the prevalence of anemia (Badham et al 2007, Abbasi *et al* 2013, Kalaivani 2009). For instance, the dietary pattern of the pregnant women showed that the diets were predominantly plant-based with little consumption of ASF. Although cereal consumption was positively associated with maternal hemoglobin concentrations (P= 0.001), consumption of legumes was negatively associated with maternal hemoglobin in this study. Hence, it is recognized that, non-heme (plant) iron, unlike that of heme is susceptible to the effects of chelating agents such as phytate and polyphenols and is thus of lower bioavailability (Brune *et al* 1992). Likewise, this is also supported by this study in that consumption of legumes was found to be important predictor of maternal hemoglobin concentration justifying poor iron bioavailability. Besides the high coffee and tea consumption by the pregnant women, especially for those who consumed them right before or after a meal, is likely to further compromise the bioavailability of minerals like iron. Increased tea consumption reduces iron absorption (Speedyp 2003). Indeed, consumption of tea in this study was an important predictor of maternal hemoglobin. In contrast, sources of iron absorption enhancers such as vitamin-A rich fruits and vegetables, ascorbic acid rich foods such as citrus fruits were rarely consumed. Unlike ASFs, fruits were available in the community, but did not constitute the food basket of the pregnant women possibly because it is more expensive than staples like cereals and legumes or possibly due to the monotonous food habit of the community. This suggests that education on nutrition during pregnancy is required, especially given the fact that significant proportions of the pregnant women have reported not having any information on optimal practices during pregnancy. Such nutritional education should recommend consumption of a diversified diet, especially given the fact that DDS and number of meals were important predictors of hemoglobin concentration in this study.

Apart from maternal dietary pattern, there are also other maternal factors that showed association with hemoglobin concentration and these include; BMI, level of education and fasting habits of the mothers'. Optimal weight gain during pregnancy can help mothers achieve favorable pregnancy outcomes and can contribute to the wellbeing of the fetus as well. The association of level of education with hemoglobin concentration in this study is in line with previous findings (Sharma *et al* 2013, Abbasi *et al* 2013) and may indicate that women with better education may have better understanding of optimal feeding during pregnancy. Therefore, women's education must be addressed, particularly during adolescence. Studies also indicated that multiparty may lead to anemia by reducing maternal iron reserves at every pregnancy and by causing blood loss at each delivery (Karaoglu *et al* 2010). However, no statistical significance was obtained between anemia and parity in this study. This may be due to two reasons; first, more than 79% of the multiparous women in this study have only 1-3 children among whom more than 80% of the women had experienced birth spacing of greater than three years. Hence, repeated bleeding during delivery would be minimal. Secondly, compared to primiparous women most multiparous women had been taking iron or iron folate supplements. Yet, the combined effect of the factors discussed above resulted in 35.8% of anemia prevalence in this study community which is relatively higher compared to the studies from African countries like Nigeria (Buseria 2008, Erhabor 2013). Although it is less than the prevalence of anemia in Galgal gibe of Ethiopia (Getachew

2012) it is greater than the report from South east Ethiopia (Kefiyalew 2014). This might be due to geographical differences, dietary patterns and other confounding factors such as communicable and non-communicable diseases.

The timing of prenatal intakes or supplementations of specific micronutrients is vital as pregnancy is characterized by different stages that represent a continuum, up to lactation and beyond and hence applying to different requirements. Consequently, nutrition during pregnancy might have long-lasting effects on the well-being of the mother and the fetus, and may further influence the health of the baby at a later age (Berti et al 2010). Nonetheless, very few subjects in this study had been taking supplements and only 0.8% of them started to take iron folic acid supplement during first trimester. Even though most women have good antenatal follow up they quit taking the supplements and this requires further study to understand the cause of the problem. In the mean time, appropriate strategies to reduce anemia through synergistic effects of improved food intake, nutrition education, and supplementation is crucial.

On the other hand, one important factor to consider is the placental transfer of nutrients from the mother to the fetus, which can be predicted through analyses of these micronutrients in cord blood. Several factors such as age, BMI, preeclampsia, amount of coffee and tea taken per day, consumption of roots and tubers and inter-micronutrient interactions were associated with cord blood micronutrient concentration in this study. Accordingly, mothers' BMI was found associated with calcium concentration in the cord blood, which indicates that maternal nutritional status predicts placental calcium transfer. Such calcium transfer is needed since the fetus in utero grows the most during the last trimester of pregnancy (Fall *et al* 2003).

Moreover, Preeclampsia complicates 2-8% of all pregnancies and has been associated with considerable maternal serum zinc depletion (Al-Jameil *et al* 2014, Gupta S *et al* 2014). Likewise, this study showed a significant negative association between cord blood zinc concentration and pre-eclampsia. Hence, a decrease in cord blood zinc may suggest the inability of the mother to compensate fetal zinc need at her own expense. This is unfortunate, given zinc's important role for growth and the immune system, filling this gap through food fortification or supplementation may be beneficial and thus need further investigation.

