Dietary practices of pregnant women and its associations with maternal and perinatal outcomes in rural Central Ethiopia

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This is to certify that the thesis prepared by Taddese Alemu Zerfu entitled: ***" Dietary practices of pregnant women and its associations with maternal and perinatal outcomes in rural Central Ethiopia"*** submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Dietary practices of pregnant women and its associations with maternal and perinatal outcomes in rural Central Ethiopia

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Addis Ababa University, 2016

Abstract

Maternal and child under nutrition is a serious developmental challenge contributing a large share to the global disease burden. It is a major reason for increased risk of adverse pregnancy outcomes, poor infant survival, and elevated risks of chronic diseases at later stages of life. Ethiopia has an unacceptably high burden of malnutrition and its consequences, and yet little is known about the determinants and responses to under nutrition during pregnancy. The few cross-sectional studies and the periodic national surveys are often less appropriate to investigate nutritional problems during the dynamic periods of pregnancy. Using a mixed-method approach, the present thesis examined the association of maternal nutrition during pregnancy with maternal and perinatal outcomes in rural Central Ethiopia. Applying a weighed-record (n= 55), the most frequently consumed foods were identified, sampled and analyzed for their nutrient composition and nutrient densities estimated. By enrolling pregnant mothers to a prospective cohort study, risk of anemia and adverse pregnancy outcomes were determined. Using a focus-group discussion (FGD), in-depth interviews (IIs) and passive observations, food consumption-related behaviors and perceptions were identified.

Dietary patterns adopted by the pregnant women were largely plant-based, predominantly cereal and legume-based. The nutrient-density of the foods were low and the overall dietary diversity was low. The overall incidence of maternal anemia increased from 28.6% to 32.4% during the follow-up period and twenty percent of the mothers experienced at least one of the adverse pregnancy outcomes (APO): low birth weight (LBW; 9.1%), preterm (13.6%), and stillbirth (4.5%). Mothers who consistently consumed $\geq$ 4 food groups (out of nine) had a lower risk of anemia [adjusted RR (ARR: 2.29; 95% CI: 1.62, 3.24], LBW (ARR: 2.06; 95% CI: 1.03, 4.11), and PTB (ARR: 4.61; 95% CI: 2.31,9.19) but not of stillbirth (ARR: 2.71; 95% CI: 0.88, 8.36) deliveries than those who consumed undiversified diets. Low or inconsistent consumption of dark green leafy vegetables(adjusted odds ratio (AOR), 2.012; 95% confidence interval (CI): 1.04; 3.87), dairy products (AOR, 2.64; 95% CI: 1.11; 6.30), and fruits and vegetables (AOR, 2.92; 95% CI: 1.49; 5.67) were associated with increased risk of APO. The presence of widespread misconceptions about weight gain during pregnancy, the food taboos among some population segments, and the relatively low awareness about maternal nutrition may be some of the underlying reasons.

Therefore, efforts to enhance maternal awareness about nutrition in general, and promoting dietary diversity ($\geq$ 4 food groups) to increase animal sources foods (ASF), fruits and vegetables in particular, could help improve maternal nutrition and prevent the associated adverse outcomes. Population based controlled trials of various options to improve dietary diversity and intake of selected limiting food groups are recommended.

Keywords: *Dietary diversity, anemia, low birth weight, preterm, stillbirth food taboo, intakes, women's nutrition, pregnancy*

Dedication

This work is dedicated to my father, the late Alemu Zerfu Abeje, who passed away before seeing the end of this work. Dear my beloved dad (Abaye), I know, you always had vision and trust in me which I promise to fulfill or die trying. Abaye you are a model and a good father. You were challenged by countless obstacles to educate me and bring me up, but you passed just a little bit before you see part of your dream come true. May the blessing and care of the Almighty God with all the Saints be with you and the whole family bereaved by your untimely death. Rest in peace my lovely dad!.

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Last but not least, I thank all study participants and data collectors who were the key for the conduct of my research in rural kebeles and villages.

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Abbreviations and Acronyms

AI	Adequate Intake
APHRC	African Population and Health Research Center
APO	Adverse Perinatal Outcomes
AOR	Adjusted Odds Ratio
ASF	Animal Source Foods;
ANC	Ante Natal Care
AOAC	Association of Official Analytical Chemists
BMR	Basal Metabolic Rate
BMC	Bio Med Central
BMI	Body Mass Index
CHO	Carbohydrate
CSA	Central Statistical Authority
CI	Confidence Interval
DHS	Demographic and Health Survey
DRI	Dietary Reference Intakes
DVD	Digital Optical Disc Storage
EDHS	Ethiopian Demographic and Health Survey
FGD	Focus group discussions
FAO	Food and Agriculture Organization
IOM	Institute of Medicine
IDRC	International Development Research Center;
IDA	Iron deficiency anemia
IFA	Iron Folic Acid

Hb	Hemoglobin
KII	Key informant interviews
LBW	Low Birth Weight
LMIC	Low and Middle Income Countries
Kg	Kilogram
PTB	Pre-tem Birth
MUAC	Mid Upper Arm Circumference
SPPS	Statistical package for Social Sciences
LNS	Lipid-Nutrient Supplements
MAM	Moderate Acute Malnutrition
NGO	Non-Government Organization
SAM	Severe Acute Malnutrition
SD	Standard Deviation
TEE	Trans Esophageal Echocardiography
TV	Television
UN	United Nations
UNFPA	United Nations Fund for Population Authorities
UNICEF	United Nations International Children's Emergency
WCC	White Blood Cell Count
WHO	WHO World Health Organization
WDDS	Women's Dietary Diversity Score

1

Chapter 1- Introduction

1. Chapter One: Introduction

1.1. Background

Pregnancy is the time during which one or more gametes develops into an embryo then to a fetus. It is a unique and a critical stage of life during which extensive anatomical, physiological, biochemical and several other related changes take place (Williamson 2006; Chandraharan & Arulkumaran 2012; Heidemann & Mcclure 2003). Maternal individual, genetic and environmental factors determine whether these dynamic changes ends with healthy or adverse outcomes (Ouzounian & Elkayam 2012; Ding *et al.* 2014; Lisonkova *et al.* 2010; Yao *et al*. 2014).

Nutrition during pregnancy is among the leading environmental factors strongly associated with pregnancy and perinatal outcomes (Abu-saad & Fraser 2010). Requirements for both macro and micronutrients increases sharply to accommodate the high demands of the growing fetus and maternal tissue deposition. During the third trimester of pregnancy in particular, the requirements for some nutrients like energy, iron, zinc, calcium, folic acid and others increases, exceptionally (Kramer & Kakuma, 2003).

Malnutrition has been identified as the major underlying contributing factor for nearly half (45%) of all child and a fifth of maternal deaths (Middleton et al. 2013). Maternal and child undernutrition, is the leading global developmental challenge affecting nearly half of the world's population and responsible for the death of 3.5 million mothers and children annually (Abu-saad & Fraser, 2010; Blencowe et al., 2013; Robert E Black et.al, 2008). Malnutrition accounts for 7% of the global disease burden and contributes to increased probability of poor pregnancy outcomes (Khoushabi & Saraswathi 2010; Persson et al. 2012). It has been strongly linked to functional consequences like increased risk of adverse pregnancy outcomes, poor infant survival and risk of chronic diseases at later stages of life (Khoushabi & Saraswathi, 2010; Muthayya, 2009; Saaka, 2012). Studies showed that nutrition during pregnancy was the single most important factor predicting preterm birth(Christian et al. 2009), intrauterine growth restriction (Behrman & Butler 2007; Zhang et al. 2009) and reproductive loss through still births (Abu-saad

& Fraser 2010; Mori et al. 2012). It is also the major determinant factor for the risk of giving birth to low birth weight infants (Potdar Ramesh D et al 2014; Muthayya 2009).

Worrisomely, the burden of both maternal and child undernutrition along with the adverse outcomes are skewed to few countries in low and middle income regions in Africa and Asia (Ahmed, Hossain, & Sanin, 2012; World Health Organisation, 2013). Near to one-third of children in these countries are either underweight or stunted, and more than 30% of the population suffers from micronutrient deficiencies (Yakoob & Bhutta 2011; Yuan et al. 2014). The sub-Saharan Africa and South Asia together stands for almost three-fourth of the world's stunted children; whereby, the former contributing for more than 40% and 10% of stunted and wasted children, respectively (Bain et al. 2013; Kramer & Allen 2015; UNICEF 2012). Sub-Saharan Africa is home to 98% of the 2.6 million annual third trimester still births (Lawn & Kinney 2015; Fretts 2010).

A major reason for the widespread malnutrition and the associated consequences in Sub-Saharan Africa is the monotonous, plant-based diets that may be inadequate to provide adequate nutrition during pregnancy (Imamura et al. 2015). Diets in such setting consists mainly of cereal or root staples; with very little intake of animal source proteins, vegetables, and fruits and thus are poor sources of bioavailable mineral and vitamins critical for healthy pregnancy. These have been identified as significant contributors to global burden of maternal malnutrition (Saaka 2012; Pinto et al. 2008). Further compromising efforts to improve dietary quality is the relatively high cost of nutrient-dense foods like Animal Source Foods (ASF), fruits and vegetables (Acham, Oldewage-theron, & Egal, 2012; Bain *et al.,* 2013). Besides, the mother is often the last to benefit in a household even when there is an improvement in household income, but the first to sacrifice. This creates a cycle of poverty, disease, and illness (Mason, John, & Shrimpton, 2010).

Even when enough food is available, the majority of women do not receive adequate nutrients during pregnancy attributable to poor knowledge on what constitutes an adequate diet (Ahmed et al., 2012; Black & Hopkins Bloomberg, 2008). Simple, rapid, and useful proxy measures and indicators like the FAO's scoring system for measuring Women's Dietary Diversity Score

(WDDS) have been shown to be valid proxy indicators for various nutritional monitoring activities. It is strongly linked with dietary energy availability at household level (Hoddinott et al. 2002), micronutrient adequacy of diets of young children and women of reproductive age (Arimond & Ruel 2004; Arimond & Wiesmann 2010; Daniels 2009) and predictors of food security and nutritional status (Onyango 2003; Ruel 2006). However, little is known on whether the WDDS is associated with maternal anemia and pregnancy outcomes.

Ethiopia has an unacceptably high burden of maternal and neonatal morbidity and mortality rates (WHO, 2013). High level of multiple micronutrient deficiencies and thinness (underweight) among mothers, childhood stunting and wasting are key malnutrition related features signifying the extent and depth of nutritional problems in the country. High burden of adverse perinatal outcomes such as low birth weight, preterm and still birth are not uncommon (Negera et al. 2013; Kedir et al. 2013; Gebremedhin et al. 2014; Gebremedhin et al. 2012; Assefa et al. 2012; Melku et al. 2014; Abriha et al. 2014; Alene & Mohamed 2014).

However, little is known about the causes, determinants and responses to maternal malnutrition and the associated adverse outcomes in Ethiopia. Furthermore, available studies in the country are often cross-sectional studies that are less appropriate to characterize dietary patterns and changes happening during the dynamic period of pregnancy.

Therefore, the present thesis employed a mixed methods that include systematic reviews of global evidences, analyses of secondary datasets, a prospective cohort study, a cross-sectional qualitative study, and laboratory analyses of food composition.

1.2 Objectives

General objective

To investigate the assosation of maternal dietary diversity, nutritent composition and nutrient density during pregancy with maternal anemia and perinatal outcomes in a rural setup in Ethiopia.

Specific Objectives

1. To review global evidences on the effect of micronutrinet supplments during pregancy on pregnancy and pregancy outcomes
2. To identify, characterize, and evaluate the adequacy of most commonly consumed foods during pregnancy
3. To determine the association of maternal dietary diversity during pregancy with maternal anemia and key prenatal outcomes in rural and resource limitted settings of Ethiopia.
4. To investigate the association of the consumption of specific food groups with the risk of adverse pregnancy outcomes in rural Arsi, Central Ethiopia
5. To analyse the prevalnce and predictors of anemia from national and demographic data
6. To determine the association of the consumption of specific food groups with the risk of anemia during pregnancy.
7. To explore maternal food taboos, dietary habits and cultural beliefs of weight gain during pregnancy in rural Ethiopia.

Thus, the PhD thesis is organized as follows:

Chapter Two presents the literature review that covers a description of the available evidences on key issues addressed by the study. The review mainly focused on a brief description of the physiological changes and nutritional requirements occurring during pregnancy. It also describes about role of dietary diversity during pregnancy and the outcomes. In addition, two published manuscripts were also included to be part of the review as one is a systematic review published on BMC Nutrition Journal and the other is a finding from analysis of secondary dataset published on Anemia Journal.

Chapter Three gives a description of the experimental designs, and the materials and methods applied in the present studies.

Chapter Four presents the findings of the different studies in the form of published papers, submitted manuscript, or manuscript prepared for publication. The chapter is organized as follows:

Section 4.1 (Paper published in BMC Nutrition Journal, First view Jan 31, 2013) presents findings of a systematic review of global literature. The review results showed that supplying micronutrients, mainly multiple micronutrients have beneficial effect in reducing the risk of low birth weight and other complications.

Section 4.2. (Draft paper entitled: *Nutrient composition and micronutrient densities of commonly consumed foods by pregnant women in rural Arsi, central Ethiopia*).

The paper presents findings from laboratory based analysis, for nutrient composition and density, of common staple foods of pregnant mothers in the study area. A number of major and commonly consumed foods were identified and analyzed for their composition in macronutrient and selected key micronutrients' (Fe, Zn and Ca). Energy and nutrient intakes were estimated, and the nutrient intake and densities were compared to recommendations.

Section 4.3 (Paper published in the American Journal of Clinical Nutrition, First view May 2016) presents findings of a prospective cohort study data. The study concluded that the FAO's simple tool for measuring women's dietary diversity score (WDDS) is when ≥

4 food groups associated with lower risk of maternal anemia and perinatal adverse outcomes including low birthweight (LBW), preterm birth (PTB), but not stillbirth.

Section 4.4. (Draft article entitled: *Consumption of dairy, fruits and dark green leafy vegetables during pregnancy is associated with lowered risk of adverse perinatal outcomes at birth: prospective cohort study in rural Ethiopia).*

This work builds up on the paper published and presented under section 4.2 and investigate the association of specific food groups with maternal and perinatal outcomes. This section highlights that the consumption of certain food groups like animal source foods, particularly dairy products, fruits, and dark green leafy vegetables are exceptionally associated with the perinatal adverse outcomes and maternal anemia

Section 4.5 (Paper published in Anemia Journal, First view August 2015) presents findings of data from analysis of secondary data of the latest Ethiopian demographic and health survey (EDHS). The findings showed that anemia during pregnancy is a moderate public health problem in Ethiopia and Maternal age, region, pregnancy trimester, number of under five children, previous history of abortion (termination of pregnancy), breastfeeding practices, and number of antenatal care visits were key independent predictors.

Section 4.6. (Paper published in the Journal of BMC Health, Population and Nutrition, first view July 25, 2016). The findings showed that misconceptions about weight gain during pregnancy and food taboos were widespread, particularly among older and illiterate rural communities.

Chapter Five gives a general discussion on the findings of the studies.

Chapter Six presents the summary of the results, conclusions and recommendations

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2

Chapter 2 - Literature Review

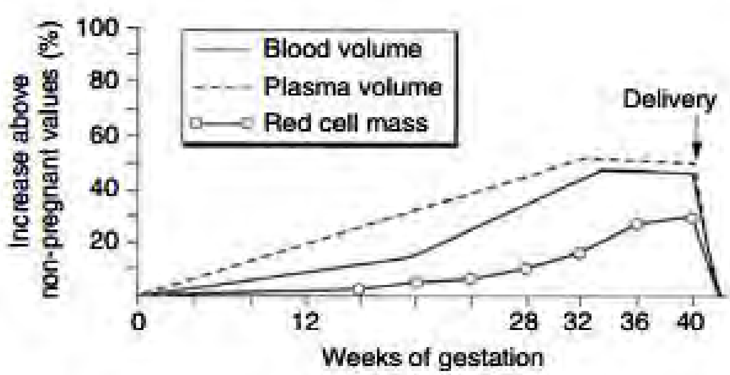
2. Chapter Two : Literature Review

2.1. Physiological Changes During Pregnancy

Pregnancy is associated with profound anatomical, physiological, biochemical and endocrine changes that affect multiple system in the body. Rapid and visible physiologic changes mainly involves hematologic, cardiovascular, respiratory, gastro-intestinal, renal, endocrine and the integumentary (skin) systems. Hormonal changes from very early on in pregnancy and mechanical changes are the driving forces. Though some complications arise, these changes are essential to help the woman to adapt to pregnancy state and to aid fetal growth and survival. The changes also support the requirements of fetal homeostasis without jeopardizing maternal well being (Rhoades & Bell 2012; Hall 1946- 2011).

a) Hematological changes

Hematological changes during pregnancy allow the mother to adapt for possible problems like infection, circulatory collapse and excessive bleeding during pregnancy and labor. Thus, the White Blood Cell Count (WCC) increases up to 10.5 $\times 10^9$ / l in late pregnancy and peaking to 20 $\times 10^9$ / l during labor (Abdel-Razeq & Buhimschi 2010). Unlike a 20 - 30% increase of the red blood cell volume, the plasma volume increases up to 50% which is too large to cause a dilutional anemia. Consequently, the hematocrit values decreases on lab analysis, reading a pseudo physiologic anemia. In response, the total concentration of plasma protein is reduced. This results in a drop in the colloid oncotic pressure, and may account for the oedema seen in pregnancy (Hall 1946- 2011; Rhoades & Bell 2012). Similarly, the platelets count stay normal or show slight decrease **(Figure 2.1).** Clotting factors like fibrinogen and factor VIII significantly due to increased production by the liver. This happens to facilitate clotting at the time of placental separation and prevent bleeding during pregnancy. Nevertheless, it has also a risk hypercoaglable state that is associated with increased risk for developing blood clots and embolisms during pregnancy (Hall & GUYTON 2010; Rhoades & Bell 2012).



(Source : ABC of Antenatal Care, 4th edn 2002)

Figure 2.1: Increases in volume of constituents of blood during pregnancy, indicating that the largest component is expansion of plasma volume

b) Cardiovascular Changes

During the course of pregnancy, cardiac output increases by about 40 - 50% or from 4 to 7 liters. This is accompanied by an increase of heart rate (15 beats/min more than usual), stroke volume, heart rate and cardiac output. There is also a decrease in total peripheral resistance. This will automatically displace the heart upward and to the left by the gravid and enlarging uterus.

c) Respiratory Changes

Major respiratory changes in pregnancy occur mainly due to mechanical, consumption and stimulation factors. Mechanical factors involve effects of the enlarging uterus with diaphragm displacement up to 4 cm upwards; consumption is related to increased need for oxygen whereby the consumption of oxygen increasing by 15-20%. The major (50%) of additional oxygen consumption goes to the uterus. Stimulation factors are due to the act of progesterone hormone as a respiratory stimulant and increasing the sensitivity of the respiratory centers to CO_2. Dyspnoea (difficulty of breathing) and hyperventilation is a common complaint in pregnancy affecting some 60 -70% of women at some stage. It occurs as a result of physiologic respiratory changes taking place during pregnancy.

d) Changes to the Gastro-Intestinal system

During pregnancy, risks of reflux and aspiration of gastric contents and nausea and vomiting increases. On the other hand, though normal during pregnancy, gastric emptying is delayed during labor. There is also prolonged gastric empty time, decreased gastro esophageal sphincter tone, which can lead to acid feflux and decreased colonic motility which ultimately causes constipation during pregnancy. All Physiologic changes to the gastrointestinal system are meditated by the smooth muscle relaxant effects of progesterone(Hall & GUYTON 2010).

e) Endocrine and Metabolic Changes

The Pituitary Gland enlarges up to 135% and there is an increased production of plasma growth hormone and prolactin. The Thyroid gland also suffers from relative iodine deficiency states which may lead to a glandular hypertrophy. Ref? Explain this effect on pregnancy

f) Changes to the Immune System

There is decreased cellular immunity, with increase in humoral, or antibody mediated immunity. This results in increased susceptibility to intracellular pathogens. The fetus inside may be viewed as an unusually successful allograft since it genetically differs from the woman (Hall & GUYTON 2010). But, cell mediated auto-immune diseases often improve during pregnancy.

2.2. Changes in Body Composition & Weight Gain during pregnancy

Maternal nutritional status at the time of conception is an important determinant of fetal growth and development. According to the latest and revised recommendation of the Institute of Medicine (IOM), women planning pregnancy are recommended to have a healthy bodyweight (BMI of 20 – 25) during the preconception and gain, on average, up to 12.5Kg during pregnancy. This level of weight gain was shown to be associated with optimal reproductive outcome and was used as the basis for estimating components of weight changes in healthy pregnant women(Committee on Obstetric Practice, 2013; IOM, 2009).

Pregnant women entering to pregnancy with a body mass index (BMI) of < 18.5, 18.5 - 24.9, 25 - 29.9 and ≥ 30 kg/m^2 are advised to gain total weight of 12.5 - 18, 11.5 - 16, 7 - 11.5 and 5 - 7kgs, respectively (Table 2.1) (IOM 2009). These range of weight gain are associated with lowest prevalence of the outcomes of greatest interest : cesarean delivery, postpartum weight retention, preterm birth, small- or large-for-gestational age birth, and child hood obesity. The fetus, placenta, amniotic fluid, maternal body water being major contributors of normal weight gain in pregnancy (Table 2.1) (Rasmussen et al. 2009).

Table 2.1. Components of weight gain during pregnancy

Body component	Increase in weight (Kg) at term (40 Wks)	Percentage (%) of total weight gain
Products of Conception		
• Fetus	3.40	27.2
• Placenta	0.65	5.2
• Amniotic Fluid	0.80	6.4
Maternal Tissues		
• Uterus	0.97	7.8
• Mammary gland	0.41	3.3
• blood	1.25	10.0
• Extracellular, extra-vascular fluid	1.68	13.4
Total weight gain	12.5	100
Assumed fat deposition	3.35	26.8

Source: adapted from (Williamson 2006)

Epidemiologic studies, in the absence experimental studies, showed that low and irregular gestational weight gain had a linear and direct relationship with birth weight for gestational age, predicting small for gestational age babies, but week correlation with still birth (Rasmussen et al. 2009; Imdad & Bhutta 2011; W.B.Saundres 1998; Viswanathan et al. 2008; Muthayya 2009). Low and irregular gestational weight gain is moderately associated with failure to initiate breastfeeding in some studies (Winkvist et al. 2015), while non-related in others (Bartok et al. 2012) . Given the obligatory weight gain in the maternal tissues (uterus, breast, blood) and the fetal-placental unit, a weight gain less than ~7.5-8.5 kg would also imply mobilization of maternal adipose tissue and possibly protein stores (Einerson et al. 2011). Conversely, excessive weight gain is associated with complications during pregnancy and an increased likelihood of overweight and obesity in the mother after the birth. According to the Institute of Medicine (Committee on Obstetric Practice 2013), a normal weight mother with BMI of 18.5 - 24.9 kg/m^2 should gain 11.5 - 16 Kg, the other non normal weight women: under weight (BMI < 18.5), overweight (BMI 25 - 29.9 Kg/m^2) and obese (BMI > 30 Kg/m2) are advised to gain between 12.5 - 18, 7 - 11.5 and 5 - 9 Kg with advisable rate of weight gains, respectively, (Table 2.2).

Table 2.2. : Recommendations for Total and Rate of Weight Gain During Pregnancy, by Pre-pregnancy BMI

Pre-pregnancy BMI	**Total weight gain** **Range in kg**	**Rates of weight gain, 2nd and 3rd Trimester** **Mean (range) in Kg/week**
Underweight *(< 18.5 kg/m^2)*	12.5 - 18	0.51 (0.44 - 0.58)
Normal weight *(18.5 - 24.9/m^2)*	11.5 - 16	0.42 (0.35 - 0.50)
Overweight *(25.0 - 29.9 kg/m^2)*	7 - 11.5	0.28 (0.23 - 0.33)
Obese *(≥ 30.0 kg/m^2)*	5 - 9	0.22 (0.17 - 0.27)

Modified from Institute of Medicine (US). Weight gain during pregnancy: reexamining the guidelines. Washington, DC. National Academies Press; 2009.

It is also harder for the mother to return to her pre-pregnancy weight. Retaining more weight postpartum, especially among women who are already overweight or obese, can lead to lifelong obesity and its cardiovascular consequences. Excessive weight gain in subsequent pregnancies can accentuate this pattern. Furthermore, a higher body mass index (BMI) at the start of a subsequent pregnancy is associated with increased risk of adverse health outcomes for the baby in both short and long term(Gillman 2012; Rasmussen et al. 2009).

In addition to the well reported physiological changes, the total amount of weight gain during pregnancy is determined by several factors including : psychological, behavioral, family, social, cultural, and environmental factors (Institute of Medicine 2009). The rate of weight gain is influenced by pre-pregnancy bodyweight. Approximately, 5% of the total weight gain of a pregnant woman occurs during the first trimester or 10 –13 weeks of pregnancy, while the rest is gained evenly throughout the second and third trimesters of pregnancy. During the later stages of pregnancy period, the mean rates of weight gain for well-nourished women with uncomplicated singleton pregnancies were reported as approximately 0.45 kg per week during the second trimester and 0.40 kg per week during the third trimester. However this can vary depending on maternal preconception BMI, ethnicity and age (Rasmussen 2009; Institute of Medicine 2009; Rasmussen et al. 2009).

The average total increase in body water during pregnancy is also about 8.5 liters, and this is the same for women in a first or subsequent pregnancy. It consists of fetal fluid, amniotic fluid, placental fluid and maternal fluid (Dundas 2003; Heidemann & Mcclure 2003).

2.2. Nutritional Requirements During Pregnancy

In a woman's life cycle, there is no time where nutrition is more important than before and /or during pregnancy. However, nutritional needs during this critical anabolic phase of life change. Pregnant women need to consume extra vitamins and minerals, increase their calorie intake, and avoid certain foods such as and chemicals to optimize the growth and development of their baby along with supporting alterations in maternal tissues and metabolism.

Requirements for almost all nutrients are increased during pregnancy compared to adult or non-reproducing women. Particularly, the need for macronutrients (energy and protein) and several micronutrients, including: Iron, Iodine, Zinc Magnesium, Selenium Folate, Vitamin B_6, Niacin, Riboflavin, Thiamine, Pantothenic acid, Vitamin C, , Vitamin A, Vitamin B-12 and Choline) is increased from 6 - 50%, in descending order. But, the need for few micronutrients like Biotine, Vitamin D, Vitamin E, Vitamin K, Calcium, Phosphorus and Fluoride remain the same (Ladipo 2000; Potdar Ramesh D et al 2014) (Table 2.3.) Details of specific nutrient requirements, health benefits and their amount during pregnancy is also presented as follows.

Table 2.3. Recommended daily nutrient intakes of adult, lactating & pregnant women

Nutrient	***Dietary Reference Intakes (DRI) for each Physiologic /reproductive state***			***Percentage ↑ (pregnancy over adult women)***
	Adult women	*Lactation*	*Pregnancy*	
Energy, kcal	*2200*	↑500 kcal/d 0–6 mo ↑1400 kcal/d 7–9 mo	↑340 (2nd) - 452 (3rd) kcal/d trimesters	15.4^{2nd} / 20.5^{3rd}
Protein, g	46	71	71	54.35
Vitamin C, mg	75	120	85	13.33
Thiamin, mg	1.1	1.4	1.4	27.27
Riboflavin, mg	1.1	1.6	1.4	27.27
Niacin, ng	14	17	18	28.57
Vitamin B-6, mg	1.3	2	1.9	46.15
Folate, g	400	500	600	50.00
Vitamin B-12 g	2.4	2.8	2.6	8.33
Pantothenic acid, mg	5	7	6	20.00
Biotin, g	30	35	30	0.00
Choline, mg	425	450	550	5.88
Vitamin A, g	700	1300	770	10.00
Vitamin D, g	5	5	5	0.00
Vitamin E, mg	15	19	15	0.00
Vitamin K, g	90	90	90	0.00
Calcium, mg	1000	1000	1000	0.00
Phosphorus, mg	700	700	700	0.00
Magnesium, mg	310	310	350	12.90
Iron, mg	18	9	27	50.00
Zinc, mg	8	12	11	37.5
Iodine, g	150	290	220	46.67
Selenium, g	55	70	60	9.09
Fluoride, mg	3	3	3	0.00

Modified from Institute of Medicine (US): Journal of Human Nutrition and Dietetics. 2003 Jun 1;16(3):199-200

2.2.1. Macronutrients

2.2.1.1. Energy and Carbohydrate

Maternal diet during pregnancy is expected to provide sufficient energy to ensure the delivery of a full-term and healthy infant of adequate size and appropriate body composition (Brion et al. 2010). Energy need during this time is estimated to be the sum of total energy expenditure (same as non-pregnant adult woman), plus the median change in total energy expenditure of 8 kcal/gestational week, plus the energy deposition during pregnancy of 180 kcal/d (McGowan & McAuliffe 2012).

Energy requirements during pregnancy increase by about 12 percent, mainly during the second and third trimester of pregnancy. In response, women should get additional energy during these late periods of pregnancy. No need to take extra energy during the first trimester, as the total energy expenditure does not change greatly and weight gain is minimal during this time. During the second trimester of pregnancy, a pregnant women should get 340kcal extra energy and increase the same to 450 kcal during the last (third) trimester (UN University & WHO 2004; Butte et al. 2004a; Byrne et al. 2011).

For most women, extra energy needs are easily met by adding a small snack during the day (Brion et al. 2010). The focus should be on increasing the consumption of nutrient-dense foods and minimizing empty-calorie foods that may provide the extra energy needed but do not provide micronutrients that are needed in much higher amounts compared with increased caloric needs (Brion et al., 2010).

The extra energy need is mainly used by the body to build increased mass of active tissue (fetal, placental, and maternal), to replace the cost of increased maternal effort (e.g., cardiovascular and respiratory work), and compensate the cost of tissue synthesis. Furthermore, increase in maternal body weight, raise of increase in basal metabolic rate (an average 10–15 percent), the energy costs of the growing foetus, and maternal physiological changes in pregnancy also contribute for the increased energy requirements (Butte et al. 2004b; Nowicki et al. 2011). Additional energy cost of pregnancy in women with an average gestational weight gain of 12 kg is given in **Table 2.4.**

Table 2.4. : Additional energy cost of pregnancy in women with an average gestational weight gain

A. Rates of tissue deposition					
	1st trimester g/d	**2nd trimester** g/d	**3rd trimester** g/d	**Total deposition** g/280 d	
Weight gain	17	60	54	12 000	
Protein deposition[a]	0	1.3	5.1	597	
Fat deposition[a]	5.2	18.9	16.9	3 741	
B. Energy cost of pregnancy estimated from the increment in BMR and energy deposition					
	1st trimester *kJ/d*	**2nd trimester** *kJ/d*	**3rd trimester** *kJ/d*	**Total energy cost**	
				MJ	*kcal*
Protein deposition[a]	0	30	121	14.1	*3 370*
Fat deposition[a]	202	732	654	144.8	*34 600*
Efficiency of energy utilization[b]	20	76	77	15.9	*3 800*
Basal metabolic rate	199	397	993	147.8	*35 130*
Total energy cost of pregnancy (kJ/d)	**421**	**1 235**	**1 845**	**322.6**	***77 100***
C. Energy cost of pregnancy estimated from the increment in TEE and energy deposition					
	1st trimester *kJ/d*	**2nd trimester** *kJ/d*	**3rd trimester** *kJ/d*	**Total energy cost**	
				MJ	*kcal*
Protein deposition[a]	0	30	121	14.1	*3 370*
Fat deposition[a]	202	732	654	144.8	*34 600*
Total energy expenditure[c]	85	350	1 300	161.4	*38 560*
Total energy cost of pregnancy (kJ/d)	**287**	**1,112**	**2 075**	**320.2**	***76 530***

Source :WHO/FAO, 2004

The table shows that additional energy cost of pregnancy during the last two trimesters are much more higher than the first trimester, in all activities be it for tissue deposition or BMR or TEE. During the first trimester, no energy is expended for protein deposition for all the three activities, but 5.1 and 121g/day of energy is expended for the same purpose during the last, third trimester. The same is true for others activities and physiologic functions (Table 2.4).

The extra dietary energy requirement in pregnancy also depends on the extent to which mothers can and do reduce their physical activity. Estimates of energy requirements and recommendations for energy intake of pregnant women should be population-specific, because of differences in body size, lifestyle and underlying nutritional status (Millward & Jackson 2004). Well-nourished women raised in affluent or economically developed societies may have different energy needs in pregnancy than women from low-income developing societies; pregnancy energy requirements of stunted or undernourished women may differ from those of overweight and obese women; and physical activity patterns may change during pregnancy to an extent that is determined by socio-economic and cultural factors (UN University & WHO 2004; Butte & King 2005).

More importantly, severe energy restriction during pregnancy, either to lose weight (Tardif et al. 2004)or poor dietary habits (Kedir et al. 2013) is not appropriate This is because inadequate energy and nutritional intake is associated with an increased risk of low birth weight infants and other adverse outcomes of pregnancy (Brion et al. 2010; Tardif et al. 2004). Energy restriction can lead to the production of ketone bodies and other metabolites in the body that can create metabolic stress detrimental to foetal development and maternal health(Imdad & Bhutta 2011; DeLany et al. 2014).

Glucose is a major fuel used by most cells in the body. Requirements for starch, sugar and non-starch polysaccharides (dietary fiber) during pregnancy are not increased. However, pregnancy results in an increased metabolic rate and thus an increased fuel requirement. Women with low intakes of non-starch polysaccharides may benefit from increased intakes, to within a range of 12–24 g per day, along with increased fluid intakes to encourage regular bowel movement. In spite of the recognized need for increased energy-yielding substrates imposed by pregnancy, the magnitude of need, as well as how much of the increased requirement needs to be met from exogenous sources, remains incompletely understood and is highly variable (Butte NF 2000; Trumbo et al. 2002).

2.2.1.2 : Proteins

Proteins are macromolecules consisting of long chains of amino acid molecules. Due to enormous effect and benefits as well as unclear evidence on guidance, the influence of maternal dietary protein during pregnancy on offspring phenotype and health is under continuous investigation.(Stephens et.al, 2015) During pregnancy, the mother must consume adequate protein to meet the needs of her growing fetus in addition to meeting her own increased needs as she physically grows in size to carry her baby. It is also useful for accretion in maternal tissues like the heart, blood, breast, and uterus, and fetal-support tissues including the placenta and extra-embryonic membranes (Blumfield et al. 2012).

The amount of nitrogen accreted due to a pregnancy involving 12.5 kg of maternal weight gain is about 148 g. It is predicted by a summation of the protein components of the fetus, uterus , expanded maternal blood volume, placenta, extracellular fluid, and amniotic fluid. Protein is not gained at a constant rate, the rate at which protein is deposited increases as pregnancy progresses. Estimates for the first, second, third and fourth quarters are 0.64, 1.84, 4.76 and 6.10 g of protein per day, respectively (Trumbo et al. 2002; Blumfield & Collins 2014; King 2000).

Despite the generally high level of protein intake globally, several special populations should be carefully monitored for adequate protein intake and quality during pregnancy: vegetarians (Foster et al. 2015; Lee & Krawinkel 2011), vegans (Kristensen et al. 2015; Bradbury et al. 2014), low-income women experiencing food insecurity (Nochera et al. 2011; Fowles et al. 2012), and women experiencing severe nausea and vomiting(Gadsby et al. 1993).

Vegetarian and vegan women are at high risk for protein deficiency both before and during pregnancy. They need to compensate the lost nutrients by consuming all essential amino acids from plant sources to create the protein necessary for the fetus's growth. Although it is possible for such mothers to have a healthy pregnancy, careful planning and monitoring to ensure the increased protein demands is essential (Kristensen et al. 2015; Lee & Krawinkel 2011).

Mothers from low income countries are also at high risk for protein deficiency during pregnancy because of potential issues with food insecurity. A woman who is food insecure may lack adequate resources to obtain protein-rich, nutrient-dense foods, which often cost more compared with less nutritious foods. Therefore, these food-insecure women should be helped by identifying low-cost protein sources, such as canned tuna, beans, eggs, and limited amounts of meat. Woman experiencing food insecurity should also be referred to nutrition assistance programs available in their locality (R. et al. 2011; Imamura et al. 2015).

Even if limit and dangers for excess protein intake are not established, experimental studies have showed that there no beneficial effects on any of the maternal or fetal outcomes and a statistically non significant increase in neonatal death was observed(Stephens et al. 2015a). One study found that protein from animal sources, primarily meat products, consumed during pregnancy may increase risk of overweight in offspring, especially when the offspring is a female one (Maslova et al. 2014).

2.2.1.3. Lipids and Fats

Fat is a major source of fuel energy for the body. If intakes of fat, along with carbohydrate and protein, are inadequate to meet energy needs, then negative energy balance occurs. Fats also aid in the absorption of the fat-soluble vitamins A, D, E, and K and carotenoids.

During organogenesis which occurs at early phases of pregnancy, lipids are involved for the formation of cell membranes and hormones and proper eye and brain development, especially during the prenatal period and into the first few years of the child's life. Hence, pregnant women need an adequate dietary intake of essential fatty acids and their longer-chain derivatives, also called omega fatty acids like the DHA and AA.

Fat is a source of concentrated energy providing substantial amounts of calories per gram of food eaten and outstandingly beneficial to women at risk of energy malnutrition while pregnant. Maternal fat intake has also been identified as a major contributor to a healthy fetal environment, with a beneficial role for unsaturated fats during development as well as a beneficial impact on cell membrane physiology(Wood-Bradley et al. 2013).

Contrarily, pregnant women who are not at risk should avoid the intake of excess fat because it can easily lead to undesired weight gain and several complications. Maternal diets high in fat were seen to affect maternal metabolic parameters, fetal growth and development and metabolic status (Reynolds et al. 2014). High intake of total fat and saturated fatty acids was also positively related to depressive symptoms during pregnancy(Miyake et al. 2013).

2.2.1.4. Dietary Fiber

Fiber is a very important component of the prenatal diet(Anderson et al. 2009). The development of the fetus is not dependent on an adequate supply of fiber, but a high-fiber diet significantly increases the comfort of the pregnant mother by helping to reduce constipation, a common side effect of pregnancy(Kim & Je 2014). Although the Adequate Intake (AI) during pregnancy is 28 g/day, this level of fiber intake has never been met even by the diets of women who depend on consuming fiber rich diets (Trumbo et al. 2002). Inadequate intake of fiber could lead to health problems and may worsen discomforts of pregnancy (Blumfield et al. 2012). It can lead to adverse effects on health, including constipation, hemorrhoids, and diverticulitis, It is even more important that pregnant women get enough fiber because they are at higher risk for these problems (Lattimer & Haub 2010).

Higher fluid needs, reduced exercise, and hormonal changes within the woman's body designed to allow the baby more room for growth may all contribute to problems with constipation and hemorrhoids during pregnancy. Pregnant women should be given the standard advice to reduce these problems, including increasing non-caffeinated fluids, moderate exercise, and high fiber intake. Fruits, vegetables, beans, whole grains, seeds, and nuts are all good dietary sources of fiber.

2.2.2. Micronutrients

Micronutrients are the minerals and vitamins that are in food. They have important influences on the health of pregnant women and the growing fetus (Christian & Stewart 2010). Discrepancies between the intake of certain micronutrients such as iodine, iron, vitamin A and folic acid and the recommended quantities during pregnancy are to be expected. This is related to the crucial benefit of the micronutrients to ensure the health and proper development of a fetus and growing child (Seshadri 2001). A lack of micronutrients can cause slowed physical growth (stunting) and weaken immune systems to the point of endangering a child's life.

Micronutrient malnutrition is among the leading and commonest health problems globally, accounting for 7% of the global disease burden and comes with a global cost of $180 billion each year. One in three people have at least one form of micronutrient deficiency (FAO 2013). Iron deficiency anemia (IDA) alone affects more than 2 billion people globally and contributes to over 100,000 maternal and almost 600,000 perinatal deaths each year (Kassebaum et al. 2014). Contrarily, malnutrition related to micronutrient deficiency is one of the most widespread, yet largely neglected nutrition challenges faced by women living in the developing world (Bain et al. 2013).

The recommended intakes for many essential nutrients increase during pregnancy and breast-feeding. But, the nutrients of particular concern are those for which there is an increased requirement during pregnancy (vitamin A, riboflavin and folate), as well as iron, and these are discussed in more detail here.

a) Vitamin A

Vitamin A is a fat-soluble vitamin essential for normal cell development, embryonic development, cell division, fetal organ and skeletal growth and maturation. It is also important for the maintenance of the immune system strengthening defenses against infection, and development of vision in the fetus as well as maintenance of maternal eye health and night vision. Adequate maternal status of vitamin A is critical for a healthy pregnancy (Who 2009; Ladipo 2000).

Dietary sources of vitamin A are both animals and plants (fruits and vegetables). Preformed forms of vitamin A or the unsaturated nutritional organic compounds like retinol, retinal and retinoic acid are found in animals, while vegetables and fruits provide carotenoids (beta-carotene). Beta-carotene is converted to retinol in the body. The conversion of beta-carotene to retinol is regulated by the vitamin A needs of the individual and level of preformed vitamin A in the diet (Lemke et al. 2003).

Vitamin A deficiency is most common in populations consuming most of their vitamin A needs from pro-vitamin carotenoid sources and where minimal dietary fat is available (Lartey 2008; Sherwin et al. 2012). Periods of general food shortage coincide with peak incidence of deficiency and common childhood infectious diseases (e.g. diarrhoea, respiratory infections, and measles). In some communities, food habits and taboos often restrict consumption of potentially good food sources of vitamin A culture specific factors for feeding children, adolescents, and pregnant and lactating women are also common(Gao et al. 2013; Oluwafolahan et al. 2014).

Vitamin A helps the health of the mother as well as the development of the fetus. It is also important for cell division, fetal organ and skeletal growth and maturation, maintenance of the immune system to strengthen defenses against infection, and development of vision in the fetus as well as maintenance of maternal eye health and night vision (Reifen & Ghebremeskel 2001; Downie et al. 2005)

The need for vitamin A increase slightly by about 10% during pregnancy. Studies in animals and humans showed that congenital anomalies can result if the fetus is exposed to severe deficiency or large excesses of vitamin A (Ross 2002; Downie et al. 2005). The prevalence of night blindness is also more common in the third trimester of pregnancy, and populations with a prevalence $\geq$ 5% are considered to have a significant public health problem with regard to vitamin A deficiency. Vitamin A deficiency affects about 19 million pregnant women, mostly from the WHO regions of Africa and South-East Asia (Christian et al. 2001).

b) Vitamin D

Vitamin D is needed during pregnancy to maintain proper levels of calcium and phosphorus, which helps to build the bones and teeth of the growing baby in utero (Kulie et al. 2009; Hollis & Wagner 2004). Nutritionally, most vitamin D is synthesized from a precursor in the skin after exposure to ultraviolet light from the sun. But, it can also be naturally obtained from food sources like fish liver oil, fatty fish, and fortified milk, egg, and cereal product. (World Health Organization 2012)

The daily dietary requirement for vitamin D is highly dependent on exposure of the skin to ultraviolet light. If a pregnant women is not exposed to adequate sunlight; however, the National Academy of Sciences currently recommends 200 IUs (5 micrograms) of vitamin D each day. Some researchers also recommend pregnant women to take additional supplement of 4,000 IU of vitamin D a day (Institute of Medicine 2002).

Studies showed that provision of vitamin D supplements during pregnancy improves the women's vitamin D levels at term (Taylor et al. 2008). Deficiency state of vitamin D during pregnancy can cause growth retardation and skeletal deformities. It may also have an impact on birth weight (Medeiros et al. 2015). Lack of vitamin D during pregnancy, may also affect the fetal length at birth result resulting a higher risk for rickets (which can lead to fractures and deformity), abnormal bone growth, and delayed physical development.

Studies have also revealed that it can also affect bone development and immune function from birth through adulthood. Vitamin D deficiency also correlates with preeclampsia, gestational diabetes mellitus, and bacterial vaginosis, and an increased risk for C-section delivery (Shin et al. 2010). A greater risk of pregnancy complications, including preeclampsia and certain autoimmune diseases (type 1 diabetes, multiple sclerosis, and rheumatoid arthritis), osteoarthritis, cancers (especially colon cancer), gum disease, and high blood pressure were also reported (Medeiros et al. 2015; World Health Organization 2012; Lee et al. 2015).

c) B Vitamins

Vitamin B-6 is a water-soluble vitamin contained in many foods including meat, poultry, fish, vegetables, and bananas. Evidence has accumulated that vitamin B-6 is required for protein, carbohydrate, and lipid metabolism as well as for erythrocyte, immune, and hormonal functions. It is plays vital roles in numerous metabolic processes in the human body and helps with the development of the nervous system.

During pregnancy, it is thought that vitamin B6 may play a role in the prevention of pre-eclampsia, where the mother's blood pressure is high with large amounts of protein in the urine or other organ dysfunction, and in babies being born too early (Salam et al. 2015). It may also be helpful for reducing nausea in pregnancy(Smith et al. n.d.)

Increased protein intake during pregnancy necessitates a modest increase in vitamin B6 intake, because of the major role of the vitamin in amino acid metabolism. (Brown 2011). Thus, an adequate intake to meet the requirements of virtually the whole population is generally considered to be 15 micrograms/g dietary protein or an RDAs of 1.5 and 2.2 mg/d. There is little evidence that pharmacological doses of vitamin B6 have any beneficial effect. Rather, neurological damage has been reported in excess (500 mg/d) as well as modest (50-100 mg/d) doses (Bender DA 2011).

Looking to its effect on pregnancy and pregnancy outcomes, some studies on maternal Vitamin B6 and folic acid supplementation showed that patients with a history of preeclampsia or fetal growth restriction and hyperhomocysteinemia appears to be favorable.

d) Folic acid/ Folate

The term “folate” is derived from the Latin word “folium” to mean the leaf which is derived because the main sources for the vitamin are green leafy vegetables. Folate is a water-soluble vitamin of the B complex group, and is required for optimal health, growth, and development. Nutritionally, human beings can't synthesize folate in the body and hence are completely dependent on dietary sources. Good sources are green leafy vegetables; in fact, the term “folate” derives from the Latin word “folium” or the leaf (Djukic 2007).

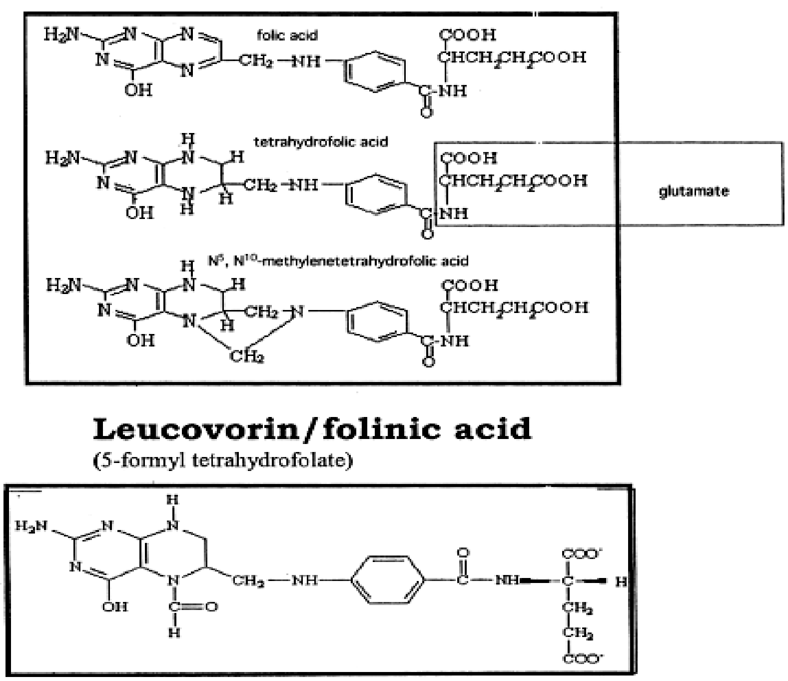


Figure 2.2. : Chemical structure of various forms of folate

Sources: (FAO & WHO 2005)

Folate plays key biological roles in a variety of physiologic processes as a cofactor or coenzyme for: maintenance and repair of the genome, regulation of gene expression, amino-acid metabolism, neurotransmitter synthesis, and the formation of myelin. Dietary folates must undergo multiple, tightly regulated absorption and metabolic processes before their cellular utilization occurs (Djukic 2007; Rondo & Tomkins 2000).

The clinical conditions range from genetic syndromes defined prior to conception (e.g., Down syndrome), to malformations that develop during embryogenesis (e.g., neural tube defects), to disorders that are acquired postnatally and may be progressive (e.g., cerebral folate deficiency).

During pregnancy, increased folate intake is required for rapid cell proliferation and tissue growth of the uterus and the placenta, growth of the fetus and expansion of the maternal blood volume. Folate requirements are 5- to 10-fold higher in pregnant than in non-pregnant women, therefore pregnant women may be at risk for folate deficiency (Djukic 2007; Sciences 2010; Rondo & Tomkins 2000).

d) Calcium

Calcium is essential for strong bones and teeth. Calcium deficiency can lead to disorders like osteoporosis (brittle bones). Good dietary sources of calcium include: milk and dairy products, green leafy vegetables, fish containing soft bones, pulses and foods made with fortified flour. But, milk and dairy products are the best dietary sources as they contain high as well as most bio-available calcium content.

All adults, including pregnant and lactating mothers, need about 700 mg of calcium per day. Likewise, even if demands of calcium during the latter stages of pregnancy are high; human body adjusts to this by increasing intestinal absorption, decreasing renal excretion and increasing resorption from the maternal skeleton to compensate the elevated demand (Williamson 2006; Olausson et al. 2012).

Babies born at full-term contain approximately 20 – 30g of calcium, most of which is laid down during the last trimester of pregnancy (Prentice 2000; Hacker et al. 2012). The fetal-placental unit has also a remarkable ability to meet its needs irrespective of maternal calcium or vitamin D levels (Kovacs & Kronenberg 1997) .

Calcium supplementation during pregnancy for women with low (not adequate) dietary calcium intake up to 2 g/day during the second and third trimesters, can increase fetal bone mineralization. A recent randomized clinical trial by WHO suggests that a 1.5-g calcium/day supplement did not prevent preeclampsia but did reduce its severity, maternal morbidity, and neonatal mortality, albeit these were secondary outcomes.

e) Zinc

Zinc is also among the key trace elements with significant effect on human health and nutrition. Physiologically, it is involved in many biological functions including DNA and protein synthesis, cell division, growth, gene expression, and stabilization of molecular structures. Deficiency states are common during pregnancy with other micronutrient due to increased nutrient requirements of the mother and the developing fetus. These deficiencies can negatively impact pregnancy outcomes including the health of the mother and newborn infant (Donangelo & King 2012; Swanson & King 1987; Shah & Sachdev 2006)

Poor maternal zinc status is associated with adverse pregnancy outcomes as it is essential for normal foetal growth and development(Hess & King 2009). Evidence is accumulating indicating that zinc supplementation during pregnancy may help to reduce preterm births in low-income settings, and other suboptimal pregnancy outcomes including low-birth-weight or pre-eclampsia (Mori et al. 2012; Zerfu & Ayele 2013; Shah & Sachdev 2001; Jønsson et al. 1996; Donangelo et al. 2005).

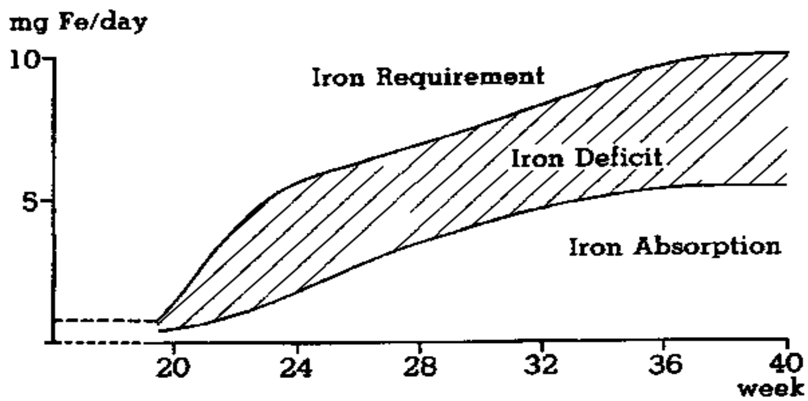
On the other hand, there is paucity of evidence from available studies on the impact of zinc supplementation weather it is alone or in combination with other micronutrients for better outcomes before specific recommendations can be made (Mori et al. 2012; Castillo-Durán & Weisstaub 2003).

f) Iron

There is no as such determinant and important micronutrient as iron in human life in general, and during pregnancy and lactation, in particular. Iron serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, acts as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues (Pena-Rosas JP & Viteri. 2012). It is needed to supply the growing fetus and placenta as well as increase the maternal red cell mass (Horowitz et al. 2013). Both the mother and the growing fetus have to absorb substantial amount of iron than is lost from the body, and both are at a considerable risk of developing iron deficiency under ordinary dietary circumstances (Gambling et al. 2011).

i. **absorption**

There are marked changes in the fraction of iron absorbed during pregnancy. During the first trimester, there is relative reduction in iron requirements as compared with the non-pregnant state and this results in a marked and somewhat paradoxical decrease in the absorption. In the second and third trimesters; however, iron absorption is increased by about 50 percent and up to four folds. This ultimately leads to a state in which the balance where the need for iron cannot be achieved, with a deficit of about 400-500 mg even with marked increase in iron absorption (FAO & WHO 2005) (Figure).



Source:(FAO & WHO 2005)

Figure 2.3. Daily iron requirements and daily dietary iron absorption in pregnancy.

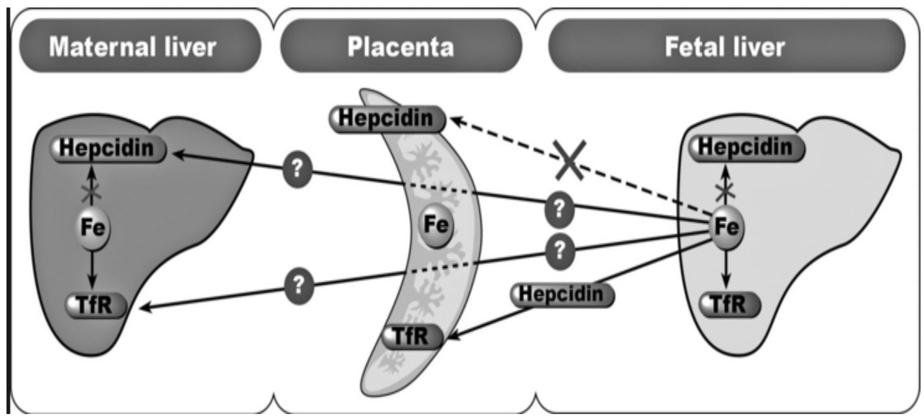
The fraction of iron absorbed from the amount ingested is typically low, but may range from 5% to 35% depending on circumstances and type of iron. Iron is found embedded within the heme component of each hemoglobin molecule, which is known to be vital in transporting oxygen and carbon dioxide in our blood. The hemoglobin molecule itself is made of four protein molecules (globulin chains) that are connected together by a peptide bond.