Cord blood iron concentration was not affected by maternal hemoglobin concentration in this study. Studies suggest that, iron transfer from mother to fetus is a regulated process involving iron uptake from the maternal circulation, its transport across the placenta and subsequent transfer into the fetal circulation (Srai *et al* 2002). Since mothers maintain their fetus' iron demand even at their own expense, the lack of association between maternal hemoglobin and cord blood iron is not surprising. However, the fact that maternal age was negatively associated with cord blood iron concentration may suggest that iron transfer could be affected by age and would require further study with more accurate markers such as serum ferritin. In the mean time, given the risk of iron store depletion with parity (Karaoglu *et al* 2010), reproductive health education along with nutrition education is essential. Likely, there is a tendency of parity to negatively affect the neonatal birth weight as revealed in this study.

In the current study, a negative statistically significant association (β=-0.158, P=0.005) was observed between cord blood iron and fetal weight, which means for every one unit increase in the neonatal birth weight there was a 0.158 unit decrease in iron concentration in the cord blood. This can be explained in two ways. First, as birth weight increase, placental iron is depleted due to higher transfer to the fetus to meet the increased demand related to body size. Because, the iron needs of the fetus take priority over maternal requirements (O'Brien *et al* 2003). The second reason may be that heavier fetus are iron-replete and thus down-regulate the placental transfer which decreases the concentration of iron in the placenta. It is evident that at a term iron transfer from mother to fetus is directed against concentration gradient (Balesaria *et al* 2012). This warrants a more thorough study, preferably through a randomized controlled trial, involving iron metabolism markers like hepcidin.

5.2 Limitation of the study

Some of the study participants did not accurately recall what they ate in the past two weeks, and this might have affected the results from the two-week food frequency. Some of the mothers initially recruited at the start, delivered in the health facility, and some were lost because they either delivered at home, or were refereed to other health centers/hospitals. Due to financial concerns, the iron concentration in cord blood was analyzed by Atomic absorption spectrophotometry; however, it would have been preferable to analyze serum feritin than serum iron alone.

Chapter six

6. Conclusion and recommendation

6.1 Conclusion

About one-third of the pregnant women in this study were anemic and this was due to dietary pattern of the pregnant women. The diet of the pregnant women was predominantly plant-based with little consumption of ASF, fruits and vegetables. Tea consumption was an important predictor of maternal hemoglobin concentration. In addition, maternal BMI, level of education and fasting habits are strongly associated with hemoglobin concentration. Even though the women had good antenatal follow up they do not have good adherence to iron or iron-folate supplements. On the other hand, umbilical cord blood micronutrient concentration was affected by BMI, preeclampsia, amount of coffee and tea taken per day, consumption of roots and tubers and inter-micronutrient (i.e. calcium and iron) interactions. Placental iron transfer depletes due to high iron demand of the fetus or movement of the iron against concentration gradient at late stage of pregnancy. Besides, foods low in nutritional contents such as roots and tubers negatively affect the neonatal birth weight.

6.2 Recommendation

Maternal micronutrients requirement increases due to increased physiological demand to enable the development of the fetus. Pregnant women should reduce the intake of foods like roots and tubers that simply fill their satiety, but do not supply adequate nutrients for maternal weight gain and fetal development or these foods must be complemented with other nutrient rich foods. It is imperative to avoid consumption of tea and coffee whenever possible or decrease the amount consumed per day to prevent anemia. In addition, foods like legumes should not be taken in bulk as they impair bioavailability of iron. Women should seek ANC as early as they realize that they are pregnant and should adhere to recommended supplements like iron/iron-folic acid to prevent congenital abnormalities (i.e. neural tube defect), both mother and neonatal anemia and hence prevent the associated adverse effects on child development and cognition. Further research is needed to investigate why adherence to recommended micronutrient supplement is low. It is also vital for ANC attendants to check the hemoglobin status of pregnant women regularly and follow up the proper supplementation and adherence. In addition, it is also crucial to make follow up on their gestational weight gain. The health educators must also teach the mothers about adequate nutrition throughout pregnancy. Besides, strong and persistent reproductive health education is essential to ensure birth spacing which will be of benefit to maternal, fetal and child well being in early and later life.