In human diets, we get iron comprising both heme and non-heme iron. It is the heme iron, mainly obtained from animal source foods which is better absorbed than non-heme iron. Non-heme iron is absorbed by intestinal luminal cells through a specific transporter and released into the circulation where it binds to transferrin. These receptors on erythroblasts accept iron-transferrin complexes; these undergo endocytosis and the iron is incorporated into Hb (West & Oates 2008).

Iron absorption is facilitated by iron deficiency state and increased erythropoiesis, while the absorption is slowed down during inflammation and iron repletion, mediated by the recently described regulator of iron homeostasis, hepcidin, which blocks iron release from enterocytes and macrophages (Ganz 2005).

Body iron stores are regulated through iron absorption. Non-heme iron is best absorbed in the ferrous form (Fe^{2+}). Non-heme iron absorption is inhibited by simultaneous consumption of anti-nutritional factors like phytates and tannins as well as mineral calcium. Contrarily, the consumption of heme-iron sources together with ascorbic acid enhances absorption. Only less than 20% of available iron is absorbed in a typical balanced diet and even lower amount is absorbed in vegetarian diet or monotonous plant based diets (Bothwell TH 2000).

During pregnancy, iron is transported from mother to fetus across the placenta. The iron, after being absorbed across the maternal gut, it is carried to the liver and goes to the serum to bound to transferrin. This has 2 iron-binding sites with approximately equal affinities. The transferrin also binds to the transferrin receptor on the placental microvillar membrane surface. After binding is completed, the complex is incorporated into clathrin-coated vesicles and internalized.



Source : (Gambling et al. 2011)

Figure 2.4.: Regulation of iron transport across the placenta..

ii. Diagnosis during pregancy

Anaemia is defined as hemoglobin (Hb) concentration below the reference range which varies across age, sex and gestation (Kraemer, K. and Zimmermann 2007). WHO defines anaemia as a Hb level below 130 g/L in men, 120 g/L in women, and 110 g/L in pregnant women and preschool children (WHO 2001). During pregnancy, hemoglobin and serum ferritin are used as the most common indices of iron status. In line with increase of gestational age, the cutoff values (g/L) for anemia for pregnant women in first, second and third trimesters are hemoglobin levels of 110, 105, and 110; or hematocrit level of 33, 32, and 33, respectively.

Complete blood count test that is usually ordered to provide information about iron deficiency anemia. In this test we can collect data about the hematocrit (the percent of blood volume that is made up of RBCs) level or the hemoglobin level and the size of your RBCs (Scholl & Reilly 2000; Xiong et al. 2000; Clark 2009).

iii. Prevalence and predictors

Iron deficiency and iron deficiency anemia are the most prevalent and the leading nutritional problems of pregnancy and childhood than any other population group. Globally, some 1.62 billion people or 24.8% of the population are affected by anemia.

The prevalence of anemia is exceptionally high among pregnant women, school children and all women of the reproductive age group, whereby nearly 293 million (47.4%) of school age children, 56 million (41.8%) of the pregnant and 468 million (30.2%) non-pregnant women are affected. Geographically, people living in Asia and Africa are at greatest risk: almost two-thirds of preschool-aged children and living in Africa are anemic.

The prevalence also varies widely between the developing and developed world. In the former, the prevalence averages nearly 56%, ranging between 35% and 75%, while it is as low as 18% in the latter, developed world.

In Ethiopia, even if there is limited evidence on the level of iron deficiency, the prevalence of iron deficiency anemia among pregnant women was around 23% (CSA I 2012a; Alemu & Umeta 2015). Nonetheless, the level varies across various reproductive, socio-demographic and economic factors.

The in-depth analysis conducted using the national demographic and health survey. indicated that maternal age, region, pregnancy trimester, number of under five children, previous history of abortion (termination of pregnancy), breastfeeding practices and number of antenatal care visits were key independent predictors of anemia during pregnancy

iv. Consequences

a) Effects on the mother

Mild anemia *(hemoglobin levels of 10–10.9 g/dL)* may not have any effect on pregnancy and labor except that the mother will have low iron stores and may advance to become moderate to- severe anemia in subsequent pregnancies. On the other hand, moderate anemia *(hemoglobin levels between7 – 9.9 g/dL)* may cause increased weakness, lack of energy, fatigue and poor work performance.

Severe anemia *(hemoglobin level <7 g/dL),* however, is associated with multiple poor outcomes. It overstresses the mother by causing medical problems like palpitations, tachycardia, breathlessness, increased cardiac output leading on to cardiac stress which can cause de-compensation and cardiac failure which may be fatal. Furthermore, there is a possibility of increased incidence of pre-term labour

(28.2%), pre-eclampsia (31.2%) and sepsis (Horowitz et al. 2013; Allen 2000b; Van den Broek 1998; Peleg & Ben-Ami 1998)

b) Effects on the fetus

Interestingly, irrespective of maternal iron stores, the fetus continues to obtain iron from maternal transferrin, which is trapped in the placenta and which, in turn, removes, and actively transports iron to the fetus (Heidemann & Mcclure 2003; Gambling et al. 2011). Gradually, however, such fetuses tend to have decreased iron stores due to depletion of maternal stores. This may lead to several adverse perinatal outcomes in the form of pre-term and small-for-gestational-age babies and increased perinatal mortality rates (Scholl & Reilly 2000; Allen 2000b; Hanieh et al. 2013; Levy et al. 2005; Atuahene et al. 2015).

Anemia, mainly iron deficiency anemia do also affect fetal mean birth weight(Kumar et al. 2013), APGAR score at birth (Preziosi et al. 1997), mean length(Preziosi et al. 1997; Yakoob & Bhutta 2011; Milman et al. 1999). and haemoglobin levels (Salam et al. 2015; Arija et al. 2014).

v. Supplementation with iron folic acid

There is little doubt that iron supplementation in pregnancy improves maternal iron status in both low income and industrialized countries (Cogswell et al. 2003; Siega-Riz et al. 2006). This, in addition to improving maternal iron status ante partum, it also continues to significantly improve postpartum (Christian et al.

2009). Furthermore, respective of maternal iron stores, the fetus manages to obtain and store normal amounts of iron even when its mother is iron deficient, in part because of the increase in placental transferrin receptors in iron deficient women. Studies shows that maternal iron deposits and level of anemia are strongly correlated to infants' serum ferritin concentrations.

Contrarily, the main challenge here is compliance to iron folic acid tablets. For instance, during the period of 2011, among pregnant women with a live birth eligible to take IFA supplement, only 17 percent of Ethiopian reported taking iron folic acid tablets during their last pregnancy. The reported rate of minimal compliance (taking IFA tables for 90 or more days) was less than one percent (0.4 %) (CSA I 2012b).

g) Multiple micronutrients

According to the sythematic review (Zerfu & Ayele 2013) we conducted, supplying micronutrients, mainly multiple micronutrients have beneficial effect in reducing the risk of low birth weight and other complications.

2.3. Dietary diversity during pregnancy and the outcomes

Dietary diversity is the number of individual food items or food groups consumed over a given period of time. It is a qualitative measure of food consumption that reflects household access to a variety of foods, and is also a proxy for nutrient adequacy of the diet of individuals (Bukania et al. 2014; Kennedy et al. 2011). Most often it is measured by counting the number of food groups rather than food items consumed

Dietary diversity can be measured at the household or individual level through use of a questionnaire. The household dietary diversity score (HDDS) is meant to reflect, in a snapshot form, the economic ability of a household to access a variety of foods. On the other hand, Individual dietary diversity scores aim to reflect nutrient adequacy(Kennedy et al. 2011).

Dietary diversity scores have been shown to be valid proxy indicators for dietary energy availability at household level (Hoddinott et al. 2002), micronutrient adequacy of diets of young children and women of reproductive age (Arimond & Ruel 2004; Arimond & Wiesmann 2010; Daniels 2009) and predictors of food security and nutritional status (Onyango 2003; Ruel 2006). Studies have also found assosation with chilhood gowth and stunting (Rah et al. 2010; Kabahenda et al. 2014; Moges et al. 2013).

FAO has published operational guidelines for measuring dietary diversity in a standardized way, based on a tool originally developed by FANTA (Kennedy et al. 2011; Swindale A & Bilinsky P 2006). The data collection tool developped by FAO uses an open recall method to gather information on all food and drinks consumed by the household or individual over the previous 24 hours. The foods and drinks recalled by the respondent are then recorded into one of 16 standardized food groups. Probing is used to capture consumption of any food groups not mentioned in the open recall (Kennedy et al. 2011).

Maternal dietary diversity indicators are known to reflect population-level adequacy for mothers (United et al. 2012). It has been also related to average household nutrient adequacy, and to infants in the same (Nguyen et al. 2013; Saaka 2012). More importantly, it is cheap, rapid and effective to study nuttional quality at population level (UN Standing Committee on Nutrition 2008). Therfore, in this thesis we used dieatry diversity as a key tool to investgate maternal nutrtional quaity and its assosation with both pregnaney and perinatal oputcomes in rural resource limmitted setups of Ethiopia. The details of objectives, methods followed and findings are presneted hereafter.

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3

Chapter 3 - Materials & Methods

3. Chapter Three: Materials and Methods

3.1 - Study Area

The study was conducted in Arsi Zone of Oromia region, Ethiopia. Oromia is the largest and most populous region in Ethiopia accounting for one third of the nation. Arsi Zone is located in the heart of the Ethiopia. The Zonal capital, Asella, is located only 167Kms from the national Capital, Addis Ababa. The Zone is neighbored by Major Zones like Bale, West Arsi, and East Showa Zones as well as the Afar regional state (Etefa & Dibaba 2011; Arsi Zone Administration Office 2010).

Arsi Zone has also four agro-climatic areas mainly due to variation in altitude. It is dominantly characterized by moderately cool (40 %) and cool (34 %) agro-climatic zones, while the remaining 1/4th accounts moderately warm (20%) and cold (6%) temperature zones. According to the latest national population projection from the central statistical authority(CSA 2013), the Zone had over 3,202,689 population; which is dominated by rural (86.6%) population.

The major food crops produced in the Zone are cereals, pulses, oil seeds and others. Among cereals, teff, barley and wheat are the pre-dominant. Area under cereals covers the largest part of the total area of the Zone. Among pulses, horse beans and field peas are grown widely. Though cultivated on small fragmented farm lands seasonally; vegetables, fruits, root crops and stimulants are also grown (Etefa & Dibaba 2011; Arsi Zone Administration Office 2010.

Husbandry and cattle keeping is common in the zone; nevertheless, reports shows that production is geared towards quantity than quality due to lack of awareness and animal health infrastructure and hence benefits from the sector is not adequate.

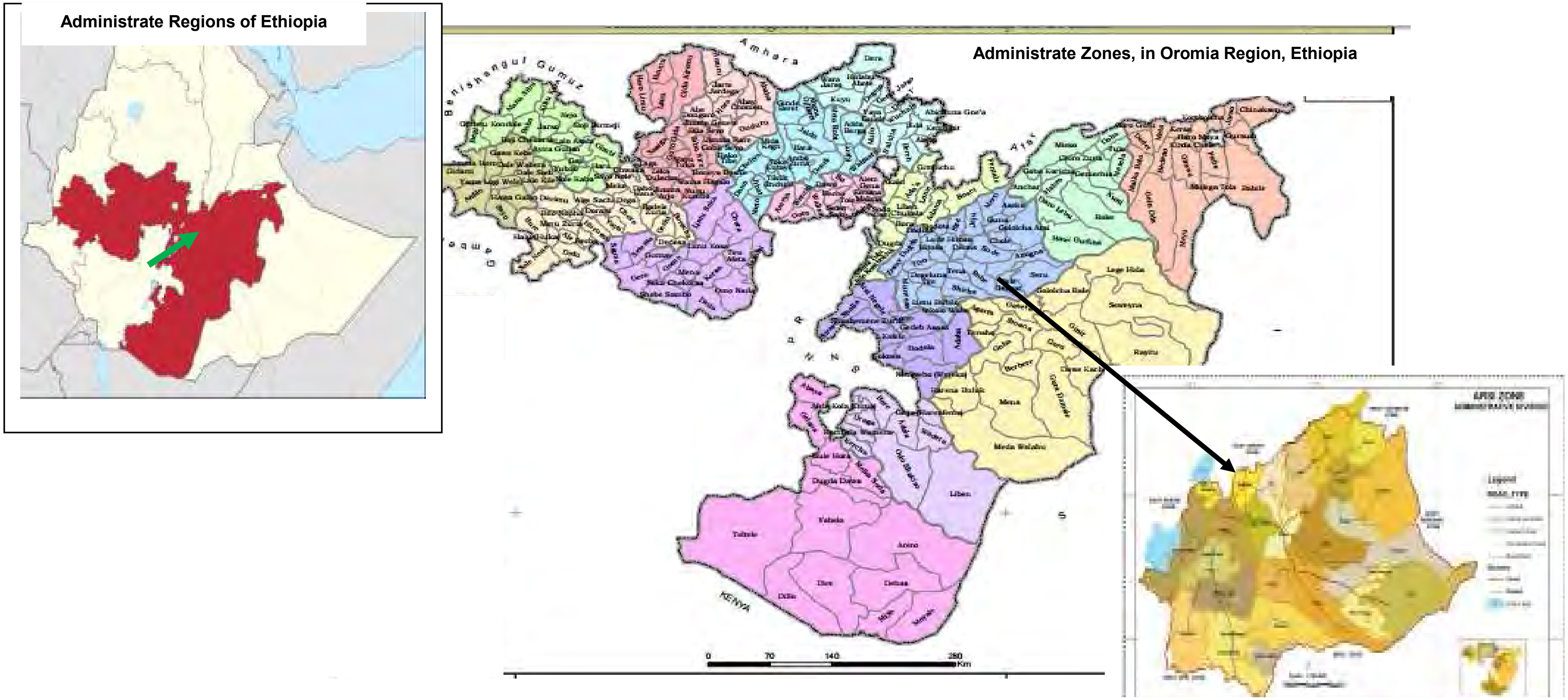

Figure 3.1 : Map of Ethiopia and Oromia administrative region showing the study sites in Arsi Zone.

Source : Arsi Zone Administration Annual Report, 2013

3.2. Study design

The study employed mixed methods design approach (a combinations of community based and laboratory research methodologies). It included various study designs including: cross sectional nutritional studies, observational (Cohort) follow-up studies and laboratory analysis of foods. Table 1 one summarizes the designs, population & the study periods of all the sub-studies included to these thesis.

Table 3.1. Summary of the studies conducted: the design, population & the study period.

Paper/ Manuscript	**Paper Titles**	**Study design**	**Study Population**	**Study Period**
Paper I	*Micronutrients and pregnancy; effect of supplementation on pregnancy and pregnancy outcomes: a systematic review*	Systematic Review	Global literatures	
Manuscript I	*Nutrient composition and micronutrient densities of commonly consumed foods by pregnant women in rural Arsi, central Ethiopia*	Lab based Food Analysis	Food Samples from 55 mothers	Oct.2014 - May, 2015
Paper II	*Dietary diversity during pregnancy is associated with reduced risk of maternal anemia, preterm delivery, and low birth weight in a prospective cohort study, in rural Ethiopia.*	Prospective Cohort study	374 pregnant women	August 2014 - March 2015
Manuscript II	*Consumption of animal source foods (mainly diary), fruits and dark green leafy vegetables during pregnancy is associated with lowered risk of adverse perinatal outcomes at birth: prospective cohort study in rural Ethiopia*			
	Dietary and nutrition sensitive characteristics of pregnant mothers associated with risk of anemia at term; a multicenter prospective cohort study in rural Ethiopia			
Paper III	*Maternal food taboos, dietary habits and cultural beliefs of weight gain during pregnancy; a qualitative study in rural central Ethiopia*	Cross-sectional qualitative	38 KII 8 FGDs	Oct.2014 - May, 2015
Paper IV	*Reproductive and Obstetric Factors Are Key Predictors of Maternal Anemia during Pregnancy in Ethiopia: Evidence from Demographic and Health Survey (2011)*	2^0 data analysis	EDHS	

EDHS : Ethiopian demographic and health survey

Description of Methods and Study Designs

3.2.1. *Laboratory analysis of common and "ready to eat" foods*

i. Dietary Assessment

Dietary intake data were collected from pregnant mothers (n= 55), representing all the agro ecologies of the zone, using repeated 24 hours dietary recall and weighing the total amount of daily food intake. Two days before intake was assessed, feeding cups and plates were distributed to the mothers and all were instructed not to change their dietary patterns. Food sizes were estimated by direct weighing of salted replicas of actual foods prepared locally.

For the 24 hours dietary recall, an interactive quantitative recall was conducted using the technique adapted and validated for use in developing countries (Gibson & Ferguson 1999). The respondents were asked to recall the exact food intake of the previous day. They were also asked to list the detailed descriptions of all foods including recipes and beverages consumed and to estimate portions and quantities based on pictures and locally used measuring tools like cups or teaspoons.

All days of the week (including fasting and feasting days) were equally represented in the sample. Midwives assisted by other experienced data collectors were locally recruited and trained in a classroom setting. This was followed by a pilot test on a group comparable to that of the actual study. Data was collected considering the two distinct seasons (pre-harvest of the main harvest season, which occurs commonly between August to October and the peak harvest season, November - January) in the area, which can have impact on the results and interpretations.

ii. **Calculation of DD score :** Dietary diversity scores are calculated by summing the number of food groups consumed by the individual respondent over the 24-hour recall period. First a new food group variables were create for those food groups that need to be aggregated then a new WDDS variable was formed. Finally, we computed values for the dietary diversity variable by summing all food groups included in the dietary diversity scores.

iii. Laboratory Analysis of Food

a) Moisture analysis:

Prepared samples were mixed thoroughly and about 5.00g of fresh samples were transferred to the dried dish and weighed dishes. The dishes and their contents were placed in the drying oven and dried at 105^{o}C for 3hrs. Then the dishes and their contents were cooled in desiccator to room temperature and reweighed. This process was repeated until constant weight was obtained. Duplicates of each sample were determined.

The amount of water present in a sample is considered to be equal to the loss of weight after drying the sample to constant weight at a temperature near the boiling point of water.

b) Analysis of total protein content

The crude protein content of major staple foods were determined by employing the Kjeldahl method (AOAC, 1995) and results were expressed as g of BSA/ 100 g dry weight.

c) Analysis of total carbohydrate content

The total and available carbohydrate content was determined by difference. Addition of the percentages of crude protein, crude fat and ash was subtracted from 100.

- Carbohydrate (CHO) (g/100g) = [100-(protein + fat +ash in grams)].
- Available CHO (g/100g) = [100- (crude protein + crude fat + total ash + crude fiber) in grams]

d) Analysis of total lipid content

Lipids were extracted from the biomass samples using AOAC 200 method. To determine total lipid content, a 2: 1 mixture of chloroform and methanol (14 mL) was added to powdered food sample (2 g) in a Pyrex tube. The tube was closed and vortexed (2 min) and then the residue were re-extracted (mixed with 5 mL of a 2: 1 mixture of chloroform and methanol and vortexed for 30 s). The two supernatants were combined, filtered through Whatman 41 paper and concentrated to dryness under N2 (g). Then, total lipids were determined gravimetrically for triplicate aliquots of each lipid extract.

e) Determination of energy intake:

Gross energy was also determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors;

- 16.7 KJ/g (4 kcal) for protein,
- 37.4 kJ/g (9 kcal/g) for fat and
- 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories using the following formula:

Total energy (in kcal/100g) = (9*crude fat + 4*crude protein + 4* utilizable carbohydrate)

f) Determination of adequacy of energy and macronutrients intakes

This was evaluated according to the Dietary Reference Intakes (DRI) of The Institute of Medicine of The National Academies (The National Academic Press 2003). The reported energy intakes were compared with estimated minimal energy requirements to assess adequacy. Basal Metabolic Rate (BMR) was estimated using the age specific equations of FAO/WHO/ UNU expert consultations. The BMR was then multiplied by a factor which stands for physical activity level for each individual.

3.2.2. Observational Prospective Cohort Studies.

i. Study design, sample size, and sampling procedure

This is the key component of the whole thesis whereby pregnant mothers were recruited during their antenatal care follow up and followed to delivery. It uses a longitudinal, prospective cohort study with two arms at a ratio of 1:1: adequate (unexposed) and inadequate (exposed) women's dietary diversity score (WDDS).

ii. Sample size

Sample size was calculated using the Open Epi Kelsey statistical software available at http://www.openepi.com/SampleSize/SSCohort.htm with the following assumptions: a 95 % significance level (two-sided), 80% power, and 37 % anemia prevalence (16) among exposed and an anticipated 10% lower prevalence of anemia among unexposed pregnant women. This yielded a total of 168 subjects per arm, and to allow for up to 20% attrition by the end of the study, a sample size of 420 was required.

iii. Inclusion and exclusion criteria

The inclusion criteria for the study were: pregnant women who are permanent residents of the study area, with no known medical, surgical, or obstetric problems, and who were willing to attend routine ANC visits.

iv. Sampling (Allocation to cohort groups)

Pregnant women in Ethiopia start ANC late, usually in their second trimester(17); hence, although the study enrolled women attending their first ANC visit, all were already in their second trimester (24 – 28 gestational weeks). At enrollment, a 24-hr WDDS was collected from the pregnant women using the FAO guidelines (14) and subjects were then divided into "adequate" (WDDS <4) or "inadequate" (WDDS ≥ 4) groups.

v. Dietary diversity data

Pregnant women were asked to recall the foods they had consumed in the previous 24-h, first spontaneously followed by probes to ascertain that no meal or snack was left out. A detailed list of all the ingredients of the dishes, snacks, or other foods consumed was generated to enable better classification of mixed dishes. The foods were then categorized into nine food groups:

1) Cereals, roots and tubers;

2) Vitamin-a-rich fruits and vegetables;

3) Other fruit;

4) Other vegetables;

5) Legumes and nuts;

6) Meat, poultry and fish;

7) Fats and oils;

8) Dairy; and

9) Eggs.

Overall, four 24-h WDDS from a typical day were collected each month from enrollment to delivery, meaning data were also collected in the pre-harvest (August-October) and post-harvest (November-January) seasons.

vi. Maternal Socio-Economic, Dietary, And Anthropometric Characteristics

Data on the socio-economic characteristics and food consumption patterns were collected using a pre-tested questionnaire that was adapted from the Ethiopian Demographic and Health Survey and the FAO (14, 18). The questionnaires were pre-tested in a similar setting. Data was collected by 24 well-trained and experienced midwives who work permanently in the antenatal care service provision units of health centers in the community.

The pregnant women were weighed at each visit from enrollment to delivery following the standardized procedures recommended by WHO(19) . Pregnant women were weighed to the nearest 100 g on electronic scales with a weighing capacity of 10 to 140 kg. Their height was measured to the nearest mm with a portable device equipped with calibrated and standardized height gauges (SECA 206 Body meter). The mid-upper arm circumference (MUAC) of the left arm was measured to the nearest mm with a non-stretch measuring tape.

vii. Outcome Assessment

Hemoglobin measurements were taken twice: once at enrollment and once before delivery (term) using a portable HemoCue (AB Leo Diagnostics, Helsinborg, Sweden). The readings were adjusted for altitude (20), and pregnant women with values below 11.0 g/dl were considered to be anemic.

Gestational age was estimated by midwives at the health center, by counting from the last menstrual period and fundal palpation during ANC visits.

Birth weight was measured and recorded by the midwives immediately after delivery. Stillbirth (no signs of life at birth after 24 completed weeks of gestation) and PTB, (<37 weeks of gestation) were ascertained by the same professionals at birth.

viii. Quality control

All data collectors were experienced (≥4 years) midwives with at least a diploma in nursing. They also received five-day training on participants' enrollment, follow-up, anthropometry, and the use of a HemoCue for hemoglobin measurements. The training was conducted just before the study and was followed by practical tests to ensure the skills were transferred.

In each of the health centers selected, one supervisor (usually the head) was assigned to oversee data collection. In addition, the investigator made a weekly visit to check the completeness and quality of the data collected.

Anthropometric measurements were taken only by trained personnel and all equipment was calibrated with standard weights and length rods on a daily basis. Neither the pregnant women nor the data collectors were aware the allocation into groups.

3.2.3. Cross-sectional qualitative study

For this component, we collected qualitative data using key informant in-depth interviews and focus group discussions between October, 2014 - Mid of May, 2015.

A total of 38 Key informant in-depth interviews (KII) and 8 Focus group discussions (FGDs) with purposefully identified study participants in twelve rural villages of four districts of Arsi Zone, Central Ethiopia were conducted. The villages and districts were selected to represent cultural similarities and the four agro-climatic areas of the Zone.

The interviews and focus group discussions were conducted with purposively selected pregnant mothers, grandmothers, health workers, traditional healers and other knowledgeable peoples as well as informal encounters; while the focus group discussions (FGD) were held with strategically identified and judgmentally contacted pregnant mothers and husbands of pregnant mothers. a total of seven to ten KII and two focus group discussions (one with the pregnant mothers and one with their husbands) were held in each of the districts and villages identified.

The Study Setting was Arsi Zone, which is found in the central part of the Oromia Regional State. The Zonal capital, Asela, is located at 175 km from Addis Ababa on Addis-Adama-Bale Robe main road and has the total area of 210082 Km2, accounting for about 7 percent of the total area of the Regional State of Oromia (Arsi Zone Administration Office 2010; Etefa & Dibaba 2011)

Open-ended questions were used to collect relevant information. Questions were tailored to each informant's characteristics regarding age, gender, marital status and social standing. Questions that reached saturation were removed every evening after transcribing the day's work and doing preliminary analysis. New questions were added whenever an information gap was identified. The questions included knowledge, experience and opinion. Data was collected by two of the research team members with qualitative and quantitative data collection. The interviews were tape recorded.

3.3. Data Analysis

All quantitative data from laboratory and prospective cohort data were entered and cleaned using Epi-data statistical software (version 20). Double entry and cleaning was conducted and then exported to SPPS (version 20.0) and STATA 10.0 for statistical analyses. P values less than 0.05 were considered statistically significant

Laboratory data was reported as mean ± SD daily energy intake and macronutrient composition of major food items. Percentage contribution of macronutrients to the total energy were compared by independent simple t-test. Chi square test of proportion was used to determine the percentage of participants with intakes at or below the recommended daily allowance and adequate intakes. All statistical analyses were undertaken using SPSS version 13.

Cohort data was were analyzed according to their categorization by WDDS. All continuous variables were checked for normality using the Kolmogorov-Smirnov test. Variables not normally distributed were log-transformed. Mean and SEM were used to describe continuous variables. An independent student's t-test was used to compare means in the adequate and inadequate groups. A Chi-square test or a Fisher's exact test was used to test for independence in distribution of categorical variables between the two study groups. In all comparisons, differences were considered statistically significant at $P < 0.05$.

Qualitative data was analyzed manually using the thematic framework analysis method. Preliminary manual analysis was an inherent part of the data collection. As more data emerged from everyday encounters, the meaning of certain ideas and concepts were made to take similar thought.

3.4. Ethical Considerations.

Verbal informed consent was obtained from the eligible participants in the presence of local kebele (smallest administrative unit) administrators after a detailed explanation of the purpose and methods of the study. The study procedures were in accordance with the Helsinki Declaration of 1975 as revised in 1983. The study protocol was approved by the institutional review boards of the college of Natural Sciences, Addis Ababa University and the Oromia Regional Health Bureau.

4

Chapter - 4 : Results

4. Chapter four: Results

4.1. Effect of micronutrient supplementation on pregnancy and pregnancy outcomes

4.1.1. Introduction

Deficiencies in micronutrients such as folate, iron and zinc and vitamins A, B6, B12, C, E and riboflavin are highly prevalent and may occur concurrently among pregnant women. Multiple micronutrient supplementations in pregnant women may be a promising strategy for reducing adverse pregnancy outcomes through improved maternal nutritional and immune status.

There are ample of randomized control trails and other observational studies reporting associations between micronutrient intake and pregnancy outcomes. However, synthesizing and reporting the findings of these studies is lacking. In the present section, global evidence has been synthesized together to examines the effect of micronutrient supplements on the progress of healthy pregnancy and the outcomes.

4.1.2. Micronutrients and pregnancy; effect of supplementation on pregnancy and pregnancy outcomes: a systematic review

Taddese Alemu Zerfu and Henok Taddese Ayele.

(Nutrition Journal 2013, 12:20)

Zerfu and Ayele *Nutrition Journal* 2013, **12**:20
http://www.nutritionj.com/content/12/1/20

NUTRITION JOURNAL

REVIEW Open Access

Micronutrients and pregnancy; effect of supplementation on pregnancy and pregnancy outcomes: *a systematic review*

Taddese Alemu Zerfu[1,2*] and Henok Taddese Ayele[1,3]

Abstract

Introduction: Every year more than 20 million infants are born with low birth weight worldwide. About 3.6 million infants die during the neonatal period. More than one third of child deaths are thought to be attributable to maternal and child under nutrition.

Objectives: To systematically review the effect of supplementing various combinations and types of micronutrients on the course and outcomes of pregnancy.

Methods: Electronic search of Medline, Pub Med, Health Internetwork access to Research Initiative, and Google Scholar databases was conducted. Outcomes of interest were birth weight, low birth weight, small size for gestational age, prenatal mortality and neonatal mortality. After exclusion of irrelevant /incomplete ones, 17 out of 115 articles were considered for the final analysis.

Findings: Majority of the articles reviewed favored the supplementation of micronutrients to pregnant mother. Some studies suggested calcium supplementation is associated with a significant protective benefit in the prevention of pre-eclampsia. The remaining articles reviewed, showed significant benefit of Multiple Micronutrients supplementation during pregnancy in reducing low birth weight, small for Gestational Age births as compared to the usual iron-folate supplements.

Conclusions: Supplying micronutrients, mainly multiple micronutrients have beneficial effect in reducing the risk of low birth weight and other complications. Further studies at various combination and doses of micronutrient supplements are recommended.

Introduction

Every year more than 20 million infants are born with low birth weight worldwide [1]. About 3.6 million infants die during the neonatal period [2]. Two thirds of these deaths occur in southern Asia and sub-Saharan Africa. More than one third of child deaths are thought to be attributable to maternal and child under nutrition [3]. Deficiencies in micronutrients such as folate, iron and zinc and vitamins A, B6, B12, C, E and riboflavin are highly prevalent and may occur concurrently among pregnant women [3]. Micronutrient deficiencies result from inadequate intake of meat, fruits and vegetables, and infections can also be a cause. Multiple micronutrient supplementations in pregnant women may be a promising strategy for reducing adverse pregnancy outcomes through improved maternal nutritional and immune status [4,5].

The World Health Organization (WHO) currently recommends iron and folic acid supplementation to reduce the risk of iron deficiency anemia among pregnant women. Since many developing countries already have systems in place for the delivery of iron and folic acid supplements, micronutrient supplements could be provided at little additional cost [6].

Several systematic reviews of trials examining the effects of maternal multiple micronutrient supplementation have been conducted [7-10] but they have had limitations.

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Although some researchers have raised concerns that micronutrient supplementation may increase perinatal mortality, none of the previous review articles has adequately addressed this issue [7-9,11]. None has examined the potential sources of heterogeneity in the effect of supplementation on perinatal mortality.

In addition, most of the studies and literature reviews dealing with maternal nutrition and birth outcomes have approached the issue by investigating single nutrients in isolation. On one level, this is necessary for an in-depth study of the complex issues involved. However, nutrient deficiencies are generally found in low-Socio-economic populations, where they are more likely to involve multiple rather than single deficiencies [4]; and studies that address and bring together the broader picture of multiple nutrient intakes or deficiencies are lacking [5].

The effects of maternal micronutrient supplementation on perinatal mortality and other pregnancy outcomes can differ depending on trial characteristics and study population. An updated systematic review is essential to provide the basis for future research and for a discussion of policy implications. Hence, this manuscript examines the effect of micronutrient supplements on the progress and outcomes of pregnancy by reviewing high quality literatures/articles.

Methods

The published results from high-quality human observational and experimental studies which analyzed the effects of supplying Multiple Micro Nutrients (MMN) on the course of pregnancy and pregnancy outcomes were all included to this literature based analysis. Electronic search of Medline, Pub Med, Health Internetwork Access to Research Initiative (HINARI), and Google Scholar databases up to the end of 2011 was conducted. Search was done in keywords: ("Mothers" OR "Pregnancy" OR mother* OR maternal OR pregnancy) AND ("Micronutrients" OR "multiple micronutrient*" OR multivitamin OR micronutrient*) AND (supplement*) AND (Observational OR studies OR clinical trials). A function extracting related articles as well as reference lists from research, reviews and editorials was used during the search process. The full version of the English-language analyzed articles and abstracts of most found papers were available during the selection process except for pubmed search.

All literatures, including: observational studies, quasi-randomized trials and prospective randomized controlled trials (RCTs) evaluating multiple micronutrient supplementation in women during pregnancy, published in English language, were included. There were no limits on gestational age at the time of enrolment in the study and the duration of supplementation. Other than the assessment of Small for Gestational Age and neonatal mortality, we did not specifically evaluate minor adverse effects of the supplements such as nausea and vomiting among the mothers and newborns.

In the primary search 115 records were found. After exclusion of studies/reviews which did not examine the effect of micronutrient supplements on pregnancy outcomes duplicated and outdated (published before 2005) data, 64 articles were selected. During the second selection we evaluated 17 of them as potentially relevant articles considering the effect of multiple micro nutrient supplementations on pregnancy and birth outcomes. Studies that failed to meet our criteria were not taken into consideration (Figure 1).

We defined a "multiple micronutrient supplement" as a single tablet containing more than three different micronutrients; however micronutrient-fortified powders, foods and beverages were not included in the definition.

Result and discussion

We identified 115 potentially relevant articles for detailed review (Figure 1). We excluded 51 articles during initial screening because they were not directly referring the effect of micronutrients on pregnancy. The remaining 64 articles were again analyzed for their internal consistency with the research objectives and some 47 of them were found not relevant and/or incomplete, hence excluded for the second time. The remaining 17 of were considered for final review and analysis. From these finally accommodated articles, five of them were related to the effect of Calcium and vitamin D supplementation, three of them were about Iron and iron folic acid supplementation, and the remaining majority [9] articles reviewed were about the effects of MMN supplementations on pregnancy and its outcomes.

Though some degrees of variation by type and amount of micronutrients supplied are observed, the results of the final analysis of the most articles reviewed, favored the supplementation of micronutrients to pregnant mothers.

The systematic review of clinical trials by Buppasiri P et al. from Thailand, with the objective of determining the effect of calcium supplementation on maternal, fetal and neonatal outcomes revealed that Calcium supplementation is associated with a significant protective benefit in the prevention of pre-eclampsia and improving mean infant birth weight. The same study also confirmed lack of additional benefits for calcium supplementation in prevention of preterm birth or low infant birth weight [12].

Another study with a similar finding by Hofmeyr GJ et al. concluded that Calcium supplementation appears to almost halve the risk of pre-eclampsia, and to reduce the rare occurrence of the composite outcome 'death or serious morbidity', with no other clear benefits, or harms [13].

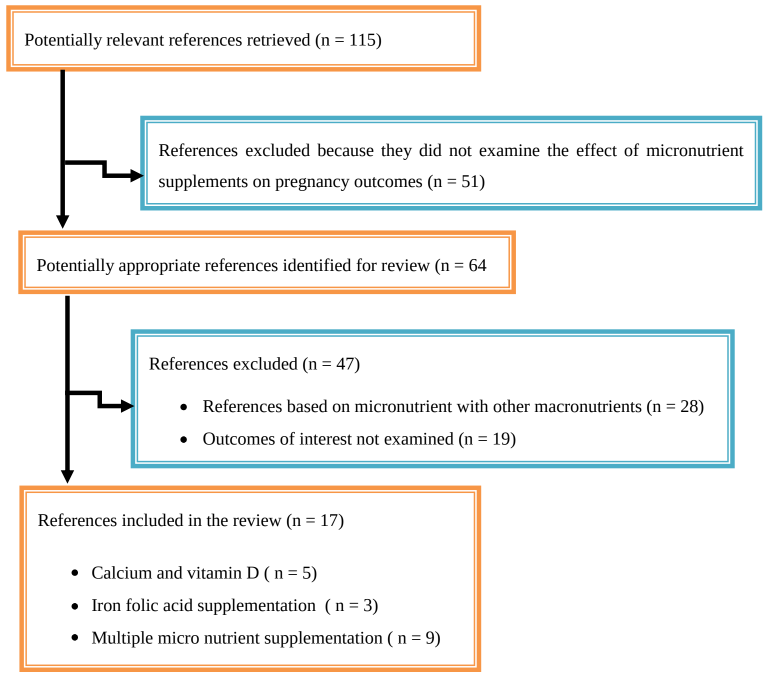

Figure 1 Selection of studies included in systematic review of randomized controlled trials and observational studies on maternal multiple micronutrient supplementation and pregnancy outcomes.

The thesis based study by Melody B. on maternal vitamin D status during pregnancy as a predictor of offspring bone mass at three years of age shows that maternal vitamin D status during pregnancy did not predict for the child at 3 years of age [14]. Another systematic review by Bruce W. on dietary vitamin D requirements during pregnancy and lactation recommends further studies to determine optimal vitamin D intakes for pregnant and lactating women as a function of latitude and race [15].

Similarly, the study by Dror DK et al. shows the existence of recent evidence supporting the role of maternal vitamin D status, particularly early in pregnancy, in modulating the risk of pregnancy complications and in sustaining fetal growth, bone development, and immune maturation [16].

On the other hand, according to the findings of the many of the final articles reviewed; though few disfavored the beneficial outcomes of supplementing MMN to pregnant mothers; majority of the others in one way or another supported the presence of significant association and benefit of MMN supplementation with at least one birth outcome.

Among the studies against the added role of supplying MMN to pregnant mothers, the study by Parul Christian, which was about the Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal, revealed that Antenatal folic acid-iron supplements modestly reduce the risk of low birth weight but MMN confer no additional benefit over folic acid-iron in reducing this risk [17,18]. Another Indian study by Umesh Kapil et al. also said MMN Supplements will not Reduce Incidence of Low Birth weight and other pregnancy outcomes [19]. The Hungarian RCT study by Czeizel AE also revealed that Periconceptional multivitamin supplementation increased fertility but had no significant effect on the rate of different groups of fetal deaths, low birth weight and preterm birth in singletons [20].

Conversely, the remaining RCT and observational studies reviewed from various parts of the world confer that oral supplementation of MMN for pregnant woman benefits the mother and the growing fetus as well as the newborn.

In line with this, the reviews and RCT studies by Prakesh S., Zulfiqar A Bhutta, Michael B Zimmermann and other scholars across the globe unanimously reported that supplementing pregnant mothers with MMN immensely benefits both the mother and the growing fetus in reducing maternal morbidity conditions and morbid neonatal birth outcomes [21-23].

Similarly, the systematic review by Prakesh S. et al. also indicates that prenatal MMN supplementation is associated with a significantly reduced risk of low birth weight and with improved birth weight when compared with iron–folic acid supplementation. However, there was no significant effect of MMN supplementation on the risk of preterm birth or small-for-gestational-age infants [21,22].

Additionally, the review by Zulfiqar A Bhutta et al. also provides evidence on significant benefit of MMN supplementation during pregnancy on reducing SGA births as compared to iron-folate, with no significant increase in the risk of neonatal mortality in populations where skilled birth care is available and majority of births take place in facilities [22].

Furthermore, other review by Kosuke Kawai et al. discusses the safety, efficacy and effective delivery of maternal micronutrient supplementation requiring further research. The other study by Dr. Kathleen Abu-Saad on the other side, declares that maternal nutrition is a modifiable risk factor of public health importance that can be integrated into efforts to prevent adverse birth outcomes, particularly among economically developing/low-income populations [24,25].

On the other hand, RCT by Philip N Baker et al. revealed that Poor micronutrient intake and status increases the risk of SGA births in pregnant adolescents and hence mothers should get prenatal micronutrient supplementation in addition to the customary iron folic acid supplementation [26].

In addition, in a poor population, the effects of maternal MMN supplementation on the fetus persisted into childhood, with increases in both weight and body size. Maternal MMN supplementation might therefore be an important part of overall strengthening of prenatal-care programmes [26-28].

Therefore, it is clear to see that given comparability of impacts on maternal anemia, the decision to replace iron-folate with MMN during pregnancy may be taken in the context of available services in health systems and birth outcomes monitored.

Conclusion

It was possible to learn that almost all available studies of RCT and other observational studies conducted in various parts of the world revealed that it is beneficial for the mother and the neonate and even to childhood with supplementation of MMN than ordinarily iron folic acid supplementation; nonetheless, there are still some equivocal findings related to the effect of MMN supplementation on the risk of preterm birth, small-for-gestational-age infants and neonatal mortality. Therefore, further studies at various combination and doses of micronutrient supplements particularly in areas where there is high prevalence of malnutrition are recommended.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TA developed the review parameters and secured support. He also undertook partial literature search, data extraction and analysis. HT made detailed and extensive literature search and involved in the write up and synthesis of the findings. He also reviewed and standardized the study. Both authors read and approved the final manuscript.

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4.2. Nutrient composition and micronutrient densities of commonly consumed foods

4.2.1. Introduction

Maternal nutrition, both macro and micronutrient, has been associated with pregnancy and pregnancy outcomes. Adequate, quality and diversified diet is needed during pregnancy. Laboratory based analysis of foods can give a good picture of dietary quality and adequacy. In the present section (4.2), common and prepared foods of pregnant mothers were identified and portion sizes were estimated using 24- hour weighed food record method.

The analysis was conducted in the laboratory aiming to identify and quantify the level of macro and micro nutrients in common and prepared maternal foods in the study area. The food samples were carefully stored and transported to the lab and analysis for all the macronutrients (protein, fat, carbohydrate and energy) as well as selected key micronutrients with significant effect on pregnancy (iron, zinc and calcium) were analyzed. Further statistical analysis was also conducted to quantify the nutrient densities in the foods. The findings showed that through maternal diets had adequate iron and carbohydrate contents, the amount and densities as well as estimated daily intakes for all the other nutrients were quite below the recommended level for pregnancy. The study findings are presented in the form of a manuscript ready for submission.

4.1.2. Nutrient composition and micronutrient densities of commonly consumed foods by pregnant women in rural Arsi, central Ethiopia.

(Paper ready for submission to a Journal)

Nutrient composition and micronutrient densities of commonly consumed foods by pregnant women in rural Arsi, central Ethiopia

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Abstract

Background: In many of the developing world including Ethiopia, energy and nutrient intake during pregnancy remain controversial because of scarcity of rigorous supplemented by laboratory based evidences.

Objective: To determine the energy, macro- and micro-nutrient densities of foods commonly consumed by pregnant women.

Setting: Rural villages in four districts of Arsi Zone, Oromia region, Ethiopia.

Methods: Pregnant women (n= 55) with singleton pregnancies and receiving ante-natal care (ANC) at the local health centers were selected. Using two-weighted food records, women's food intake were recorded at 24 –28 weeks and 32 – 38 weeks of gestation. Five representative food samples for each item were collected and stirred gently together and a representative portion from the these were used for analysis. The most-frequently consumed foods were identified and samples were collected for macro- and micro-nutrient analyses. Food intakes were converted to energy, macro and micronutrient intakes and densities (/kcal) using software. Excel spread sheet and SPSS V-20 were used for data analysis.

Results: Starchy staples and legumes were major food groups consumed by pregnant mothers. Median (q1, q3) intake for enjera and stew from shiro, which make the bulk of daily food intake were 455 (420, 580) and 176 (158, 216) g/day, respectively. Carbohydrate content of the foods ranged from 4.3 mg/100g in stews made from kale to as high as 29.93 mg/100g in enjera. Similarly, protein and fat content of foods ranged from 1.1 - 6.48 mg/100g and 0.1 - 8.01mg/100 g of edible portions, respectively. Coffee and stew from bean/pea with curd/tofu had the highest micronutrient content and densities. Enjera had the highest content of iron and calcium. The same had the highest densities of iron, zinc and calcium.

Conclusions: The most common diets of pregnant women were dominated by plant source foods lacking adequate energy, protein and other micronutrient contents. Pregnant mothers in this community could benefit from increasing consumption of protein and energy rich foods.

Key words : *Ethiopia, micronutrient densities, nutrient composition, pregnancy.*

Introduction

Malnutrition contributes to nearly half (45%) of child and 20% of maternal death (1,2). Millions more are permanently disabled by the physical and mental adverse effects of poor dietary intake in early life starting before and during pregnancy (3,4). Unfortunately, pregnant women are particularly vulnerable to inadequate energy and nutrient intakes relative to their increased physiologic demand (5). For example, during the period of pregnancy, the requirements for some key micronutrients like zinc, calcium and iron increases by 44 - 407%, 122–167% and 187 - 407%, respectively (6). Despite such increased requirements, diets during pregnancy in many low-and middle-income countries (LMIC) remain monotonous and thus of low-nutrient density. This is partly the reason for the widespread undernutrition and its associated adverse pregnancy outcomes (7).

In response, micronutrient supplementation (iron-folic acid) and more recently multiple micronutrients and lipid-nutrient supplements (LNS) have been proposed and evidence of their efficacy is growing. Nevertheless, such interventions are largely dependent on a well-performing and efficient ANC, absent in many LMIC. For example, in Ethiopia, women start ANC late, particularly in their second trimester. A recent study showed that among those who attend ANC, only 37% were supplied with IFA supplements and only 3.5 % were supplemented for the recommended three months or more (8). This suggests that along with efforts to improve the effectiveness of the ANC, dietary strategies that promote improved energy and nutrient intakes may be needed.

Unfortunately, there is paucity of evidence on the dietary practices of pregnant women in Ethiopia. The national food consumption survey didn't account pregnancy and pregnant women constrained studies that estimate energy, macro- and micro-nutrient intakes during pregnancy. This is unfortunate, given the need for such information to adequately design and implement effective food-based strategies.

Therefore, the present study aimed to identify the most frequently consumed foods by pregnant women in rural Arsi, Central Ethiopia, to analyze their energy, macro-and micro-nutrient composition.

Methods

Study settings

The study was conducted in rural Arsi Zone, Oromia region, central Ethiopia. Oromia is the largest and most populous region in Ethiopia accounting for one third of the national population. The Zonal capital, Asella is located 175 km away from the capital city, Addis Ababa (9,10).

Study subjects

The subjects of the present study were pregnant women (n = 55) selected from households randomly selected from four rural district purposively selected to represent the four agro-climatic conditions prevailing in Arsi. The inclusion criteria was for mothers to reside in the study area for at least six months, to be pregnant and be apparently healthy.

Dietary assessment and data collection

A two-day weighed dietary record was conducted with pregnant mothers (n = 55). Trained local health and agriculture extension workers collected the food samples.

Feeding cups and plates were distributed to the pregnant mothers two days before intake was assessed. All were advised not to change their dietary patterns during the study period. Food sizes were estimated by direct weighing of salted replicas of actual foods prepared locally. The respondents were asked to recall to list the detailed descriptions of all sold and liquid foods consumed to estimate portion sizes. All days of the week , excluding fasting and feasting days, were equally represented in the sample and seasonality was accounted for by conducting the dietary intake assessment in the pre-harvest (August-October) and post-harvest (November - January) seasons.

Nutrient intake estimation

Food intakes were converted to nutrient intakes using excel and SPSS for windows computer programmes. The mean ± SD / Median and 1st and 3rd quartiles (Q1, Q3) were calculated. Micronutrient (iron, zinc and calcium) levels were presented using descriptive statistics and simple frequencies. Median nutrient intakes and adequacy were compared the RNI's and desired densities, respectively. The critical nutrient densities were calculated by dividing the RNI by the respective energy requirements for each trimester of pregnancy.

Collection of the most frequently consumed foods by pregnant women

The most frequently consumed "ready to eat" foods by pregnant mothers were identified and samples were collected for analyses. A total of 24 samples including fermented (n= 8) and unfermented (n =6) *Injera* made from teff, barely, wheat, sorghum or mixes of these. Samples of commonly consumed stews (n = 6) accompanying Injera and hot drinks like coffee and tea (n = 4) were collected from households. Separate samples were collected for moisture content determination and biochemical analysis.

Collected food samples were packed in sealed polyethylene plastic bags, labeled and transferred within 4 hour of collection to a deep freeze (−20 °C) at a local health centre until sample collection in the area was completed, after which they were transported to Addis Ababa University, Center for Food Sciences and Nutrition research laboratory. All transport of foods were carried out in ice boxes with cooling elements pre-cooled to −20 °C. The samples were stored at −20 °C until analysis.

Macronutrient, energy and micronutrient composition analyses

Moisture content of foods was determined using AOAC, 2000 by weight difference after oven drying at 105° C until constant weight (11). Crude protein content of major staple foods were determined as 'N' employing the Micro-Kjeldahl method (12). The method involved three steps: 1) digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia; 2) distillation of the ammonia into a trapping solution; and 3) quantification of the ammonia by titration with a standard solution. Lipids were also extracted from the samples and determined using the using AOAC, 2000 method. The total and available carbohydrate content was determined by difference using the following formula. Addition of the percentages of crude protein, crude fat and ash and fiber was subtracted from 100.

Available CHO (g/100g) = [100- (crude protein + crude fat + total ash + crude fiber)]

Gross energy was calculated using values obtained for fat, carbohydrate and protein contents and by using the Atwater's conversion factors (13); 16.7 KJ/g (4 kcal) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories using the following formula:

*Total energy (in kcal/100g) = (9*crude fat + 4*crude protein + 4* utilizable carbohydrate)*

Values for micronutrients Ca, Fe and Zn contents, whenever possible, were determined based on results of biochemical analyses; otherwise, data were compiled from the Ethiopian food composition table and published data (14,15). Samples were digested to destroy organic matter and reduce complex molecules to their elements, using dry ashing at 550 ^{o}C (digestion). The ash was dissolved in dilute HCl and the mineral content was measured using atomic absorption method of Osborne and Voogt (1978). The AAS reading was compared against the calibration curve to estimate the concentration in mg/100g. The mineral contents was calculated by using the following formula:

$$Mineral\ content\left(\frac{mg}{100g}\right) = [(A - B)x\ V]/10W$$

Where,

W = weight of samples (g)

V = volume of extract (ml)

A = concentration of sample solution (ml)

B = concentration of blank solution (ml)

Statistical analysis

Statistical analysis was done using SPSS 18 for windows. Descriptive statistics was presented for selected socio-demographic variables. The assumption of normality was tested using Shapiro-Wilk test. Dietary intakes (per day) and energy densities (per 100 kcal) were reported as medians and inter-quartile range.

Ethical Consideration

The study was approved by the Addis Ababa University, College of Natural Sciences and the Oromia Regional Health Bureau Ethical Review Committees. Written consent was obtained from all the participants prior to the interviews.

Results

The socio-demographic, anthropometric and nutritional characteristics are presented in **Table 1**. Most mothers were in their twenties (> 70%), were from the Oromo ethnic group (~ 80%), and less than 50% had completed secondary level education. About 32% of the study subjects were anemic.

Table - 4.1.1 : Socio-demographic characteristics of participants in a prospective cohort of pregnant mothers at rural Arsi, central Ethiopia.

Maternal Socio-demographic Characteristic	Frequency	Percent
Maternal Age (Years)		
– < 20yr	5	9.1
– 20 - 24	22	39.3
– 25 - 29	20	34.8
– ≥ 30	9	16.8
Ethnic Group		
– Oromo	45	80.5
– Amhara	9	15
– Guraghe	3	4.5
Educational Status		
– No formal Education	18	32.1
– Primary Education	14	25.4
– Secondary and above	24	42.5
Land size (Hectare)		
– < One	25	44.9
– One - Two	20	36.4
– > Two	11	18.7
ANC visits completed at birth		
– One	3	4.6
– Two	17	30.3
– ≥ Three	36	65.1

Starchy crops mostly consumed in the form of *Injera* and legumes used for the preparation of stews (mostly *shiro*) were the staple. Hot beverages like coffee and tea were also widely consumed. Animal Source Foods (ASF) like dairy (27%), fruits and vegetables (23%), eggs (21%), fish and meat (4%) and Organ meat (3%) were rare **(Table 2).**

Table - 2 : Food groups, common local names, timing to eat and amount of daily intake from each food item in a sub-sample of study population (n = 56) at rural Ethiopia, Arsi Zone.

Food Item	Description of "ready to eat" food items			Estm. daily intake (g/day)*	Est.. Population consuming (%)
	General description and mode of preparation	***Local name and varieties***	***Local synonyms***		
Enjera	*Enjera is a pancake like bread prepared from cereals such as barely (Hordeum vulgare L.), teff (Eragrostis tef), maize (Zea mays L.), wheat (sinde, Triticum vulgare) or a mixture of these cereals which served as a main dish with the stews.*	• *Ye key teff enjera* • *Ye nech teff enjera* • *Yesergegna teff* • *Nech gebs injera* • *Tikur gebs enjera* • *Nech sindie, enjera* • *Tikur gebs, enjera* • *Kiyit enjera*	• *Teff, red* • *Teff, white* • *Sergegna teff,* • *Barely, white* • *Barely, black* • *Wheat, white* • *Wheat, black* • *Mixed*	455 (420, 580)	96
Bread	*Unleavened bread prepared from unfermented maize or wheat or mixed*	*Ye sindie dabo or "kita"*	• *Maize bread*	142 (128, 177.8)	96
Porridges	*Porridge prepared from cereal or mixture of cereals, served as a main dish*	*Genfo*	• *Barely porridge*		

				168 (145, 209)	67
Boiled cereals	*Prepared by boiling cereals such like wheat, or legumes such as, broad beans (Vicia faba L.), chick peas (shimbira, Cicer arietinum (L.)) or a mixture of the above in water with added salt. The cooking water is drained and the food is served as a snack or even breakfast*	*Ashuk, nifro*	• *Bean , boiled* • *wheat and bean, boiled*	107 (86, 135)	89
Stew/ sauce	*Stew prepared from ground legumes such as garden or field peas (atter, Pisum sativum), chickpea (Cicer arietinum L.), or grass pea (guaya, Lathyrus sativus L.), Kale(gommen, Brassica carinata Braun), potato (Solanum tuberosum) by roasting, decortications and grinding the grains, seasoning with spices and then cooking. Served with a main dish*	• *Yeshiro wet* • *Ye misir wet* • *Yeater kik wet* • *Ye gomen wet* • *Ye dinch wet* • *Ye doro wet* • *Alcha wet*	• *Stew, from bean or pea* • *Stew from lentils* • *Stew from pea, (Coarsely grinded)* • *Stew from kale* • *Stew from potato* • *Stew from chicken* • *Stew with curd*	176 (158, 216)	97
Hot drinks	*Prepared by boiling roasted coffee beans (Araecerus coffeae) and taken after each meal daily.* *Prepared by boiling leaves of tea (Camellia sinensis) and taken usually in the morning with or after meal*	• *Shai* • *Buna*	• *Coffee* • • *Tea*	126 (98, 156)	79

∗ median (first, third quartile)

Carbohydrate content of the foods ranged from as low as 4.3 (kale) to as high as ~30.0 mg/100g found in teff Injera **(Table 3)**.