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Appendix A: Informed consent to Participant

Date:

Dear Madam:

Title: Dietary habit and prevalence of anemia among pregnant women attending antenatal clinic during their third trimester and concentration of micronutrient in cord blood: effect on neonatal birth weight

Name of Researcher: Kefiyalew Jote

On the behalf of Addis Ababa University, Center for Food Science and Nutrition, I am writing to invite you to participate in an in depth interview and clinical specimen collection that I am conducting to determine how food, anemia and nutrition in pregnancy can affect the neonatal birth weight. The research has several objectives: 1) To assess the dietary habit of third trimester pregnant women 2) To determine the prevalence of anemia among pregnant women during their third trimester 3) To determine the neonatal birth weight 4) To analyze concentration of micronutrient in cord blood 5) To determine the link between maternal micronutrient status and neonatal birth weight

Participation is Voluntary; Right to Withdraw without Negative Consequences

You are going to be a prospective research participant and you are not under any obligation to participate in this research project; there are no negative consequences to deciding not to participate.

If you do agree to participate, you are not obliged to answer specific questions or to provide information you do not wish to give. You have the right to not answer specific questions but continue as a participant. If you choose to participate and have agreed to have the interviews; yet, you can withdraw from the interview by stating that you have decided to withdraw.

In addition, you can withdraw from the project up until the point when I provide the summary report of the interviews. There will be no negative consequences to withdrawing from the research project. You can state your intention to withdraw from the project by contacting me, Kefiyalew Jote (the researcher), whose contact information is provided at the end of this form. If you choose to withdraw from the project please indicate whether you want the previously collected data destroyed or returned to you.

Potential Harms

There are no harms associated with participating in this research. Indeed, any potential risks that may arise will be considered and treated by seeking appropriate measures to reduce the level of harm.

Potential Benefits

This research may help any authorized body understand about nutrition in pregnancy and its effects on neonatal birth weight to foster a magnificent intervention which might be very important to promote maternal health and child survival. It may also help educators understand more about food, anemia and nutrition in pregnancy.

Confidentiality and Anonymity

Your participation in this research will be kept in confidence. Confidentiality of your data is assured. All data will be transcribed by me and/or a research assistant and we will both be bound by confidentiality. We will keep all data in a secure and locked place until the project is complete. No identifying information will be included in any document resulting from this study. All the data obtained will remain confidential even after completion and dissemination of the findings of this study. Do not hesitate to ask us any questions regarding the objectives or the process of the investigation.

Contact information

Mr. Kefiyalew Jote

Faculty of life science, Addis Ababa University, Center for Food Science and Nutrition

Cell phone: +251 911 77 36 62

e-mail: naftan2006@yahoo.com

Addis Ababa, Ethiopia

Thank you for considering this request!!!

Appendix B: Consent From participant

I have received a copy of the Invitation to Participate for the research project entitled "Dietary habit and prevalence of anemia among pregnant women attending antenatal clinic during their third trimester and concentration of micronutrient in cord blood: effect on neonatal birth weight" and have had an opportunity to read the information provided or it has been explained to me, and any questions that I may have had have been answered.

I agree to participate in this research project, understanding that I am doing so voluntarily, that confidentiality will be maintained, and that I have the right to withdraw from the study at any point using the means outlined in the invitation to participate.

I would like to have a copy of the summary report from the research data (please put a check mark here) ______

Signature _________________________

Date _____________________________

Appendix C

Questionnaire to obtain dietary habit and maternal health status

A. Socio-demographic, economic, women and lifestyle characteristics

1. Personal information

Name (optional) ____________________Code ________________Age____________

Gestational age__________________ Number of children ever born _______________

Pregnancy category: 1. Multiparous 2. Primiparous

Gap between the last delivery and current pregnancy ___________________

Place of delivery_______________________

Religion:

1. Orthodox
2. Muslim
3. Protestant
4. Catholic
5. No religion
6. Other, please specify____

2. Address:

Woreda _____________________

Kebele______________________

Residency place/Locality:

1.Urban

2. Peri-urban

3. Rural/country side

3. Marital status

1. Married 2. Single

4. Ethnicity

1. Oromo 2. Amhara 3. Tigre 4. Gurage 5. Other

5. Level of education

1. Illiterate
2. Primary school
3. Secondary school
4. Tertiary/University

6. Employment status: 1. Employed   2. Unemployed

7. If employed:

1. Government employee
2. Non-governmental organization
3. Self-employed
4. Daily laborer

8. If unemployed:

1. Trader/merchant
2. Farming
3. Nil
4. Other, please specify_________________________________

9. Family size___________

10. Body mass index: ______   Weight __________   Height _________

11. What is the source of your drinking water

1. Wells water
2. River water
3. Tap water
4. Spring water
5. Other

12. Do you have latrine facilities?

1. Yes   2. No

13. What is your housing condition?

Roofing: 1. Iron corrugated sheet   2. Hatched roof

Residential house made of: 1. Brick   2. Soil   3. Cement blocks

14. Do you have access to mass media? 1. Yes   2. No

15. Do you have any source of information about nutrition in pregnancy?

1. Yes   2. No

16. If yes, what is the source of your information?

1. Radio
2. Newsletter
3. Television
4. Internet
5. Community advocacy
6. Health extension workers
7. School
8. Other, please specify ____