Table 3: Macronutrient content of common "ready to eat" foods of pregnant mothers in rural Arsi, Ethiopia.

Food Item	Carbohydrate (mg/100g)	Protein (mg/100g)	Fat (mg/100g)	Energy (kcal)
Enjera				
– *Teff enjera, red, fermented*	22.1	3.4	0.9	140
– *Teff enjera, white, fermented*	17.7	6.48	0.7	155
– *Teff enjera, sergegna, fermented*	29.93	3.00	1.5	140
– *Barely enjera, white, fermented*	16.57	2.6	0.2	138
– *Barely enjera, black, fermented*	17.9	3.6	0.9	158
– *Mixed grain enjera, fermented*		4.8	1.3	150
Stew (eaten with enjera)				
– bean or pea	7.8	2.3	6.3	143.8
– lentils	13.5	3.1	7.2	355
– pea, split	8.5	3.2	6.9	353.1
– kale	4.3	1.1	0.1	22.5
– potato	20.2	1.2	0.1	199.7
– bean/pea with curd/tofu	8.5	2.4	5.7	143.9
Other foods/drinks				
– Porridge with butter	10.78	8.93	8.01	147.62
– Roasted bean ('ashuk')	34.19	22.75	0.7	205.24
– Coffee drinks	18.9	5	2.8	169.2

Similarly, Injera had the highest protein content, whereas kale had the least. In contrast, stews made from kale and porridge with butter had the highest fat content. Wide variations in energy contents were also observed within food types. For example, the energy content of edible foods of pregnant mothers varied from 22.5 kcal/100g in kale-based stews to 355 kcal in lentil-based stews.

Zinc, calcium and iron contents of the common ready to eat foods consumed by pregnant mothers were highly variable and depended on the ingredients used to prepare them **(Table 4)**. However, the zinc contents were very low and did not exceed 2.5 mg/100g (coffee). Major staples like injera, and the stews that are consumed with, rarely provided more than 2 mg/100g of zinc. In contrast, all Injera and particularly red teff Injera had high iron contents (14.7 mg/100g). Stews made from beans and lentils had also high iron contents. Calcium contents were high in Injera ', stews prepared from beans, and teas.

The nutrient density of micronutrients expressed relative to the energy a certain food provides (mg/100 kcal) followed a similar pattern **(Table 5).** The nutrient density calculations indicated that the zinc and calcium densities of the most frequently consumed foods were below the desired densities for meeting requirements during pregnancy.

Table 5 : Nutrient density (mg per 1000 kcal) of key micronutrients (Iron, Zinc and calcium) from major "ready to eat" foods of pregnant mothers, rural Arsi Ethiopia.

Food Items	Zinc (mg/100 kcal)	Iron (mg/100 kcal)	Calcium (mg/100 kcal)
Enjera			
Teff enjera, red, fermented	4.5	55.4	188.5
Teff enjera, white, fermented	4.5	26.4	207.4
Teff enjera, sergegna, fermented	4.6	26.4	377.1
Barely enjera, white, fermented	4.5	7.9	18.9
Barely enjera, black, fermented	6.8	13.6	128.2
Wheat enjera, white, unfermented	3.3	16.6	79.2

Mixed grain enjera, fermented	4.5	10.9	49
Stew (eaten with enjera)			
· Stew, from bean or pea	3.8	6	67.9
· Stew from lentils	2.3	15.8	71.6
· Stew from pea, split	2.7	20.7	107.5
· Stew from kale	1.5	2.6	162.1
· Stew from potato	3.4	5.7	33.9
· Stew from bean/pea with curd/tofu	7.9	20.4	184.8
Other foods/drinks			
· Coffee	9.4	15.1	316.7
· Tea	3	23	633.5

In contrast, all Injera except the ones made from barley met the desired density (> 10 mg/100 kcal). Overall, Injera, coffee and tea had the highest iron, zinc and calcium densities.

Discussion

We have collected and analyzed ready to eat foods that were most frequently consumed by pregnant women in rural Arsi, Oromia region, Ethiopia. Cereals, legumes and beverages like coffee and tea were widely consumed, whereas ASF, fruits and vegetables were rarely consumed. Cereals were fermented and consumed in the form of injera along with legume-based stews. A significant proportion (> 70%) of the energy intakes were from carbohydrates. The staples had a low zinc and calcium contents, but iron contents were high enough to meet desired densities required to meet daily nutrient requirements provided that the pregnant women had adequate energy intakes.

The present study area, rural Arsi, is among the leading surplus crop producing and extensive husbandry practicing areas in Ethiopia (9). Contrarily, we found that maternal diets during pregnancy were less dense as well as less nutritious. This is in line with the fact that in sub-Saharan Africa, where socio-economic constraints, poor dietary quality, high intensity of agricultural labour and frequent reproductive cycles cause significant burden to mothers, access to high quality nutritious foods is a major challenge (14,15). Most diets, in the region consist mainly of cereal or root staples; and very little in terms of animal source proteins (16–18).

Starchy staples, mainly cereals and legumes, and beverages like coffee and tea were widely consumed in the study area, whereas ASF, fruits and vegetables were rarely consumed. Intake of animal source proteins that are nutrient dense foods and rich in bio-available micronutrients like fish, egg, meat and organ meat, was very limited. This could be associated with various factors, but requires further exploration in a separate future study. However, it is consistent with the results of several other studies from other low income countries (7) including Ethiopia (19,20).

Though varied from place to place in magnitude and severity, this level and amount of intake is not surprising as it is expected in such resource poor settings. Study findings from several other low income countries shows that inadequate macronutrient intake during pregnancy and lactation is common (19–22).

We also observed that carbohydrates dominate the proportional contribution of energy from macronutrients. It contributed around 71.3% of the daily total caloric intake, while the daily calorie contribution of protein and fat limited to 13.1%. and 16.5%. This pattern of energy contribution is in line with the national food consumption survey findings and WHO/FAO's recommendations(20,23). In the recent (2013) Ethiopia national food consumption survey, carbohydrates contributed for about 73.1% of energy in women of reproductive age(20). On the other hand WHO/FAO recommends that foods containing energy contributed from 55 - 75%, 15 - 30% and 10 - 15% by carbohydrates, fats and proteins, respectively are healthy enough to support normal life (23). In the same way, findings from studies from other African countries shows that protein's share for energy, but not fat or carbohydrate, is within the same range (24).

Though the proportional energy contribution of macronutrients was in the normal range, the overall estimated daily energy intake was quite very low. For example, the median energy intake of pregnant mothers was less than the requirement at least by one third (34%) or 780 kcal/day. This is contrary to the findings of previous studies of systematic reviews of clinical trials. The studies found that nutritional advice to increase energy and protein or balanced energy/protein supplementation (i.e. supplements in which protein provides less than 25% of the total energy content) during pregnancy has been found effective in reducing several risks. It reduced the risk of preterm birth and low birth weight; while increasing head circumference, birth weight and protein intake. Balanced protein energy supplementation also improved fetal growth, and reduced the risk of stillbirth, low-birth-weight and small-for-gestational age infants, especially among undernourished pregnant women (25–29).

Other studies have also reportedly proved that adequate energy and protein intake were strongly and positively associated with fetal growth, and possibly associated with a reduced risk of preterm birth (30). Malnourishment or inadequate dietary intake during pregnancy, on the other hand, leads to multiple adverse perinatal outcomes (30–32).

Analysis of the micronutrients showed that both, content as well as estimated daily intake for calcium and zinc was poor. However, the density per 1000kcal for each of these foods were comparable to other animal source foods studied in East and South African diets (33), as well as mineral rich wide plant foods common to west Africa (34–36). This shows that if mothers are able to increase their overall food intake during pregnancy, adequate micronutrients particularly be obtained from available local foods.

Conversely, iron was adequately present in many of the common food that rural mothers consume. It was also comparable with previous analysis of row and prepared cereals, enset starchy foods and legumes and other foods in Ethiopia (14,15). Nevertheless, in contrarily to the present finding; where maternal diets contain relatively adequate densities of micronutrients, iron deficiency anemia and micronutrient malnutrition is common in Ethiopia including the study area (37–40). This could be attributed to a couple of key reasons; firstly, the overall low total food intake (20,41) and very rare or infrequent intake of animal source foods (20,42) containing highly bio-available micronutrients including iron and zinc (43). Secondly, it may be due to traditional practices and feeding habits that affect nutrient bioavailability, i.e. routine and regular consumption of foods (like coffee and tea) containing high anti-nutritional factors.

The present study has several limitations that should be taken into account while interpreting the results. Firstly, data was collected with the objective of quantifying nutrient contents and micronutrient densities of common and "ready to eat" food items of pregnant women. thus, the daily intakes estimated given in the result are just simply indicative, not conclusive, as these were not collected from mothers with adequately powered sample. Secondly, data shown were obtained from both laboratory analysis in our center as well as compiled from other sources like food composition tables, for some micronutrients and not analyzed food items. This could affect comparability and accuracy of nutrient values, and hence needs caution in interpreting the findings. Thirdly, even if the two peak pre-harvest and harvest seasons were considered during data collection, this can't guarantee the whole picture of dietary intake in the study area, as this may vary across the year while we collected data for some months only.

Generally, acknowledging the above limitations, the study provides the first of its kind laboratory based data quantifying nutritional content, densities and food items commonly consumed in rural Ethiopia. Pregnant mothers in the study area consume foods mainly from by plant source starchy staples, with limited intake from animal proteins. As a result, it was showed that even if pregnant women had comparably adequate carbohydrate intake, the level of intake for other macronutrients including energy, protein, fat and key micronutrients like iron, zinc and calcium seems below the recommended values for pregnancy. Promisingly, even if the overall intake of macronutrients was low and source of energy is highly skewed to carbohydrates, the proportional share of carbohydrates, proteins and fats was within WHO/FAO recommended range.

Thus, mothers in the study community could benefit most if interventions of proven effectiveness like: advices of increasing consumption of protein and energy rich foods, improving fat intake or getting direct balanced protein energy supplements. Mothers also need to increase consumption of animal source foods which are dense enough and contain highly bio-available micronutrients.

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4.3. Association of dietary diversity with maternal and perinatal outcomes

4.3.1. Introduction

Dietary diversity has been linked to several nutritional indicators like dietary quality and adequacy as well as improved nutritional status. In resource limited settings of the sub-Saharan African, including Ethiopia; however, diets are usually monotonous arising from plant sources with little diversity. The role of dietary diversity during pregnancy, was hardly investigated in relation to maternal and perinatal outcomes .

In this section (4.3), results from a longitudinal follow-up study conducted in rural districts of Arsi Zone Ethiopia are presented in the form of a published paper accompanied by supplementary results. The study was conducted in 8 randomly selected health centers in 4 rural districts that were representative of the different agro ecologic zones of the Zone. Pregnant mothers were enrolled to prospective cohort study during their first antenatal care visit (usually second trimester of pregnancy) with 2 arms at a ratio of 1:1 adequate (unexposed) and inadequate (exposed) WDDSs and were followed to term. Maternal and perinatal outcomes were assessed during pregnancy (anemia) and at birth (pre-term, low birth weight and still birth babies). Based on the findings of the study, A WDDS of ≥ 4 food groups during pregnancy was shown to be associated with lower risk of maternal anemia, LBW, and PTB.

4.3.2. Dietary diversity during pregnancy is associated with reduced risk of maternal anemia, preterm delivery, and low birth weight in a prospective cohort study in rural Ethiopia. *American Journal of Clinical Nutrition. 2016;103:1482–8*

Dietary diversity during pregnancy is associated with reduced risk of maternal anemia, preterm delivery, and low birth weight in a prospective cohort study in rural Ethiopia[1]

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ABSTRACT

Background: Anemia during pregnancy is a leading nutritional disorder with serious short- and long-term consequences for both the mother and the fetus.

Objective: The objective was to investigate the association between dietary diversity during pregnancy and maternal anemia, low birth weight (LBW), preterm birth (PTB), and stillbirth in rural Ethiopia.

Design: We conducted a prospective cohort study and enrolled 432 pregnant women in their first antenatal care visit (24–28 gestational weeks); 374 women completed the follow-up. By using the FAO Women's Dietary Diversity Scores (WDDSs), subjects were categorized into "inadequate" (WDDS <4) and "adequate" (WDDS ≥4) groups and were followed until delivery. Primary outcomes were maternal anemia, birth weight, term delivery, and stillbirth.

Results: The attrition rate was 13.7% and was balanced across the 2 groups. The proportion of women consuming dairy, animal-source foods, fruits, and vegetables including vitamin A–rich ones was higher in the adequate than in the inadequate WDDS group ($P < 0.05$). The overall incidence of maternal anemia increased from 28.6% to 32.4% during the follow-up period. The overall proportion of LBW, PTB, and stillbirth were 9.1%, 13.6%, and 4.5%, respectively. After control for baseline differences, women in the inadequate group had a higher risk of anemia [adjusted RR (ARR: 2.29; 95% CI: 1.62, 3.24], LBW (ARR: 2.06; 95% CI: 1.03, 4.11), and PTB (ARR: 4.61; 95% CI: 2.31, 9.19) but not of stillbirth (ARR: 2.71; 95% CI: 0.88, 8.36) than women in the adequate group ($P < 0.05$).

Conclusions: A WDDS of ≥4 food groups during pregnancy was shown to be associated with lower risk of maternal anemia, LBW, and PTB. Population-based controlled trials of various options to improve dietary diversity are needed before conclusive recommendations can be made. This trial was registered at clinicaltrials.gov as NCT02620943. *Am J Clin Nutr* 2016;103:1482–8.

Keywords: anemia, low birth weight, dietary diversity, pregnancy outcome, preterm, stillbirth

INTRODUCTION

Maternal and child undernutrition accounted for 45% of all child deaths in 2011 (1). Poor maternal nutrition, both before and during pregnancy, is associated with adverse pregnancy outcomes including intrauterine growth restriction, which greatly increases the risk of neonatal deaths (2), low birth weight (LBW),[5] preterm birth (PTB), and stunting (3). Thus, improving the dietary pattern and nutritional status both before and during pregnancy can play a major role in preventing anemia, intrauterine growth restriction, and the associated short- and long-term adverse effects (4, 5). This is in line with the current emphasis on the first 1000 d of life (conception through 24 mo of age) as a window of opportunity to promote healthy child growth.

Unfortunately, diets of pregnant women in low- and middle-income countries (LMICs) are monotonous and predominantly plant-based with little consumption of micronutrient-dense animal-source foods, fruits, and vegetables (6, 7). Recognizing that such poor diets are likely to be deficient in multiple micronutrients, supplementation, particularly with iron or iron-folic acid (IFA), has been the mainstay of nutrition interventions targeting pregnant women. Despite the WHO's clear recommendation, the coverage and compliance of iron and IFA supplementation remain unsatisfactory in most LMICs including Ethiopia (8, 9). Therefore, in these settings, complementary strategies that address the underlying poor dietary practices are needed.

Very few studies have investigated the effect of improved diets in reducing the risk of maternal anemia and adverse pregnancy outcomes in a LMIC setting (10–12), and those that did came up with inconsistent findings showing positive as well as no effects. In a recent cross-sectional study among pregnant women, Agrawal et al. (12) showed that IFA supplementation and dietary diversity, measured by the use of the food groupings described

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[5] Abbreviations used: ANC, antenatal care; ARR, adjusted RR; IFA, iron folic acid; LBW, low birth weight; LMIC, low- and middle-income country; MUAC, midupper arm circumference; PTB, preterm birth; WDDS, Women's Dietary Diversity Score.

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in the WHO infant and young child feeding guidelines (13), are associated with reduced symptoms suggestive of pre-eclampsia or eclampsia. However, to our knowledge, no longitudinal study has investigated the association of the FAO's scoring system for measuring Women's Dietary Diversity Score (WDDS) with maternal anemia and adverse pregnancy outcomes. The WDDS is a simple, rapid, and useful proxy measure of micronutrient intake adequacy (14, 15), which, if found to be associated with reduced risk of maternal anemia and pregnancy outcomes, can easily be integrated in public health interventions targeting pregnant women.

Therefore, to investigate the association between dietary diversity during pregnancy and maternal anemia, LBW, PTB, and stillbirth, we conducted a prospective cohort study that followed pregnant women from their first antenatal care (ANC) visit to term, in a rural, low-income setting in Ethiopia. The women were separated into adequate (WDDS ≥4) and inadequate (WDDS <4) dietary diversity groups, and the relative risks of maternal anemia, LBW, PTB, and stillbirth were estimated.

METHODS

Study setting

The present prospective cohort study was conducted in 8 randomly selected health centers in 4 rural districts that were representative of the different agroecologic zones of the Arsi zone, Oromia region, Ethiopia. The Arsi zone is one of the surplus-producing agricultural areas of Ethiopia, with major production of wheat and barley.

Study design, sample size, and sampling procedure

The study was designed to investigate the association of dietary diversity during pregnancy with pregnancy outcomes. The study was a longitudinal, prospective cohort study with 2 arms at a ratio of 1:1 adequate (unexposed) and inadequate (exposed) WDDSs. Sample size was calculated by use of the Open Epi Kelsey statistical software available at http://www.openepi.com/SampleSize/SSCohort.htm with the following parameters and assumptions: a 95% significance level (2-sided), 80% power, and 37% anemia prevalence (16) among exposed pregnant women and an anticipated 10% lower prevalence of anemia among unexposed pregnant women. This yielded a total of 168 subjects/arm, and to allow for ≤20% attrition by the end of the study, a sample size of 420 was required. The inclusion criteria for the study were as follows: pregnant women who are permanent residents of the study area, with no known medical, surgical, or obstetric problems and who were willing to attend routine ANC visits.

Ethics

Verbal informed consent was obtained from the eligible participants in the presence of local *kebele* (smallest administrative unit) administrators after a detailed explanation of the purpose and methods of the study. The study procedures were in accordance with the Helsinki Declaration of 1975 as revised in 1983. The study protocol was approved by the institutional review boards of the College of Natural Sciences of Addis Ababa University and the Oromia Regional Health Bureau.

Allocation to study arms

Pregnant women in Ethiopia start ANC late, usually in their second trimester (17); hence, although the study enrolled women attending their first ANC visit, all were already in their second trimester (24–28 gestational weeks). At enrollment, a 24-h WDDS was collected from the pregnant women by use of the FAO guidelines (14), and subjects were then divided into "adequate" (WDDS <4) or "inadequate" (WDDS ≥4) groups (**Figure 1**). Briefly, the pregnant women were asked to recall the foods they had consumed in the previous 24 h, first spontaneously followed by probes to ascertain that no meal or snack was left out. A detailed list of all the ingredients of the dishes, snacks, or other foods consumed was generated to enable better classification of mixed dishes. The foods were then categorized into 9 food groups: *1*) cereals, roots and tubers; *2*) vitamin A–rich fruit and vegetables; *3*) other fruit; *4*) other vegetables; *5*) legumes and nuts; *6*) meat, poultry, and fish; *7*) fats and oils; *8*) dairy; and *9*) eggs. Overall, four 24-h WDDSs from a typical day were collected each month from enrollment to delivery, meaning data were also collected in the preharvest (August–October) and postharvest (November–January) seasons.

A pregnant woman was assigned and remained in the adequate or inadequate group if her WDDS remained in that category for ≥3 of the 4 visits. Otherwise, the woman was excluded from the analysis (*n* = 8, 4/group).

Maternal socioeconomic, dietary, and anthropometric characteristics

Data on the socioeconomic characteristics and food consumption patterns were collected by using a pretested questionnaire that was adapted from the Ethiopian Demographic and Health Survey and the FAO (14, 17). The questionnaires were pretested in a similar setting. The data were collected by 24 well-trained and experienced midwives who work permanently in the antenatal care service provision units of health centers in the community.

The pregnant women were weighed at each visit from enrollment to delivery following the standardized procedures recommended by WHO (18). Pregnant women were weighed to the nearest 100 g on electronic scales with a weighing capacity of 10–140 kg. Their height was measured to the nearest millimeter with a portable device equipped with calibrated and standardized height gauges (SECA 206 body meter). The midupper arm circumference (MUAC) of the left arm was measured to the nearest millimeter with a nonstretch measuring tape.

Outcome assessment

Hemoglobin measurements were taken twice: once at enrollment and once before delivery (term) by use of a portable HemoCue (AB Leo Diagnostics). The readings were adjusted for altitude (19), and pregnant women with values <11.0 g/dL were considered to be anemic.

Gestational age was estimated by midwives at the health center, by counting from the last menstrual period and fundal palpation during ANC visits. Birth weight was measured and recorded by the midwives immediately after delivery. Stillbirth (no signs of life at birth after 24 completed weeks of gestation) and PTB (<37 wk of gestation) were ascertained by the same professionals at birth.

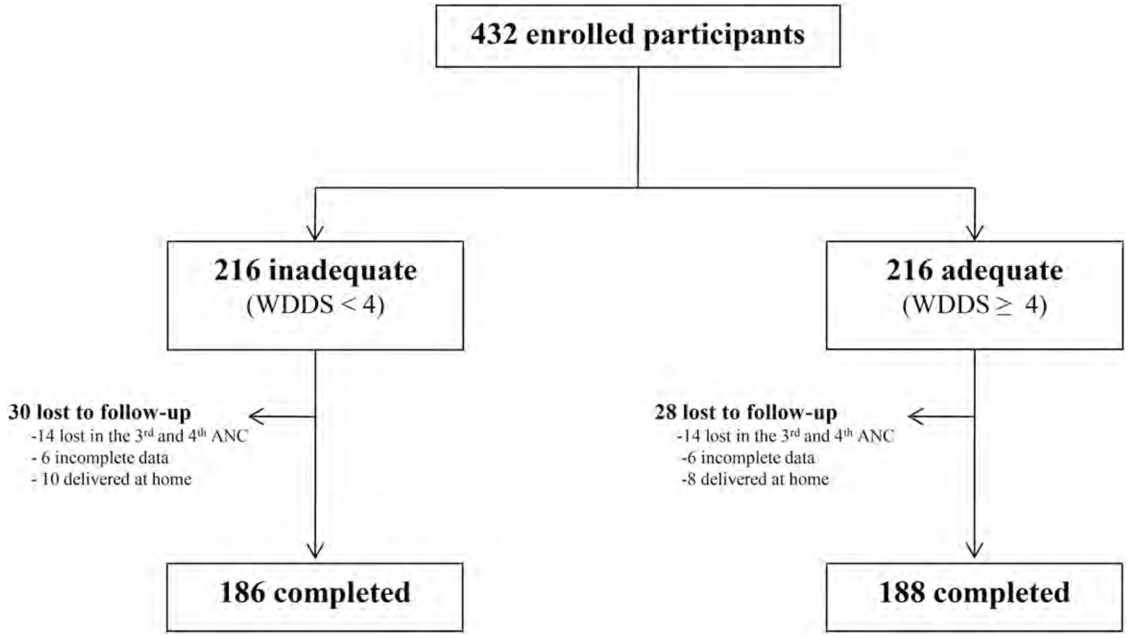

FIGURE 1 Study participant flow of the prospective cohort of pregnant mothers in rural Arsi, Central Ethiopia. ANC, antenatal care; WDDS, Women's Dietary Diversity Score.

Quality control

All data collectors were experienced (≥4 y) midwives with at least a diploma in nursing. They also received 5-d training on participants' enrollment, follow-up, anthropometric measurements, and the use of a HemoCue for hemoglobin measurements. The training was conducted just before the study and was followed by practical tests to ensure the skills were transferred.

In each of the health centers selected, one supervisor (usually the head) was assigned to oversee data collection. In addition, the investigator made a weekly visit to check the completeness and quality of the data collected.

Anthropometric measurements were taken only by trained personnel, and all equipment was calibrated with standard weights and length rods on a daily basis. Neither the pregnant women nor the data collectors were aware of the allocation into groups.

Statistical analyses

Data were entered by use of Epi-data statistical software (version 20). The data were double entered and cleaned and then exported to SPPS (version 20.0) for statistical analyses. All data on singleton pregnancies were analyzed according to their categorization by WDDS. All continuous variables were checked for normality by use of the Kolmogorov-Smirnov test. Variables not normally distributed were log-transformed. Means and SEMs were used to describe continuous variables. An independent Student's *t* test was used to compare means in the adequate and inadequate groups. A χ^2 test or a Fisher's exact test was used to test for independence in distribution of categorical variables between the 2 study groups. In all comparisons, differences were considered statistically significant at $P < 0.05$.

The association between adequacy of WDDS and pregnancy outcomes (anemia, LBW, PTB, and stillbirth) was investigated after adjusting for baseline differences. A log-binomial model adjusting for baseline group differences MUAC, level of education, hemoglobin, age, height, and gestational age was run, and the adjusted RRs (ARRs) and 95% CI values are reported.

RESULTS

A total of 432 eligible pregnant women (216 from each group) were enrolled, of whom 373 completed the study. The reasons for dropping out were mainly discontinuation of the ANC visits (*n* = 28), incomplete data (*n* = 12), or not delivering in a health facility (*n* = 18) (Figure 1). Overall, the dropout rate was 13.7% and was balanced across the 2 groups. The baseline characteristics of the dropouts and the women who completed the study were not statistically different (data not shown).

At enrollment, the mean duration of pregnancy was 25–27 wk, but women in the adequate group had their first ANC ∼1 wk before those in the inadequate group ($P < 0.05$) (**Table 1**). The 2 groups had similar monthly income, land size, parity, and rate of previous abortions ($P > 0.05$). However, women in the adequate WDDS group had a higher number of completed school years, had higher hemoglobin concentrations and MUAC values, and were taller and younger than their counterparts in the inadequate WDDS group ($P < 0.05$). Women in the adequate WDDS group consistently consumed more dairy, animal-source foods, fruit, and vegetables throughout the follow-up period than those in the inadequate group (**Figure 2**).

At the end of the follow-up, several of the outcome variables differed across groups (**Table 2**). Pregnant women in the adequate group had significantly higher weight, higher weight gain, longer duration of follow-up, and higher hemoglobin concentration and gave birth to heavier babies ($P < 0.05$). The proportion of pregnant women taking IFA tablets was very low, and although the number was slightly higher in the adequate group, the difference was not statistically significant. In addition, very few IFA tablets were taken by any of the women, and the number did not differ across the groups.

TABLE 1
Baseline sociodemographic, anthropometric, and biochemical characteristics of a cohort of pregnant women in rural Arsi, Ethiopia[1]

	WDDS[2]		
Characteristics	Inadequate (n = 186)	Adequate (n = 188)	P value
Maternal age, y	25.54 ± 0.347	24.44 ± 0.30	0.017*
Educational status, y	3.9 ± 0.288	5.7 ± 0.355	<0.001*
Proportion of nulliparous women, %	21.7	23	0.66[3]
Previous deliveries or parity, n	2.34 ± 0.09	2.14 ± 0.08	0.147
Previous abortions, n	0.11 ± 0.02	0.08 ± 0.02	0.369
Monthly income[4]	1507 ± 212	1566 ± 264	0.767
Land size, hectare	1.62 ± 0.12	1.36 ± 0.08	0.079
MUAC, cm	22.43 ± 0.12	22.97 ± 0.14	0.001*
Height, cm	158 ± 0.11	159.24 ± 0.39	0.039*
Weight, kg	51.95 ± 0.43	52.59 ± 0.52	0.358
Gestational age, wk	26.59 ± 0.22	25.32 ± 0.26	<0.001*
Hemoglobin,[5] g/dL	10.95 ± 0.12	11.73 ± 0.12	<0.001*

[1]Values are means ± SEMs. *Different between groups, $P < 0.05$ (independent t test). MUAC, midupper arm circumference; WDDS, Women's Dietary Diversity Score.
[2]Inadequate means a WDDS <4, and adequate means a WDDS ≥4.
[3]Value determined by Fisher's exact test.
[4]Ethiopian currency (*birr*).
[5]Hemoglobin concentration was adjusted for altitude.

At enrollment, 37.6% of women in the inadequate group were anemic compared with 19.7% in the adequate group (**Figure 3**). The proportion of anemia in the inadequate group continued to increase and reached 44.6% of anemic women at the end of the follow-up, whereas it remained fairly stable in the adequate group (20.2% at term). After control for baseline differences, pregnant women in the inadequate WDDS group had a ∼2-fold higher risk of being anemic (ARR: 2.29; 95% CI: 1.62, 3.24), a 4.7-fold higher risk of PTB (ARR: 4.61; 95% CI: 2.31, 9.19), and a 2-fold risk of LBW (ARR: 2.06; 95% CI: 1.03, 4.11) than their counterparts in the adequate WDDS group (**Table 3**). There were no differences in the risk of stillbirth between the 2 groups

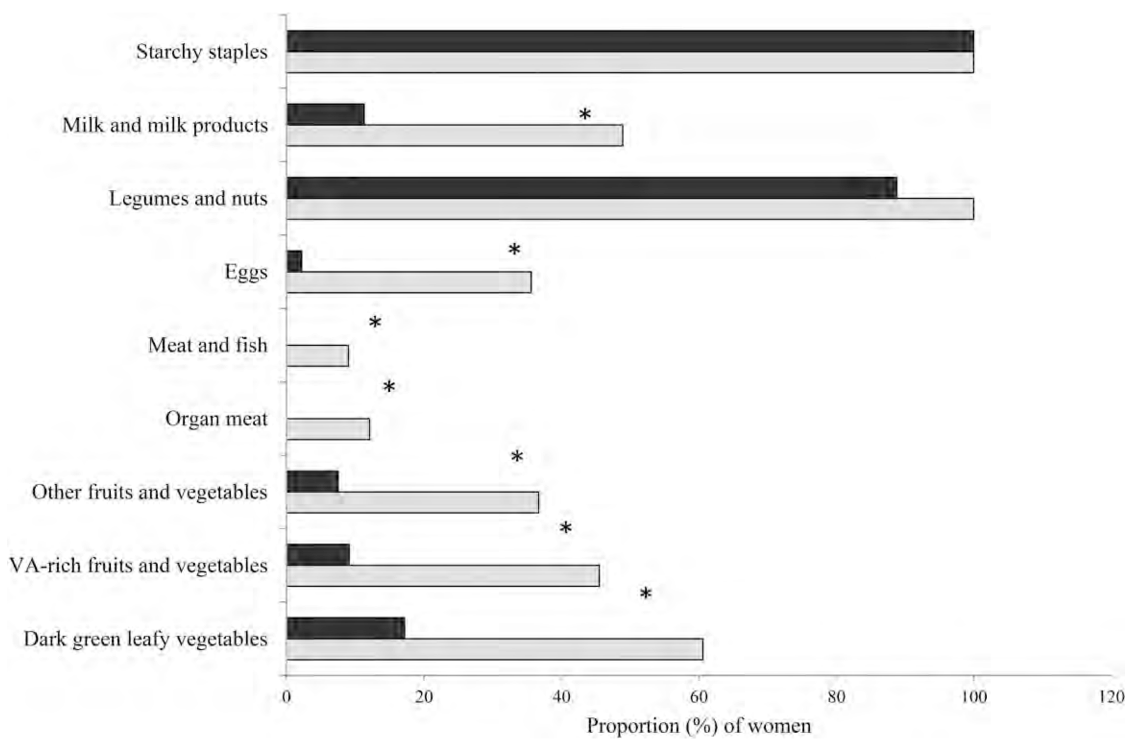

FIGURE 2 Food groups consumed by category of WDDS. Dark shaded bars indicate inadequate diet (WDDS <4), and light shaded bars indicate adequate diet (WDDS ≥4). *Statistically significant difference between adequate and inadequate groups measured by use of Fisher's exact test (P <0.05). VA, vitamin A; WDDS, Women's Dietary Diversity Score.

TABLE 2
Pregnancy and birth outcomes of pregnant women by dietary diversity group at end line (term)[1]

Outcome variable	WDDS[2] Inadequate (n = 186)	Adequate (n = 188)	P value
Birth weight, g	2690.9 ± 20.65	3192.3 ± 33.3	<0.001*
Weight, kg	61.32 ± 0.51	62.65 ± 0.53	0.072
Changes from baseline	9.42 ± 0.16	10.02 ± 0.18	0.01*
Gestational age, wk	36.86 ± 0.07	37.39 ± 0.08	<0.001*
Duration of follow-up, wk	10.76 ± 0.25	12.05 ± 0.27	<0.001*
Hemoglobin,[3] g/dL	10.96 ± 0.14	12.19 ± 0.13	<0.001*
Changes from baseline	0.00 ± 0.11	0.46 ± 0.13	0.009*
Number of IFA tabs taken	35.39 ± 4.38	41.72 ± 3.25	0.345
IFA, proportion taking[4]	23.7	33.7	0.659

[1]Values are means ± SEMs. *Different between groups, $P < 0.05$ (independent t test). IFA, iron-folic acid; WDDS, Women's Dietary Diversity Score.
[2]Inadequate means a WDDS <4, and adequate means a WDDS ≥4.
[3]Hemoglobin concentration was adjusted for altitude.
[4]Value determined by Fisher's exact test.

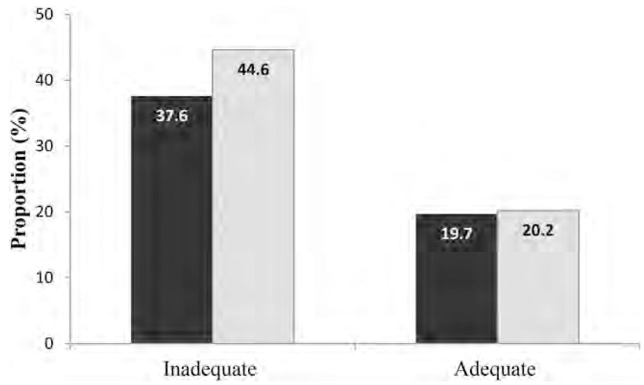

FIGURE 3 Incidence of anemia by WDDS category at baseline and endpoint. Dark shaded bars indicate baseline measurements, and light shaded bars indicate measurements at endpoint. Inadequate means a WDDS <4, and adequate means a WDDS ≥4. WDDS, Women's Dietary Diversity Score.

(ARR: 2.71; 95% CI: 0.88, 8.36). The incidence of LBW, PTB, and still birth at the end of the follow-up period was significantly higher in the inadequate than in the adequate group **(Figure 4)**.

DISCUSSION

The present multicenter prospective cohort study was conducted in a rural setting in Ethiopia. The study enrolled pregnant women from their first ANC visit (second semester) and followed them until delivery to investigate the association between the dietary diversity of pregnant women and the risk of LBW, PTB, stillbirth, and anemia during pregnancy. To our knowledge, this is the first study to examine the relation between the FAO WDDS and maternal anemia and pregnancy outcomes in a developing country setting. After adjustment for baseline differences, the study found that pregnant women in the inadequate WDDS group had a higher risk of anemia. Compared with their counterparts in the adequate WDDS group, women in the inadequate group had also a higher risk of LBW and PTB but not of stillbirth.

The higher risk of anemia among pregnant women in the inadequate WDDS group is not surprising and can be partly explained by a lower energy and nutrient intake than in the adequate group. Studies conducted among women of reproductive age and young children in various countries showed nutrient intakes increased with an increase in dietary diversity (15, 20). This was the basis for the validation and operationalization of dietary diversity tools as proxy indicators of dietary quality. Although we were unable to find any such studies among pregnant women, the nutrient adequacy of the diets is similarly expected to improve with dietary diversification, especially because the women in the adequate WDDS group consumed more nutrient-dense foods such as animal-source foods, fruits, and vegetables.

In the present study, women in the inadequate WDDS group had an increased risk of PTB (ARR: 4.61; 95% CI: 2.31, 9.19) and LBW (ARR: 2.06; 95% CI: 1.03, 4.11) compared with women in the adequate WDDS group. Poor nutrition in general and anemia in particular have been associated with various pregnancy complications including PTB and LBW (21). Most of this evidence comes from trials that involved supplementation with IFA, multiple micronutrients, and, more recently, food supplementation (22, 23). Most of these studies compared multiple micronutrients with iron or IFA and suggested that multiple micronutrient supplementations have several advantages in reducing LBW and preterm. We were only able to find a few observational and randomized controlled trials that investigated the effect of improved diet on birth outcomes (10, 24, 25). Taken together, these studies indicate that diet quality is associated with birth weight and PTB. In a prospective cohort study involving 66,000 pregnant women in Norway, "prudent" dietary patterns that consisted of vegetables, fruits, oils, water as a beverage, whole-grain cereals, and fiber-rich breads were associated with a lower risk of preterm delivery (26). Another prospective cohort study in the United States showed that the Dietary Approach to Stop Hypertension diet, which is also rich in dairy, fruit, and vegetables, was found to be associated with reduced risk of preterm delivery (25).

However, a recent randomized controlled trial in India reported that an intervention that increased consumption of dairy, fruits, and green leafy vegetables before and during pregnancy through a specially formulated snack had no effect on birth weight (11). In contrast, in the present study, the risk of LBW was lower in the adequate than in the inadequate group. Compared with women in the inadequate group, those in the adequate group consumed more dairy, fruits, and vegetable, but also other animal-source foods such as meat and eggs. The latter are rich sources of bioavailable micronutrients and thus may partly explain the difference in findings between the study in India and ours. It should also be noted that unlike the study in India, our study lacks evidence for causality.

It is worth noting that inconsistent findings among studies investigating micronutrients and pregnancy outcomes are common. For instance, the provision of lipid-nutrient supplement rather than IFA or multiple micronutrients during pregnancy in Ghana improved fetal growth (23), whereas it did not in Malawi (27). Such inconsistencies suggest that other factors in addition to improved nutrient intakes can come into play. In our study, baseline differences in maternal age, level of education, hemoglobin concentrations, and anthropometry could all have affected the incidence of maternal anemia and pregnancy outcomes (28). However, the association between dietary diversity, maternal

TABLE 3
Risk of maternal anemia and adverse pregnancy outcomes by dietary diversity group[1]

Outcome assessment	*n*	RR (95% CI)[2]
Anemia		
Anemic		
Inadequate	70	2.29 (1.62, 3.24)
Adequate	37	1
Total	107	
Nonanemic		
Inadequate	116	
Adequate	151	
Total	267	
Birth weight		
LBW		
Inadequate	23	
Adequate	11	2.06 (1.03, 4.11)
Total	34	1
Normal weight		
Inadequate	163	
Adequate	177	
Total	340	
Term delivery		
PTB		
Inadequate	42	4.61 (2.31, 9.19)
Adequate	9	1
Total	51	
Term birth		
Inadequate	144	
Adequate	179	
Total	323	
Birth		
Stillbirth		
Inadequate	13	2.71 (0.88, 8.36)
Adequate	4	1
Total	17	
Live birth		
Inadequate	173	
Adequate	184	
Total	357	

[1]Total N = 374: inadequate group, n = 186; adequate group, n = 188. LBW, low birth weight; PTB, preterm birth.

[2]Values were estimated by using log-binomial regression adjusted for baseline differences: maternal educational status, age, height, midupper arm circumference, and hemoglobin concentration.

anemia, and the birth outcomes remained significant even after adjusting for these baseline differences. Another confounding factor is the IFA supplementation supplied by the health facilities. However, the proportion of women who took IFA supplementation was similarly low in the 2 groups, and those who took the supplements did not take more than a third of the WHO-recommended regimen of minimum 90 tablets. Such low compliance and adherence to IFA supplementation is common among pregnant women in many developing countries including Ethiopia (8, 9). At enrollment, ∼30% of the pregnant women were already anemic, and a closer look at these data showed that 20% more women in the inadequate group were anemic than in the adequate group. Considering the equally low compliance, the inadequate amount of tablets taken, and the late start of IFA supplementation in the 2 groups, we do not think this is the main reason for the observed differences in the risk of maternal anemia and pregnancy outcomes. There was also no reported

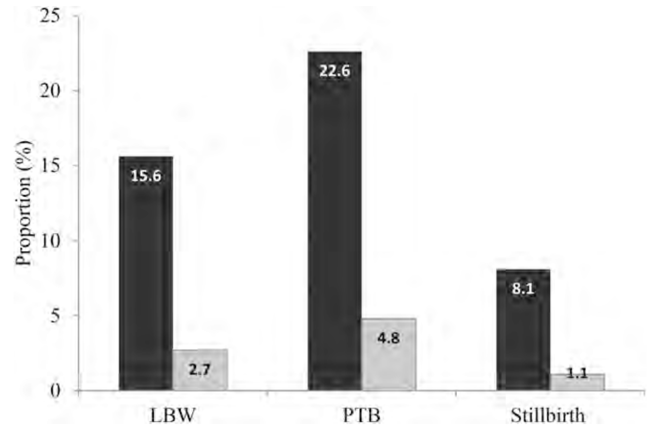

FIGURE 4 Incidence of adverse pregnancy outcomes by WDDS category at the end of follow-up. Dark shaded bars indicate inadequate diet (WDDS <4), and light shaded bars indicate adequate diet (WDDS ≥4). LBW, low birth weight; PTB, preterm birth; WDDS, Women's Dietary Diversity Score.

case of malaria during the follow-up period, and none of the women were smokers.

In addition to improved micronutrient intakes, a plausible way by which dietary diversity could improve pregnancy outcomes would be through improved antioxidant and fiber intakes (29, 30). This is particularly true for women in their second trimester of pregnancy, because oxidative stress reaches a peak at this time (31). Such oxidative stresses and the associated inflammation have been linked to pregnancy complications (32, 33). Therefore, increased dietary diversity could also mean higher intakes of antioxidants and fiber through increased consumption of fruit and vegetables, which could counteract oxidative stress-related pregnancy complications (34).

The present study has several limitations that need to be taken into consideration when interpreting the findings. Although it is recognized that both pre- and early pregnancy nutrition are associated with pregnancy outcomes, we were only able to follow the women starting from their second trimester, mainly because of the late start of ANC visits. Nevertheless, given the consistency of WDDSs in the 4 monthly assessments conducted during the follow-up period, we expect that the pre- and early pregnancy WDDSs would have been similar. We used the FAO WDDS, which included 9 food groups and assumed consumption of ≥4 food groups as the minimum adequate dietary diversity score. Unfortunately, our study was not adequately powered to test several thresholds of WDDS. Neither did we impose a minimum amount restriction in counting food groups. However, our dichotomous categorization of dietary adequacy based on a cutoff point of 4 food groups was a good predictor of maternal anemia and the various adverse pregnancy outcomes. By assessing the dietary diversity of the women at monthly intervals (a total of 4 times) and hence in both pre- and postharvest seasons, this study was able to account for possible seasonal variations. Although efforts were made to control for baseline differences when calculating the relative risks of adverse pregnancy outcomes, the effect of some factors like the level of education cannot be completely controlled. Also, the present finding should be interpreted with caution, because it does not provide evidence for causal relation.

Notwithstanding the above-mentioned limitations, our study is to our knowledge unique in showing that a WDDS ≥4 is associated with reduced risk of maternal anemia, LBW, and preterm among pregnant women in rural Ethiopia. This finding suggests that the FAO WDDS could be used as a simple tool to assess the dietary quality of pregnant women and could thus be used to support programs in further defining recommendations of a "balanced diet" at this vulnerable life stage. However, the present findings suggest association and not causality. Thus, future studies should explore the validity of the WDDS as a predictive tool for maternal anemia and adverse pregnancy outcomes in other low- and middle-income countries.

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The authors' responsibilities were as follows—TAZ, MU, and KB: developed the analyses parameters and objectives and secured financial support; TAZ and KB: conducted detailed analyses and wrote the manuscript; and all authors: read and approved the final version of the manuscript. The authors declared that they had no conflicts of interest.

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4. 4. Food groups associated with lower risk of adverse pregnancy outcomes

4.4.1. Introduction

The prospective cohort study showed us that WDDS of four or more groups were associated with better pregnancy and perinatal outcomes and reduced risks. Though dietary diversity, just in count of food group are linked to strong proxy measure of quality and adequacy, may not be informative enough programmatically and policy wise. This is because certain food groups may have outstanding role in explaining the observed benefits which prompted the current analysis.

In this section (4.4), further in-depth analysis of the same dataset was conducted to illustrate the role of food groups and other nutritional characteristics on both the risk of adverse perinatal outcomes (low birth weight or preterm birth or still birth, computed as one variable) and anemia of pregnancy. The results are presented in the form of a manuscript prepared for publication.

4. 4. Consumption of dairy, fruits and dark green leafy vegetables is associated with lower risk of adverse pregnancy outcomes in a *prospective cohort study in rural Ethiopia*

(Paper ready for submission to a Journal)

Consumption of dairy, fruits and dark green leafy vegetables is associated with lower risk of adverse pregnancy outcomes in a *prospective cohort study in rural Ethiopia.*

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Abstract

Background: Maternal dietary and nutritional factors have been linked to pregnancy and the outcomes. Nevertheless, there is paucity of evidence on the association of specific food groups with risk of adverse perinatal outcomes (APO) in resource poor settings.

Objective: The aim of this study was to determine the associations between dietary and nutritional factors with risk of key APO: preterm birth, low birth weight and still birth in rural Ethiopia.

Setting: Rural health centers in four districts of Arsi Zone, Oromia region, Ethiopia.

Design: A multi-center prospective cohort study

Subjects: A total of 432 eligible pregnant women were recruited. Mothers were then classified into exposed (n = 216) versus unexposed (n = 216), based on women's individual dietary diversity (WIDD) score and followed-up since their enrolment, during second antenatal care visit, until the end of pregnancy.

Findings: A total of 374 pregnant women were retained at the end of the study. Among these, one in every five,74(19.8%), experienced at least one of the APO: 34 (9.1%) gave birth to low birth weight babies, 51(13.6%) had preterm births and 17 (4.5%) had still birth babies. Poor or inconsistent consumption of dark green leafy vegetables (adjusted odds ratio (AOR), 2.012; 95% confidence interval (CI): 1.04; 3.87), dairy products (AOR, 2.64; 95% CI: 1.11; 6.30), and fruits and vegetables (AOR, 2.92; 95% CI: 1.49; 5.67) were dietary factors independently associated with APO. Similarly, being non-anemic at term (AOR, 0.24; 95% CI: 0.12; 0.48) showed up as a single independent nutritional factor predicting APO, in a rural resource limited settings of Ethiopia.

Conclusions: Risk of adverse pregnancy outcome was associated with poor or inconsistent dietary consumption of animal source foods, mainly dairy products, fruits and dark green leafy vegetables.

Key words: *Adverse Perinatal Outcome, Dietary Diversity, Birth Weight, Preterm Birth, Still Birth.*

Introduction

In the last couple of decades, our planet has witnessed a remarkable reduction in child mortality. The rate of under five mortality has declined substantially since 1990from 12.6 to6.6 million deaths in 2012(1,2). Nevertheless, the incidence of adverse perinatal outcomes, such as preterm birth, low birth weight and still birth has continued to be a leading cause of neonatal and under five child deaths (2–4). In 2010, approximately 15 million babies were born preterm, and more than one million died due to complications in the first month of life, more from indirect effects, and millions have a lifetime of impairment (10). Similarly, every year, at least 2.6 million stillbirths occur globally, of which 98% are happening in low and middle income-countries.

Recent estimate suggest that about 11% have low birth-weight, 46 in every 1,000 pregnancies experience prenatal death after seven or more months of gestation(15). Ethiopia has ranks 5th in the global burden of maternal death, 6th in neonatal death, and 7thin the incidence of still births. This is unfortunate, considering that low birth weight (LBW) and preterm lead to mortality and morbidity of neonates and infants, which includes developmental delays that can impose enormous cost to families and the society in large(6,8,9).

Inadequate dietary intake is among the leading cause of these adverse pregnancy outcomes. Trials investigatingthe effect of micronutrient supplementation on adverse pregnancy outcomes have come up with inconsistent findings, depending on the type and regimen of the supplementation. In a prospective cohort study in Central Arsi, Ethiopia we have recently reported that pregnant women with a dietary diversity score > 3 had lower risk of adverse pregnancy outcomes including LBW, preterm, and maternal anemia. However, these associations may depend on certain food groups more than others. To our knowledge, there is no published literature on food group level associations with adverse pregnancy outcomes. This is unfortunate, given that such information could be useful for interventions that aim to improve pregnancy outcomes.

Therefore, the aim of the present study was to investigate the association between consumption of specific food groups and adverse pregnancy outcomes including LBW, preterm and stillbirth.

Methods

Details on the methods part including: study setting, study design, sample size, and sampling procedure, Ethics, Socio-demographic and anthropometric characteristics, Dietary diversity and food group classification and outcome measurements for the study is given elsewhere. Briefly, The study was conducted in eight randomly selected health centers of four rural districts in Arsi Zone, central part of the Oromia Regional State, Ethiopia. The four rural districts were selected purposefully to represent cultural similarities and the four agro-climatic areas of the Zone. This is based on the assumption that peoples of similar agro-climatic area produce and consume similar foods (19–21).

The sample size was calculated using the Open Epi Kelsey statistical software which yielded a total of 168 subjects per arm, and was increased to 432 to allow for up to 20% attrition by the end of the study. We enrolled pregnant women who are permanent residents of the study area, with no known medical, surgical, or obstetric problems, and who were willing to attend routine ANC visits.

Data on socio-economic variables like age, educational status, land ownership and others were obtained through questionnaire-based face-to-face interviews. Anthropometric data was also taken during each visit by the midwives from all the health centers included to the study. The pregnant women were weighed at each visit from enrollment to delivery following the standardized procedures recommended by WHO(19) to the nearest 100 g on electronic (Salter Brecknell) scales with a weighing capacity of 10 to 140 kg. Their height was also measured to the nearest mm with a portable device equipped with calibrated and standardized height gauges (SECA 206 Bodymeter). The mid-upper arm circumference (MUAC) of the left arm was measured to the nearest mm with a non-stretch measuring tape.

Four 24-h Women Dietary Diversity Scores (WDDS)were conducted each month from enrollment to delivery. A nine food group classification was used as follows: 1) cereals, roots and tubers; 2) Vitamin-A-richfruitsandvegetables;3)other fruit; 4) other vegetables; 5) legumes and nuts; 6) meat, poultry and fish; 7) fats and oils; 8) dairy; and 9) eggs.

Outcome assessment

Hemoglobin measurements were taken twice: once at enrollment and once before delivery (term) using a portable HemoCue (AB Leo Diagnostics, Helsinborg, Sweden). The readings were adjusted for altitude (20), and pregnant women with values below 11.0 g/dl were considered to be anemic (24).

Birth weight was measured and recorded by the midwives immediately after delivery. Stillbirth (no signs of life at birth after 24 completed weeks of gestation) and PTB, (delivery <37 weeks of gestation) were ascertained by the same professionals at birth. Gestational age was estimated by midwives at the health center, by counting from the last menstrual period and fundal palpation during ANC visits (25).

Statistical analyses

Data was entered using Epi-data statistical software (3.1). Data was double entered and cleaned and then exported to SPPS (version 20.0) for statistical analyses. Continuous variables were checked for normality using the Kolmogorov-Smirnov test.

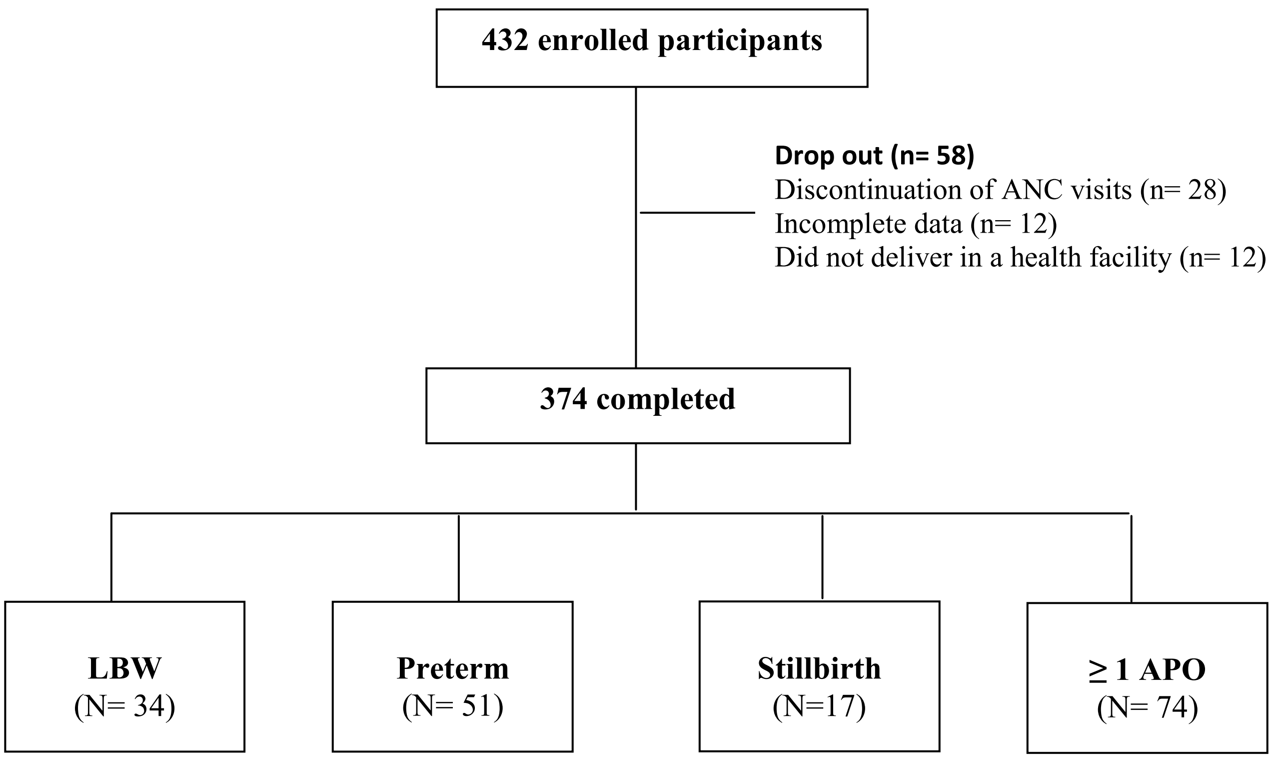
The association between specific food groups consumed and the odds of adverse pregnancy outcomes were investigated using bivariate and multivariate logistic regression analyses. Differences with P-values < 0.05 were considered statistically significant.