17. Have you had any abortion?

1. Yes 2. No

18. Was it unintentional?

1. Yes 2. No

19. Are you taking any medication?

1. Yes 2. No

20. If yes, what kind of medication?

1. Iron supplement
2. iron-folic acid supplement
3. Anti-acid
4. other (specify) ___________________

21. If you are taking iron/iron-folic acid supplement, when did you start taking it?

____

22. How often do (did) you take it?

1. one tablet daily
2. Two tablets daily
3. Three tablets daily
4. One tablet three times/week
5. Two tablets three times/week
6. Three tablets three times /week
7. Other, please specify ____

23. Did you notice any changes in your appetite since you became pregnant?

1. Yes 2. No

If yes, specify *(please note any increase/ decrease)*

____

____

24. Do (did) you experience some symptoms like:

1. Persistent swelling of feet, hands or face
2. Increasing breathless, especially on routine activity
3. Headaches
4. Blurring of vision
5. Fever (temperature > 38 C)
6. High coloured urine in the past two weeks.

B. Twenty four hour recall food questionnaire

25. Did you practice any fasting since you became pregnant?

1. Yes 2. No

26. Level of fasting?

1. Deprivation of animal source food except fish
2. Deprivation of all animal source food
3. Deprivation of animal source food + no breakfast
4. Deprivation of animal source food + fasting until 3.00 pm

27. Number of meals per day? ________

28. How often do (did) you eat the following meals?

28a. Breakfast	1. Always	2. Sometimes	3. Do not eat
28b. Lunch	1. Always	2. Sometimes	3. Do not eat
28c. Dinner	1. Always	2. Sometimes	3. Do not eat
28d. Snack	1. Always	2. Sometimes	3. Do not eat

29. Please take time and try to fill the following table accordingly.

<table>
<tr><td colspan="3">Interviewer____________________
ID number__________
Interview date:________________
Mon Tue Wed Thru Fri Sat Sun</td><td colspan="4">Location _________________________________</td></tr>
<tr><td>Food/drink</td><td>Time</td><td>Place eaten</td><td>Description, cooking method</td><td>Amount</td><td>Weight equivalent (g)</td><td>Food code</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="7">Probe for sickness: 1. Yes 2. No
If yes, did the sickness affect appetite? 1. Yes 2. No
If yes, 1. Increase 2. Decrease</td></tr>
<tr><td colspan="7">Was it a feast day? 1. Yes 2. No
Was it a fasting day? 1. Yes 2. No</td></tr>
</table>

30. Did you eat the following foods in the past two weeks?

a. Iron rich foods such as; Meat and meat products, eggs, bread, green leafy vegetables, pulses and fruits

   1. Yes 2. No

b. Animal source foods such as; milk, yogurt and cheese

   1. Yes 2. No

c. Vitamin A-rich foods such as; (papaya, mango, tomato, carrot and green leafy vegetables

   1. Yes 2. No

d. Calcium rich foods such as; dairy products, cabbage, eggs and cereals

1. Yes   2. No

e. Zinc rich foods such as; maize, meat, fish and beans

1. Yes   2. No

31. Did you drink coffee daily in the past two weeks? 1. Yes   2. No

32. If yes, how many cups did you drink on average per day?________________

33. If yes, to question number 31, how often did you practice to drink?

   1. Before meal
   2. With meal
   3. Immediately after meal
   4. Approximately 1hour after meal
   5. Do not know

34. Did you drink tea daily in the past two weeks?

35. If yes, how many cups did you drink on average per day?________________

36. If yes, to question number 35, how often did you practice to drink?

   1. Before meal
   2. With meal
   3. Immediately after meal
   4. Approximately 1hour after meal
   5. Do not know

37. Did you drink the following beverages in the past two weeks? If your answer is yes, please specify the number of times and amount you had.

   a. Beer   1. Yes   2. No   If yes, specify__________________
   b. Liquor   1. Yes,   2. No   If yes, specify__________________
   c. Local liquor (Areqe)   1. Yes   2. No   If yes, specify__________________
   d. Carbonated beverages   1. Yes   2. No   If yes, specify__________________
   e. Tella   1. Yes   2. No   If yes, specify__________________
   f. Wine   1. Yes   2. No   If yes, specify__________________

Thank you for taking time to fill this survey!!!!

Appendix D

Clinical sample and data collection form

Code	Client name	Age	Card no	Phone number	Bp	Blood sugar	Hb	Remark

Appendix E

Cord blood sample collection form

Code no	Client name	Neonatal birth weight	Concentration of MN in the cord blood			Remark
			Iron	Zinc	Calcium	