Ethics

Verbal informed consent was obtained from the eligible participants in the presence of local *kebele*(smallest administrative unit) administrators after a detailed explanation of the purpose and methods of the study. The study procedures were in accordance with the Helsinki Declaration (22).The study protocol was approved by the institutional review boards of the College of Natural Sciences, Addis Ababa University and the Oromia Regional Health Bureau.

Results

A total of 432 eligible pregnant women (216 from each group) were enrolled, of whom 374 completed the study. The reasons for the dropping out were mainly discontinuation of the ANC visits (n= 28), incomplete data (n= 12) or not delivering in a health facility (n=18) (**Figure 1**). Overall, the dropout rate was 13.7 % and was balanced across the two groups.



ANC, ante-natal care; APO, adverse pregnancy outcomes;

FIGURE 1: Study participant flowchart– prospective cohort of pregnant mothers, in rural Arsi, Central Ethiopia.

Table 1 presents aggregated and classified percent distribution of maternal socio-demographic characteristics. Accordingly, a larger proportion of the mothers (n=159; 42.5%) of them have completed primary education, 147 (39.3%) were the age group of 20 - 24 years, 166(44.9%) owned two or more hectares of land, and 241 (65.1%) have attended three or more antenatal care visits. Nearly two thirds of them were non-anemic at enrollment (n=267; 71.4%)and, at term, 254 (67.9%)(Table 1).

Table 1 Selected socio-demographic, anthropometrics, and nutritional characteristics of pregnant mothers in rural Ethiopia, stratified by adverse pregnancy outcomes (n = 374).

Selected characteristics	**All** (n = 374)	**LBW** (n = 34)	**Preterm** (n = 51)	**Stillbirth** (n = 17)	**APO** (n = 74)
Maternal age (yrs)					
< 20	34 (9.1)	1 (2.9)	1 (2)	0	2(2.7)
20 - 24	***147 (39.3)***	12 (35.5)	13 (25.5)	2 (11.8)	23 (31.1)
25 - 29	130 (34.8)	***15 (44.1)***	***27 (52.9)***	***8 (47.1)***	***33 (44.6)***
≥ 30	63 (16.8)	6 (17.6)	10 (19.6)	7 (41.2)	16 (21.6)
Ethnicity					
Oromo	***301 (80.5)***	***27 (79.4)***	***33 (64.7)***	***13 (76.5)***	***53 (71.6)***
Amhara	52 (13.9)	5 (14.7)	10 (19.6)	2 (11.8)	11 (14.9)
Guraghe	17 (4.5)	2 (5.9)	8 (15.7)	2 911.8)	10 (13.5)
Other	4 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)
Educational status					
Unable to read & write	120 (32.1)	7 (20.6)	***18 (35.3)***	8 (47.1)	***25 (33.8)***
Read and write only	95 (25.4)	***17 (50)***	16 (31.4)	***5 (29.1)***	26 (35.1)
Primary education	31 (8.3)	2 (5.9)	7 (13.7)	2 (11.8)	7 (9.5)
Secondary or above	***128 (34.2)***	8 (23.5)	6 (11.7)	2 (11.8)	16 (21.6)
Land size (hectare)					
< One	***166 (44.9)***	8 (23.5)	27 (52.9)	***9 (52.9)***	30 (40.5)
One - two	136 (36.4)	***19 (55.9)***	***18 (35.3)***	4 (23.5)	***33 (44.6)***
>Two	70 (18.7)	7 (20.6)	6 (11.8)	4 (23.5)	11(14.9)
Hemoglobin (Baseline)					
Anemic (Hb < 110 g/l)	107 (28.6)	***22 (64.7)***	***28 (54.9)***	8 (47.1)	***39 (52.6)***
Non-anemic	***267 (71.4)***	12 (64.3)	23 (45.1)	***9 (52.9)***	35 (47.3)
Hemoglobin (Term)					
Anemic (Hb < 110 g/l)	121 (32.1)	***27 (79.4)***	***33 (64.7)***	***11 (64.7)***	***48 (64.8)***
Non-anemic	***253 (67.9)***	7 (20.6)	18 (35.3)	6 (35.3)	26 (55.2)
MUAC (cm)					
Malnourished (< 23)	173 (46.3)	***26 (76.5)***	***35 (68.6)***	***11 (64.7)***	***49 (66.2)***
Well-nourished (≥ 23)	***201 (53.9)***	8 (23.5)	16 (31.4)	6 (35.3)	25 (33.8)

MUAC, mid-upper arm circumference;

In addition, among the 374 mothers who remained in the study, one in every five (19.8%) of them experienced at least one of the adverse outcomes. Fifty one (13.6%) gave birth to premature baby, nearly one in every ten (9.1%) gave birth to low birth weight babies, and seventeen (4.5%) gave birth to a still birth baby that died at or immediately after birth.

A bivariate and multivariate analysis of dietary and nutritional factors was conducted to determine the level of associations and prediction level of APO. Thus, deprivation or inconsistent intake of four food groups (dark green leafy vegetables, vitamin A rich foods, other fruits and vegetables as well as milk and milk products) out of the nine were associated with increased risk of APO. However, in the final multivariate regression analysisonly two food groups (dark green leafy vegetables and dairy products) appeared in the final model, predicting risk of APO.

Pregnant mothers who were either totally deprived or inconsistently consuming dark green leafy vegetables, had a 92% (AOR, 1.92; 95% CI: 1.04; 3.58) added risk of giving birth to at least one of the APO. In the same way, pregnant mothers who did not get or seldom took milk and milk products, experiencedmore than a threefold (AOR, 3.35; 95% CI: 1.48; 7.60) risk of APO compared to those who did **(Table 2).**

Table 2: Association between specific food groups consumption with adverse pregnancy outcomes in Central Arsi, rural Ethiopia (n = 374)†.

Food groups consumed	Number (%)	Adverse Pregnancy Outcome (APO)	
		COR (95% CI)	AOR (95% CI)
Starchy staples	374 (100)	-	-
Yes	0 (0)	-	-
No			
Meat & fish			
Yes	17 (4.5)	1	1
No	357 (95.5)	1.89 (0.42, 8.47)	0.50 (0.07, 3.84)
Organ meat			
Yes	23 (6.1)	1	1
No	351 (93.9)	2.71 (0.62, 11.82)	1.21 (0.20, 7.27)
Dark green leafy vegetables			
Yes	146 (39)	1	1
No	228 (61)	2.31 (1.29, 4.31)*	**2.01 (1.04 , 3.87)***
Vitamin A rich foods			
Yes	103 (27.5)	1	1
No	271 (72.5)	2.01 (1.05, 3.84)*	1.23 (0.57, 2.65)
Other fruits & vegetables			
Yes	83 (22.2)	1	1
No	291 (77.8)	2.75 (1.26, 5.99)*	1.48 (0.57, 3.84)
Legumes			
Yes	333 (89)	1	1
No	41 (11)	1.16 (0.53, 2.55)	1.39 (0.56, 3.49)
Eggs			
Yes	71 (19)	1	1
No	303 (81)	1.88 (0.89, 3.99)	0.59 (0.21, 1.66)
Milk & Products			
Yes	113 (30.2)	1	1
No	261 (69.8)	3.35 (1.65, 6.79)*	**2.64 (1.11, 6.30)***
Animal Source foods (ASF)¥			
Yes	141 (37.7)	1	1
No	233 (62.3)	2.85 (1.54, 5.25)*	1.46 (0.69, 3.06)
All types of fruits and vegetables.			
Yes	212 (56.7)	1	1
No	162 (43.3)	3.24 (1.89 , 5.54)*	**2.92 (1.49, 5.67)** *

†Logistic regression adjusted for baseline MUAC and hemoglobin concentrations; APO, adverse pregnancy outcomes. ¥ all ASF (meat, dairy, eggs, and fish) are included

Table 3 Association between anthropometric measures and hemoglobin concentration with adverse pregnancy outcomes in central Arsi, rural Ethiopia

Nutritional Characteristics	**Number (%)**	**Adverse Pregnancy Outcome (APO)**	
		COR(95% CI)	AOR (95% CI)
Weight gained (kg)			
< 6	33 (8.8)	1	1
6 - 9	155 (41.4)	0.98 (0.41, 2.37)	1.23 (0.46, 3.32)
9.1 - 12.5	147 (39.3)	1.64 (0.66, 4.07)	1.78 (0.64, 4.99)
> 12.5	39 (10.4)	2.80 (0.76, 10.37)	2.60 (0.63, 10.78)
MUAC (cm)			
<21	90 (24.1)	1	1
21 - 23	171 (45.7)	0.31 (0.15, 0.64)	1.39 (0.72, 2.70)
23+	113 (30.2)	0.61 (0.31 , 1.21)	1.72 (0.76, 3.86)
Hemoglobin (g/dl) - at term			
<11	121 (32.4)	1	1
≥ 11	253 (67.6)	0.17 (0.10, 0.30)*	**0.23 (0.11, 0.45)***
		0.17 (0.10, 0.30)	**4.56 (2.25, 9.25)† ***

†logistic regression adjusting for baseline hemoglobin concentrations

Among the various composite food groups formed, those pregnant mothers who were totally deprived or inconsistently consuming at least one of the animal source foods and/or fruits and vegetables during pregnancy, had more than a two (AOR, 2.29; 95% CI : 1.11 - 4.73) to three Considering the association between maternal nutrition characteristics and risk of APO, it was identified that only maternal anemia status, both during the second trimester as well as at term were associated with higher risk of APO. Nevertheless, only maternal anemia status at term remained in the final model independently predicting the risk of APO. Thus, pregnant mothers whose altitude adjusted hemoglobin level werehigher than 11 mg/dl (non-anemic) at term had 77% less risk (AOR, 0.23; 95% CI: 0.11; 0.45) of experiencing APO compared to those who were anemic (altitude adjusted hemoglobin level < 11 mg/dl) **(Table 3).**

Discussion

In this prospective cohort study of pregnant women in rural Ethiopia, we found that both dietary and nutritional characteristics of women during pregnancy were associated with risk of adverse pregnancy outcomes, resulting in increased or lowered risks. The study enrolled pregnant mothers during their first antenatal care visit and followed them until delivery. Poor or inconsistent consumption of animal source foods (particularly dairy) and fruits and vegetables (dark green leafy vegetables) were found to be independently associated with APO.

To our knowledge, this is the first study that assessed the associations of the consumption of specific food groups with the risk of adverse pregnancy outcomes in resource poor settings by employing a prospective cohort study. Few of the available studies in LMIC were mostly cross-sectional (26–28). Given the dynamic nature of pregnancy and interdependence of adverse perinatal outcomes(2,4,29), available studies are ill-equipped to give the best picture of the problem. Our study comprehensively looked to the threemain adverse outcomes risksand employed a more rigorous study design.

The other strength of this study was that we investigated risk of adverse pregnancy outcomes in the most prone as well asaffected rural resource limited developing population, whilemany others focusing on urban and developed populations which have better access to information and health care services(30,31). The study also yielded a better and balanced attrition rates between study arms, exposed and unexposed, compared to recommendations and truth of other similar prospective follow-up studies(32,33).

In Ethiopia, there are few community based studies (16,17,34–36) presenting the prevalence of adverse pregnancy outcomes, mainly focusing on low birth weight. Data on preterm birth and still birth is hardly available. In this study, we found that 13.6%, 9.1% and 4.5% of mothers gave birth to preterm, low birth weight and still birth babies, respectively. Even if comparable to the findings of some local (15,16,37) and sub-Saharan African or Asian(10,15,16,38–40) estimates, all are either significantly below(17,35,36,41) or above (34)to ample of cross-sectional local estimates. None of these incidence levels were closer tomillennium development goals MDG and global targets(10,42,43).

Multivariate analysis of our results suggest that eitherpoor (complete deprivation) or inconsistent consumption of dark green leafy vegetables and dairy products during pregnancy were important predictors of APO in the study population, which is relatively homogenousin terms of ethnicityand other socio-cultural characteristics. Analysis of composite food groups has also showed cognizant findings, whereby mothers who did not or seldom consumed animal source foods and fruits and vegetables, particularly dark green leafy vegetables,had nearlytwo to four fold increased risks of APO, compared to those who regularly consumed either during pregnancy.

The finding that consumption of animal source foods, particularly dairy as important predictor of lowered risk of APO is not surprising. It is rather consistent with several other studies conducted in the developed world.Previous studies has documented that low intake of milk during pregnancy was associated with decreased birth weight and infant size at birth and vice versa (44–47). The studies has also proposed the role of cow milk, particularlyprotein fraction of milk than the fat or carbohydrate fraction, mediating acceleratedmaternal weight gain and increasing placental weight, particularly when consumed during the last trimester of pregnancy(44,48). A milk-induced increase in placental weight not only raises the transfer of nutrient to the fetus, but also increases the amount of placenta-derived growth hormones that impair maternal insulin sensitivity, thereby enhancing maternal blood glucose levels leading to fetal overgrowth and increased birth weight (44,49). In the same way, many of the studies in the Western world have revealed that maternal cow's milk consumption was not associated with the risk of preterm birth or with the risk of a small or large size for gestational age at birth in the offspring (50,51)

Our finding that consumption of fruits and vegetables, mainly dark green leafy vegetablesas another important predictor of lowered risk of adverse pregnancy outcomes is still a debatable issue. Many of the existing evidences attributes this with the fact that fruits and vegetables are rich sources of severalessentialmacro and micronutrients. Fruits and vegetables are known to be the main sources of potassium, magnesium, dietary fiber, folate, and vitamins A and C, and antioxidants, bioactive compounds, or phytochemicals. These are in turn strongly linked to better outcomes of pregnancy(51–56). Secondly, some micronutrients contained in fruits and vegetables may also contribute to optimal immune and placental functions, which are important for fetal growth(57,58).

On the other hand, a recent systematic reviews of eleven studies showed that there is limited and inconclusive evidence of a protective effect of increased consumption of vegetables and risk for small for gestational age birth, or increased birth weight among women from both developed and less developed countries. The synthesized evidence slightly support maternal consumption of a variety of fruits and vegetables as part of a balanced diet throughout pregnancy, not linked to better perinatal outcomes(51).

The present study had several limitations that need to be taken into consideration when interpreting the findings. Although it is recognized that both pre- and early-pregnancy nutrition are associated with pregnancy outcomes, we were only able to follow the women starting from their second trimester, mainly because of the late start of ANC visits.

Although efforts were made to control for baseline differences when calculating the relative risks of adverse pregnancy outcomes, the effect of some factors cannot be completely controlled.

The other limitation is related with socio-demographic characteristics of our study population. A larger proportion of the population was relatively better educated and comprised of younger women; above two out of five (42.5%) have completed primary education, as compared with 2.2% % in rural Ethiopia and 181 (45.4%)of them were youth (< 25 years). This difference may have largely been due to restriction of the study population to those who visited health facilities. Selective participation may thereby have led to biased estimates. However, it has been shown that selection bias in cohort studies primarily arises from loss to follow-up rather than to non-response at baseline(59). We prospectively collected detailed information on milk and dairy consumption, which enabled separate analyses.

The overall incidence of APO: low birth weight, preterm birth and still birth is comparable to national and regional estimates, but still above expected targets and global estimates of other developed regions. Some dietary and nutritional characteristics during pregnancy were also found to be significantly associated with lowered or heightened risks of APO. More specifically, consistent and adequate intake of animal source foods, milk and milk products (dairy), fruits and vegetables (dark green leafy vegetables) were independently associated with APO.

Therefore, we recommended further studies with larger sample size,randomized clinical trials and meta-analysis pulling the strength of association between these key factors for better policy and programming. We also recommended that policy makers, planners and concerned bodies give attention to maternal dietary and micronutrient status, emphasizing consistent and adequate consumption of animal source foods, dairy products, fruits and vegetables in their planning and programming. Special attention should also be given to the overall nutritional status of the women during pregnancy with a focus on treating anemia at later stages of pregnancy maintaining adequate weight gain to end the unacceptably high level of adverse pregnancy outcomes in these settings.

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4.3.3. Complementary results: Food groups associated with lower risk of anemia during third trimester (term) of pregnancy.

Complementary data on Food groups associated with lower risk of anemia

Among the final cohort of pregnant mothers remained in the study, majority of the mothers (267 (71.4%) at enrollment and 253 (67.9%) at end line) were non-anemic. The remaining 121 (32.4%) were anemic; whereby majority 99 (81.8%) had moderate anemia followed by mild 12(9.9%), and severe 10 (8.3%) forms **(Table 1).**

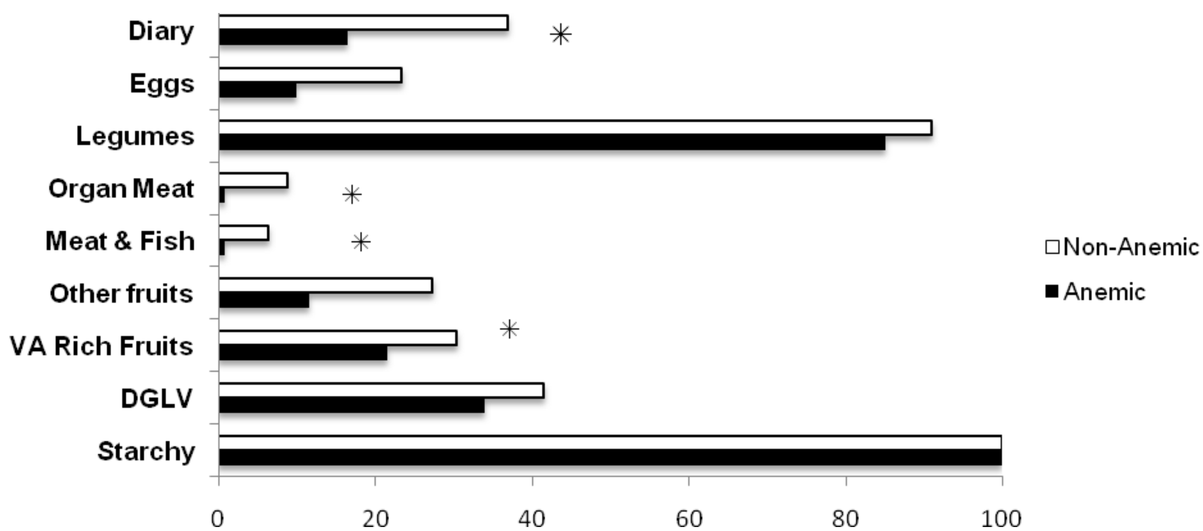

Figure 1 : Maternal dietary diversity groups vis-à-vis anemia status during pregnancy in rural Ethiopia, 2015

Table 1. Selected socio-demographic & nutritional characteristics of pregnant mothers in rural Arsi, Ethiopia; stratified by level of anemia at term (n = 374).

Socio-demographic & nutritional characteristics.	**Anemic Mothers** (#/%)					**All mothers**
	Mild (n = 57)	Moderate (n = 99)	Severe (n = 10)	All anemic (n = 121)	Non - Anemic (n =)	(n = 374)
Maternal age (Years)						
• < 20	4 (4.8)	15 (18.3)	3 (3.6)	22 (26.5)	61 (73.5)	34 (9.1)
• 20 - 24	0 (0)	27 (27.6)	0 (0)	27 (27.6)	71 (72.4)	147 (39.3)
• 25 - 29	7 (5.4)	36 (27.6)	7 (5.4)	**50 (38.5)**	80 (61.5)	130 (34.8)
• ≥ 30	1 (1)	21 (33.3)	0 (0)	22 (34.9)	41 (65.1)	63 (16.8)
Educational Status						
• No formal Educ.	7 (5.8)	30 (25)	3 (25)	40 (33.3)	80 (66.7)	120 (32.1)
• Primary Educ.	4 (4.2)	27 (28.4)	7 (7.4)	**38 (40)**	57 (60)	95 (25.4)
• Above primary	1 (0.6)	42 (26.4)	0 (0)	43 (27)	116 (73)	**159 (42.5)**
Hgb (baseline)						
• Anemic	0 (0)	7 (66.4)	10 (9.3)	81 (75.7)	26 (24.3)	107 (28.6)
• Non-anemic	12 (4.5)	28 (10.5)	0 (0)	40 (15)	227 (8.5)	***267 (71.4)***
Hgb (Term)						
• Anemic	12 (9.9)	99 (81.8)	10 (8.3)	121 (32.4)	0 (0)	121 (32.1)
• Non-anemic	0 (0)	0 (0)	0 (0)	0 (0)	253 (67.9)	***253 (67.9)***
MUAC (Cm)						
• SAM (< 21 cm)	1 (1.2)	30 (33.3)	10 (11.2)	**41 (45.6)**	49 (54.4)	90 (24.1)
• MAM (21 - 23cm)	6 (3.5)	47 (27.5	0 (0)	53 (31)	57 (60)	**171 (45.7)**
• Well-nourished (≥ 23)	5 (4.9)	22 (19.5)	0 (0)	27 (23.9)	116 (73)	113 (30.2)
Total	12 (3.2)	99 (26.5)	10 (2.7)	121 (32.1)	253 (67.9)	374 (100)

Considering maternal characteristics, those in aged in the late twenties (25 -29 years), lower schooling (primary education) and sever acute malnutrition (MUAC <21cm) carried the larger proportion of anemia compared to others **(Table 1).**

Bivariate analysis of the association between Women Dietary Diversity Score (WDDS) and anemia at term, showed that consumption of food groups from animal sources (egg, organ meat, meat or fish and diary) and vitamin A rich plant based foods were significantly associated with anemia of pregnancy at term **(Figure 1).**

Table 2: **:** Logistic regression analysis of pregnant mothers' key food diversity groups & maternal anemia, rural Ethiopia (n = 374).

Collective or independent Food groups eaten	Number (%)	Maternal anemia COR (95% CI)	AoR (95% CI)
1. Consumed Animal Source foods (ASF)			
• Yes	141 (37.7)	1	1
• No	233 (62.3)	3.25 (1.96, 5.38)*	**2.36 (1.35, 4.14)***
2. Consumed meat & meat products			
• Yes	32 (8.6)	1	1
• No	242 (91.4)	16.75 (2.26, 124.27)*	7.70 (0.97, 61.03)
3. Consumed fruits and vegetables.			
• Yes	212 (56.7)	1	1
• No	162 (43.3)	1.60 (1.04, 2.48)*	0.74 (0.38, 1.40)
4. Consumed Fruits			
• Yes	83 (22.2)	1	1
• No	291 (77.8)	2.87 (1.54, 5.34)*	1.97 (0.99, 4.13)
5. Consumed legumes			
• Yes	333 (89.5)	1	1
• No	39 (10.5)	1.91 (0.98, 3.74)	1.32 (0.56, 3.11)
6. Dietary Diversity Adequacy			
• Adequate (WIDDS $\geq$4)	186 (49.7)	1	1
• Inadequate (WIDDS $<$ 4)	188 (50.3)	3.18 (2.01, 5.03)*	**2.22 (1.09, 4.52)***

Multivariate analysis has also showed that, poor or inconsistent consumption of animal source foods and/or adequately diversified foods were independently associated with anemia at term. Thus, pregnant mothers with poor consumption of at least one of the animal source food groups had more than a twofold (AOR, 2.36; 95% CI: 1.35 - 4.14) added risk of developing anemia at term compared those who consumed of the same. Similarly, those mothers who used to consume non-diversified diets (< 4 food groups) had a twofold (AOR, 2.22; 95% CI:1.09 - 4.52) added risk of being anemic at term compared to those who consistently consumed diversified (≥ 4) food groups **(Table 2).**

Analysis of nutrition sensitive household and maternal individual characteristics showed that ownership of information communication facilities (radio or phone) in the household, maternal educational status and hemoglobin values at enrollment (second trimester of pregnancy) were associated with risk of anemia at term. Thus, mothers from households lacking a radio and phone (telecom) facilities had nearly a two (AOR, 1.93; 95 % CI : 1.12 - 3.39) to three (AOR 3.14; 95% CI : 1.75 - 6.62) fold added risk of anemia, respectively.

In the same way, maternal educational status and hemoglobin level during the second trimester of pregnancy were important individual level risk factors of anemia during the second, not third, trimester of pregnancy. Mothers who completed primary school had a 53% (AOR 0.47 ; 95% CI : 0.25 - 0.88) lower risk of anemia during the second trimester of pregnancy compared to those with no formal education. Obviously, who were anemic during the second trimester of pregnancy had over 28.56 times (AOR 28.56 ; 95% CI : 14.33, 56.79) added risk of staying anemic at term compared to those who were non-anemic during this period of time **(Table 3).**

Table 3: **:** Logistic regression analysis of pregnant mothers' selected HH and individual nutrition sensitive characteristics & maternal anemia, rural Ethiopia

Maternal house hold and individual characteristics	Number (%)	**Maternal anemia** COR (95% CI)	AoR (95% CI)
1. Land size (Hectare)			
– No Land	133 (35.6)	1	1
– <1	136 (36.4)	0.57 (0.34 , 0.96)*	0.60 (0.33, 1.09)
– 1 - 2	70 (18.7)	0.66 (0.35, 1.23)	0.77 (0.38, 1.54)
– 2+	35 (9.4)	0.66 (0.29, 1.46)	1.02 (0.42, 2.47)
2. Presence of Radio in the HH			
– Yes	270 (72.6)	1	1
– No	102 (27.4)	1.48 (0.89, 2.46)	**1.93 (1.12, 3.39)***
3. Presence of mobile/cell phone in the HH			
– Yes	286 (76.5)	1	1
– No	88 (23.5)	2.55 (1.56, 4.17) *	**3.14 (1.75, 5.62)***
4.Presence of audiovisual (TV, DVD...etc)			
– Yes	119 (31.8)	1	1
– No	255 (68.2)	1.37 (0.85, 2.21)	1.01 (0.57, 1.80)
5. Maternal Educational status			
– No formal Education	120 (32.1)	1	1
– Primary	95 (25.4)	0.75 (0.43 , 1.31)	**0.47 (0.25, 0.88)***
– Primary+	159 (42.5)	1.35 (0.80, 2.26)	0.85 (0.46, 1.57)
6. MUAC (Cm)			
– < 21 (SAM)	90 (24.1)	1	1
– 21 - 23 (MAM)	171 (45.7)	1.86 (1.10, 3.15) *	1.52 (0.75 , 3.11)
– 23+ (Normal)	113 (30.2)	2.66 (1.46, 4.85) *	1.03 (0.43, 2.34)
7. Hemoglobin (g/dl) at baseline			
– <11 (anemic)	107 (28.6)	1	1
– 11+ (Non-Anemic)	267 (71.4)	17.68 (10.14, 30.80)	**28.56 (14.33, 56.79)***

4.5. Maternal dietary habits, perceptions and food taboos

4.4.1. Introduction

The previous studies have showed that maternal diets have poor nutrient contents and densities. This was also related to the consumption of less-diversified plant based foods. On the other hand, the incidence of adverse pregnancy outcomes as well as anemia during pregnancy was high, particularly among those mothers that has inadequate dietary diversity practices which potentially represent majority of the population in the study area.

In the present section (4.5), a community based cross-sectional qualitative study was conducted involving a range of key informants and community leaders. A total of 38 key informants and four focus groups discussions were held to explore maternal dietary habits and presence of any forms of food taboos that can potentially affect nutrition during pregnancy. The findings showed that there were misconceptions about weight gain during pregnancy and food taboos were widespread, particularly among older and illiterate rural communities. The study findings are presented in the form of a published article on BMC Journal of Health, Population and Nutrition.

4.4.2. Dietary habits, food taboos, and perceptions towards weight gain during pregnancy in Arsi, rural central Ethiopia: qualitative cross-sectional study

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RESEARCH ARTICLE Open Access

Dietary habits, food taboos, and perceptions towards weight gain during pregnancy in Arsi, rural central Ethiopia: a qualitative cross-sectional study

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Abstract

Background: The nutritional status of women before and during pregnancy can be determined by maternal knowledge, attitudes, and perceptions towards certain foods. The present study aimed to explore maternal dietary habits, food taboos, and cultural beliefs that can affect nutrition during pregnancy in rural Arsi, central Ethiopia.

Methods: A qualitative, cross-sectional study, involving 38 key informant in-depth interviews and eight focus group discussions, was conducted among purposefully selected pregnant women and their husbands, elderly people, community leaders, health workers, and agriculture office experts. Participants were selected purposefully from all the major agro-ecologic areas of the study site. Data was analyzed manually using the thematic framework analyses method.

Results: The pregnant women reported that they did not change the amount and type of foods consumed to take into account their increased nutritional need during pregnancy. The consumption of meat, fish, fruits, and some vegetables during pregnancy remained as low as the pre-pregnancy state, irrespective of the women's income and educational status. Although not practiced by all, a number of taboos related to the intake of certain food items and misconceptions that can adversely affect nutritional status during pregnancy were identified. The most common taboos were related to the consumption of green leafy vegetables, yogurt, cheese, sugar cane, and green pepper. However, the frequency and extent of the practice varied by maternal age, family composition, and literacy level. Older mothers, from rural villages, and those with no formal education were more likely to practice the taboos than younger and educated ones. Almost all of the participants disfavored weight gain during pregnancy in fear of obstetric complications associated with the delivery of a bigger infant.

Conclusions: Misconceptions about weight gain during pregnancy and food taboos were widespread, particularly among older and illiterate rural communities. Thus, future nutrition programs should promote diversification of both the agricultural production and consumption.

Keywords: Food taboo, Maternal nutrition, Dietary diversity, Pregnancy, Weight gain

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Background

Access to safe, adequate, and nutritious food is a basic human right that is essential for good health [1, 2]. Unfortunately, this basic right is denied in many low- and middle-income countries (LMIC) partly due to food insecurity, poverty, and inappropriate food distribution [3]. Consequently, children, women of reproductive age, pregnant, and lactating women are disproportionately affected by under nutrition and its associated adverse effects [4]. Low dietary intakes, inequitable intra-household food distribution, recurrent infections, and poor care in general are among the leading causes of under nutrition, but food taboos and misconceptions can also contribute significantly to the high levels of under nutrition [5, 6].

Food taboos refer to the restriction of specific foods as a result of social or religious customs. In many traditional societies, cultural norms and customs govern behaviors including during critical life stages like pregnancy [7]. Pregnancy is a particular period when physiological nutrient demands are substantially increased. To meet this increased nutrient requirement for both the woman and the fetus, a pregnant woman is supposed to increase the amount and quality of foods she consumes [8, 9]. Nevertheless, when misconceptions or food taboos exist, the pregnant woman's ability to meet such increased demands can even be more compromised, hence putting the woman at a greater risk of adverse pregnancy outcomes [10].

Various forms of taboos, misconceptions, and cultural beliefs towards certain foods exist in various countries [11]. For example, foods consumed cold like fruits and vegetables were reported to be taboo among nursing mothers in Mexico [12]. Similarly, snails and grass-cutter meat are taboo among pregnant women and eggs among children in South Eastern Nigeria [13]. To what extent such food taboos and misconceptions exist and how they affect pregnancy outcomes in Ethiopia remain largely unknown.

This is unfortunate, since such information could be considered in the design of nutrition interventions targeting pregnant women. Besides, studies that involve a number of actors that can influence food consumption like health workers, the elderly, and agricultural extension workers, as well as husbands of pregnant women are rare, if not non-existent. Therefore, the present study used focus group discussions (FGDs) with pregnant women and their husbands, as well as key informant interviews (KIIs) with various health, agriculture, and community leaders to explore maternal dietary habits, food taboos, and misconceptions that can affect nutrition during pregnancy in rural Ethiopia.

Methods

Study site

The study was conducted in 12 rural villages selected from four districts of Arsi Zone, central part of the Oromia Regional State, Ethiopia. The Zonal capital, Assela, is located at 175 km from Addis Ababa and has a total area of 210,082 km^2 [14]. The study sites (12 villages) were randomly selected from four rural districts that were selected purposefully.

Study participants and design

The study employed a cross-sectional qualitative study design, mainly KIIs and FGDs. Since food taboos and dietary habits are sensitive issues, they are best explored through qualitative methods like FGDs and KIIs rather than quantitative methods. Both FGDs and KIIs were used to triangulate individual and group-level opinions. Data were collected between October, 2014 and May, 2015.

We have conducted a total of 38 KIIs and eight FGDs ($n = 6–10$) with subjects selected purposively from rural villages. The FGDs were held with pregnant women and their husbands, separately. The reason for conducting the FGDs with male and female separately was to facilitate the expression of opinions without fear of being judged by their respective partners. Individual KIIs were conducted with health workers, agricultural experts, and the elderly (grandmothers and fathers). The KIIs were held with health workers because they are mainly responsible for the delivery of nutrition education messages to the community; agricultural experts because they can provide information related to the production and availability of certain foods; and the elderly to investigate whether or not a shift away from food-taboo-related practices is present among younger pregnant women. The KIIs were conducted in an interactive manner, whereby the study participants were encouraged to take an active role in establishing the flow of the interview. FGDs and KIIs were held at the nearby health posts or health centers.

Open-ended questions were used to collect relevant information. Questions with redundant responses were considered to be saturated and were removed every evening after transcribing the day's work and preliminary analysis. New questions were added whenever an information gap was identified. These included questions about knowledge, experience, and opinion about food consumption during pregnancy. Data were collected by two of the research team members and interviews were tape-recorded.

Data analyses

Data were analyzed manually by applying the thematic framework analysis method [15]. Preliminary manual analysis was an inherent part of the data collection. As more data emerged from everyday encounters, the meaning of certain ideas and concepts were grouped into previously identified categories.

The lead author coded all the transcripts, and all the authors independently read the material and contributed by negotiating the final categories and their contents. Each audiotape interview was professionally transcribed word by word in *Afan Oromo* (local language) and then translated to English.

Ethics

Ethical clearance was obtained from the Ethics committees of the Addis Ababa University, College of Natural Sciences and the Oromia regional health bureau. Written consent was obtained from each respondent prior to data collection. Privacy and confidentiality were maintained throughout the study.

Results

We have conducted a total of 38 KIIs and eight FGDs in four districts of Arsi Zone, Oromia region, Ethiopia. Twenty three of the key informants were elderly (grandparents) and six were community leaders, while the remaining nine were health professionals. The participants represented a wide age range (23–92 years). The individual KIIs took 30 min on average, whereas the FGDs with the pregnant women and the husband, each took about an hour and half (Table 1). The findings of the FGDs are summarized as follows.

Table 1 Socio-demographic and basic data of qualitative study participants, rural Arsi Ethiopia, 2015

Socio-demographic characteristic	KII (*n* = 38) *N* (%)	FGD (*n* = 59) *N* (%)
Age		
• 23–30	6 (15.8)	*18 (30.5)*
• 31–40	2 (5.3)	17 (28.8)
• 41–50	2 (5.3)	15 (25.4)
• 51–60	1 (2.6)	7 (11.9)
• 60+	*27 (71)*	2 (3.4)
Occupation/role in the community		
• Health worker	9 (23.7)	
• Grand parent	*23 (60.5)*	
• Community leader	6 (15.8)	
Role in the family		
• Wife		*33 (55.9)*
• Husband		26 (44.1)
Sex		
• Male	*21 (55.3)*	*33 (55.9)*
• Female	17 (44.7)	26 (44.1)
Total	38 (100)	59 (100)

Footnote: The maximum frequency (%) under a given category of socio-demographic characteristic is italised for emphasis

Dietary habits

Food types consumed

The diet of the pregnant women was predominantly composed of cereals and legumes that were consumed in the form of *injera* (fermented pancake-like flat bread) and legume-based stews like *shiro*. The food consumption of the pregnant women, like that of the rest of the family, was mainly determined by seasonal variations and the households' agricultural production.

> …we (pregnant women) eat what we get at home,… we mainly produce maize, barley and beans. We eat injera and shiro (local sauce prepared from beans/peas)….., but during 'belg'(short-rainy season) and 'kiremt' (main rainy season), we also harvest and consume potato, cabbage and other vegetables…
>
> Pregnant women, 35 years, FGD participant.

The consumption of fruits and vegetables was reported to be rare. The participants reported that they produce little fruits and vegetables. This in turn limited the availability of fruits and vegetables in the market, further restraining the diets' diversity.

> … my wife is pregnant and I know that she needs to eat a variety of foods including fruits and vegetables … but in our village, we do not produce fruits and thus do not consume them…;if we want to consume fruits, we need to buy them from the district town or the zonal capital, Assela…
>
> Husband of a pregnant woman, 42 years, FGD participant from the highlands.

Nevertheless, some pregnant mothers from the lowland and warm agro-climatic areas reported to have little, and mostly seasonal productions of fruits like papaya, banana, and mangoes. However, these are often destined for the market and not for household consumption.

Agricultural production seems to be a key determinant of consumption and is dependent on seasonal variations and agro-ecologic conditions, which are partly governed by altitude differences among the study sites.

> …agricultural production and crops grown in the zone depend on weather conditions and the land settings. Lowland and midland settlers, mainly produce teff, maize, sorghum, and to some extent wheat; whereas highlanders produce barley, wheat and sometimes oats … consumption is largely determined by what is produced.... the exception is that lowlanders produce teff and sell most of it as a cash crop instead of consuming it….

Twenty-eight years, male, agricultural office expert.

Domestic animals, including cows, oxen, sheep, and chicken were owned by most households. A considerable number of households had access to milk and milk products, but have reported that they do not often consume them. The consumption of meat, fish, and poultry, whether pregnant or not, was also rare and was limited to annual religious and new year festivities or special occasions like weddings.

…though livestock rearing is common here, it is rare to eat meat; we do not slaughter animals for household consumption unless the animal is severely ill. On holidays, however, everyone slaughters at least a chicken…

Grandmother, 78 years, claimed to have lived >40 years in the study area.

Quantity of food consumed

Although pregnant women are expected to increase the amount of food they consume, they have reported to have reduced their food intake on various occasions. This was mainly ascribed to pregnancy-related discomforts like nausea, vomiting, and morning sickness. Some have also complained of poor appetite and reduced interest for food as a result of gastric irritation and dyspepsia.

… you are asking us to know if we have increased the amount of food we consume per meal during pregnancy,….yet, in many cases, we rather have poor appetite,… we take less food than usual as most of us get sick during pregnancy…

Pregnant woman, 28 years, FGD participant, speaking loud and the remaining participants nodding their heads in favor.

The action taken to cope with the reported gastric irritation was either to avoid specific food groups believed to aggravate their conditions or to decrease/modify their consumption. Among the foods mentioned in this regard are *injera* and hot stews that contain pepper or spice as ingredients.

… we usually eat a less acidic form of injera prepared by reducing the number of days of fermentation (locally known as 'aflegna'). We also avoid stews containing pepper to avoid gastric irritation …. Some mothers would even skip certain meals to escape the digestive discomforts …

Pregnant women, 31 years, FGD participant.

Such side effects, along with the lack of constant supply of the supplements, could be the reason behind the low compliance and adherence to the IFA supplementation during pregnancy.

…. The nurse at the nearby health center gave me 90 tablets in three rounds, but I couldn't take more than ten… I suffered from gastric irritation…. I have told the problem to the nurse, but she told me to take it after eating food with ample of water; I didn't get better…. I thus discontinued taking the pills…. I believe that the tablets would have helped me, but what should I do to the gastric irritation…

Pregnant women, 35 years, diagnosed with anemia.

Food taboos and perceptions related to weight gain during pregnancy

Divergent opinions were noted regarding the practice of food taboos. Some health workers and key informants believed that food taboos are becoming an old story.

….. Except for few illiterate women that live in remote rural areas, I do not think that many still believe that some foods need to be avoided during pregnancy…

Nurse, female, 34 years, born and working in the study area.

In contrast, a considerable proportion of the elderly and some pregnant women and their husbands still believed that some foods should be avoided during pregnancy.

… pregnant women should be careful and avoid certain foods, particularly towards the last trimester. Our community strongly believes that what a woman eats after her eight months of pregnancy goes directly to the womb to feed the baby. Thus, some foods can hurt the fetus…

Pregnant women, 34 years, FGD participant.

Among foods considered to be taboo are leafy vegetables like cabbage. Some study participants mentioned that in their culture, if a pregnant woman eats leafy vegetables, especially after 8 months of gestation, the leaf passes to the womb and attaches to the baby's head and form what they called "particles". These "particles" are considered harmful to the child and are even considered to cause immediate death to the newborn. Similarly, the consumption of dairy products like milk, yogurt, and cheese during pregnancy is considered harmful to the fetus.

….we old people believe that pregnant women should avoid consuming dairy products like yoghurt and cheese, particularly as the gestational age advances. This is because dairy products can pass to the womb and attach to the baby's head… I have seen this happen with my naked eye: a baby born full of milk products on the head… full of cream and cheesy substances… I have witnessed these babies dying immediately after delivery…

Husband of pregnant women, 74 years, KII respondent.

Similarly, the discussants considered foods like fruits, sugarcane, and some types of vegetables as taboo. The consumption of these foods was also perceived to be associated with having bigger babies, which is believed to lead to a difficult delivery.

… I doubt the effect, but our community strongly believe that if a pregnant woman eats sugar cane, fruits, and some other vegetables, she may have a big baby which endangers her life by making labor difficult…

A focus group discussant, pregnant woman.

Almost all of the pregnant women, their husbands, and the elderly involved in the KIIs and FGDs disfavored weight gain during pregnancy in fear of having big babies that can complicate delivery. They have said that delivering a bigger baby can be life-threatening for both the mother and the newborn.

….I know that no one dislikes to have a big and handsome baby; we in fact feed our babies to make them grow big during early childhood… but in the womb, it is very risky … we thus decrease our food intake to limit the size of the fetus and facilitate its delivery….

Pregnant women, 29 years, FGD participant

Discussion

The present KIIs and FGDs conducted in four rural districts of Arsi Zone have identified that little change occurs in the diets of the women as they transit from pre-pregnancy to pregnancy. The diets remain predominantly plant based, with little or no consumption of animal-source foods, fruits, and vegetables. The limited production and market availability, the seasonality of production, and the several taboos identified limit the consumption of fruits and vegetables during pregnancy. In addition to the limited dietary diversity, the amount of food consumed are often reduced due to gastrointestinal irritations or intentional food restrictions from fear of a possible obstructed labor associated with the delivery of a bigger baby.

Pregnancy is a critical window of opportunity when the fate of the fetus starts to be decided [16]. Organs are being formed, and the fetus grows at an extremely rapid rate [17]. This all leads to increased nutrient needs that require the pregnant women to increase both the diversity and the amount of foods consumed [8]. Contrarily, pregnant women in the study area reported that their dietary diversity (quality) during pregnancy remained similar or lower than the pre-pregnancy state. This is consistent with an earlier report from a study conducted in West Arsi, Ethiopia [18], and can partly explain the highly prevailing malnutrition observed during pregnancy [19–22]. Such undiversified diets have been associated with adverse pregnancy outcomes including anemia, low-birth weight, and preterm deliveries [23].

Indeed, the inadequate intake of various nutrients, including those of iron, folate, etc. has been closely linked to adverse pregnancy outcomes [24]. Consumption of animal source foods (ASFs), along with fruits and vegetables, can improve the intake of critical micronutrients. However, like in most rural settings in low-income countries, the consumption of ASFs, fruits, and vegetables was rare [25]. The low consumption of fruits and vegetables was partly attributed to the relatively low production and market accessibility of these produces. This is corroborated by the very low (<5 %) national fruits and vegetable production figures [26], which further highlights the need to make the agriculture sector and perhaps the overall food system more nutrition sensitive.

Increasing the production and accessibility of nutrient-dense foods alone would not be enough to improve the nutritional status of the pregnant women in light of the identified food taboos and misconceptions. Most of the identified food taboos, although their reasons are not scientifically supported, seem to have originated from the intention to prevent pregnancy complications. For example, a child may be born with meconium stain as a result of a prolonged and obstructed labor; the associated dark-green color and sometimes grey-whitish particle led to its association with the consumption of vegetables and dairy products during pregnancy, making dairy products and vegetables a taboo. Similar taboos have also been noted in a recent quantitative study conducted in West Arsi [18].

Despite the general consideration that optimal weight gain is indicative of a healthy pregnancy [27], this was disfavored in fear of complicated deliveries. This negative attitude towards weight gain during pregnancy could be related to the higher risk of obstetric complications that may be faced during the delivery of a bigger infant. A risk greatly increased by the short stature and the associated reduced pelvic capacity of the mothers [28]. However, such inadequate food intake compounded with the very low compliance to the routine IFA supplementation may lead to intrauterine growth restriction and low-birth weight [29].

In line with earlier findings from studies conducted in Ethiopia [18] and elsewhere [11–13], food taboos were found to be dominant among remote rural residents with little access to nutrition and health services, older, and uneducated women. In such circumstances, cultural believes, whether right or wrong, tend to shape behaviors. However, this finding also shades hope that such misconceptions and taboos may gradually be circumvented with increased access to education and health services.

Several limitations need to be considered when interpreting our findings. First, although the KIIs and FGDs were carefully conducted, we do not know to what extent women over-reported or underreported positive or negative behaviors. While the villages were randomly selected, they may not represent the 22 districts found in Arsi Zone. Besides, the sampling of key informants and focus group discussant was purposive. Although food taboos and food restrictions were identified as a problem in this community, their practice may depend on the age of study participants, their educational status, location of residence, etc. Thus, the findings should not be generalized to the whole study population and beyond, but should be taken as an indication that such practices are still present among some of the studied subjects.

Conclusion

The present study has shown that the dietary quality of the pregnant women remains as low as, or sometimes lower than the pre-pregnancy state, partly because of the limited access/availability of ASFs, fruits, and vegetables. This, along with the prevailing food taboos and misperception towards weight-gain during pregnancy could lead to adverse pregnancy outcomes. Interventions that diversify agricultural production to make fruits and vegetables more accessible, along with educational interventions emphasizing on the importance of adequate food intake during pregnancy, both in terms of quantity and quality, are needed. Such interventions may benefit from correcting the prevailing misconceptions, improving the health infrastructure in rural settings, and encouraging pregnant women to deliver their babies in a health facility, where help can be available in case of pregnancy complications.

Abbreviations

APHRC, African Population and Health Research Center; ASF, animal source foods; FGD, focus group discussions; IDRC, International Development Research Center; IFA, iron folic acid; KII, key informant in-depth interviews; LMIC, low- and middle-income countries; NGO, non-government organization

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Authors' contributions

All three authors designed the study and developed the analyses parameters. TAZ, MU, and KB secured financial support. TAZ conducted detailed analyses and wrote the first draft of the manuscript. All authors have read and approved the final version of the manuscript.

Competing interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Ethical clearance was obtained from the Ethics Committees of Addis Ababa University, College of Natural Sciences and the Oromia Regional Health Bureau. Consent was obtained from each individual respondent during data collection. Privacy and confidentiality were maintained throughout the study.

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5

Chapter 5 : General Discussion

General Discussion

The overall aim of the present thesis was to investigate whether or not dietary patterns adopted during pregnancy are related to maternal anemia and adverse pregnancy outcomes including LBW, preterm, and stillbirth. The dietary patterns of pregnant women was characterized, the most frequently consumed foods were identified, and their nutritional composition and nutrient-adequacy analyzed. Using a prospective cohort study, the risk of adverse pregnancy outcomes among pregnant women having inadequate relative to adequate dietary diversity scores were estimated. Specific food groups associated with a reduction in the risk of adverse pregnancy outcomes were identified. Using a focus-group discussion, in-depth interviews and passive observations, food taboos and misconceptions that have negative implications to diets adopted during pregnancies were identified.

5.1 Dietary patterns and nutrient-adequacy of frequently consumed foods during pregnancy

The first 1000 days from conception to the child's second birthday is considered as a critical window of opportunity for a child to thrive through adequate nutrition (UNICEF 2012; Burke et al. 2014). Nevertheless, the diet of the pregnant women was predominantly plant-based with little consumption of fruits, vegetables and ASFs ***(Manuscript II).*** Consequently, the most frequently consumed foods were found to be of low energy and nutrient density; hence, making it even more difficult for the pregnant women to fulfill the increased energy and nutrient requirements (Lee et al. 2013). Deficiencies in several micronutrients, and particularly in iron, are associated with maternal anemia and adverse pregnancy outcomes(Allen 2000). The low zinc and calcium density of the most frequently consumed foods suggests that deficiencies in these micronutrients are expected even if energy intakes would have been met. This suggests that the diet should be diversified with more nutrient-dense foods, should be fortified or supplements should be taken. Despite WHO's clear recommendation for IFA supplementation during pregnancy(WHO 2001), most of the pregnant women did not take supplements, and those who did have not taken for the recommended amount and duration. This highlights that alternative strategies that promote diversification of the diets are needed, and this is more evident in such settings where the health system is not efficient enough.

5.2 Dietary diversity and the risk of maternal anemia, LBW, preterm and stillbirth.

Dietary diversity (WDDS ≥ 4) was associated with reduced maternal anemia, LBW, preterm, but not stillbirth (Paper I). Several reasons can explain this finding. First, the nutrient profile of a diet is expected to improve with dietary diversification. This is in line with the finding of various studies conducted among infants, young children and women of reproductive age (Arimond & Wiesmann 2010; Daniels 2009), but no such reports were identified for pregnancy. Although not always consistent, there is a growing evidence on the impact of micronutrient supplementation in reducing adverse pregnancy outcomes (Zerfu & Ayele 2013 ***(Paper I);*** Bhutta et al. 2009). Second, pregnancy is a period of increased oxidative stress and inflammation, which can lead to adverse pregnancy outcomes (Al-Gubory et al. 2010; Poston et al. 2011; Gupta et al. 2007). Hence, a more diversified diet that includes fruits and vegetables is likely to provide more antioxidants that can help counter the damaging oxidative stress and inflammation (Grieger et al. 2013)(Jones et al. 2013). This suggests that perhaps specific food groups may have stronger associations to adverse pregnancy outcomes than a certain cut-off of a dietary diversity score.

Thus, we conducted another round of in-depth analysis to identify specific food groups having specific association with adverse pregnancy outcomes (***Manuscript II).*** This was meant to provide a clearer picture of the role of maternal nutrition during pregnancy on health and pregnancy status of women**.**

5.3 Consumption of specific food groups and the risk of adverse pregnancy outcomes

The consumption of fruits and vegetables, animal source foods in general and dairy products in particular were associated with reduced risk of adverse pregnancy outcomes (***Paper II).*** Pregnant women not consuming or consuming less animal source foods, dark green leafy vegetables and fruits had a two to three fold increased risk of giving birth to pre-term or low birth weight babies. This finding is consistent with the results of studies conducted in other developing countries that have found that the consumption of fruits and vegetables associated with increased birth weight (Murphy et al. 2014; Loy et al. 2011). Similarly, studies conducted mostly in developed countries have reported increased birth size, and hence a reduced risk of LBW and SGA (Heppe et al. 2011; Olsen et al. 2007). A few studies conducted in developing countries have also reported a lower risk of SGA and LBW with increased consumption of ASF (Grillenberger et al. 2006; E. et al. 2013; Muthayya 2009), which may not be surprising given that ASFs are good sources of bio-available minerals and vitamins (e.g. iron and folate) intrinsically related to healthy pregnancy outcomes (Ruel 2003; Grillenberger et al. 2006). This suggests that the promotion of the consumption of ASFs, dairy products, fruits and vegetables should be an integral part of nutrition interventions targeting pregnant women. Nevertheless, the success of such interventions largely depend on the perceptions, culture and food habits that underpin the food choices of the community and hence the pregnant women.

5.4 Food taboos and misconceptions adversely affect nutrition during pregnancy

Food taboos towards dairy products and green leafy vegetables were identified in the study area (***Manuscript III***). This is unfortunate given that the consumption of these food groups were found associated with a reduction in adverse pregnancy outcomes ***(Paper II).*** Further impeding the consumption of dairy and green leafy vegetables is the low production and thus low availability (Arsi Zone Administration Office 2010). This emphasizes the need to nutrition-sensitize the agriculture to diversity production by increasing production of fresh fruits and vegetables, while at the same time working on nutrition education to revert the existing food taboos. However, for such a nutrition education to be successful, understanding that the root causes of the misconceptions and taboos are grounded on the fear of obstructed labor is key. The short stature of the mother reduces their pelvic capacity, making the delivery of a healthier and normal weight baby often complicated (Dietz et al. 2003; Cheetham & Davies 2014). Consequently, pregnant women reduce their food intake to avoid weight gain ***(Paper III),*** which reduces energy and nutrient intakes (Manuscript II), in turn putting the women at risk of adverse pregnancy outcomes ***(Paper II).*** This is also compounded by economic distress that makes nutrient-dense foods like ASFs less accessible and affordable.

5.5 Strength and limitations of the studies

The study has a number of limitations as indicated in the various chapters. Briefly, the sample size used to estimate energy and nutrient intake of pregnant women (manuscript 1) is small. However, this has allowed to identify the staple foods consumed by pregnant women and although not generalisable, it has indicated that deficits in energy and nutrient intakes are likely. A composite sample was used to analyze the nutrient composition of the ready to eat staple foods. Although, this has helped reduced the cost of analyses it obscured potential intra-household variability in nutrient contents.

It is recognized that both pre- and early-pregnancy nutrition are associated with pregnancy outcomes, but our prospective cohort study was only able to follow women starting from their second trimester, mainly because of the late start of ANC visits ***(Paper II).*** Nevertheless, given the consistency of WDDS in the four monthly assessments conducted during the follow-up period, we expect that the women pre- and early pregnancy WDDS would have been similar. The use of a non-validated WDDS cut-off is a limitation that could have not been prevented given that our study was not adequately powered to test several thresholds of WDDS. We also did not impose a minimum amount restriction in counting food groups. However, our dichotomous categorization of dietary adequacy based on a cut-off point of four food groups was a good predictor of maternal anemia and the various adverse pregnancy outcomes, but needs further confirmation through population-based controlled trials. This was further elaborated by investigating food group specific associations with adverse pregnancy outcomes ***(Manuscript II)*** and maternal anemia. Nevertheless, the analyses stopped at the food group level and did not investigate possible heterogeneity within the same food group in influencing adverse pregnancy outcomes.

Notwithstanding the above limitations, the studies included in this dissertation had several strengths. The use of a mixed method approach helped generate findings that can guide public health interventions and advance current understandings on the role of maternal nutrition on perinatal outcomes in the context of a less-resourced setting. Although not exhaustive, the findings from the systematic review of global evidences on the role of micronutrients, the prospective cohort study relating dietary diversity and adverse pregnancy outcomes, the cross-sectional qualitative study that helped identify and explain dietary patterns, and the food composition analysis and dietary intake assessments that assessed the nutrient density of staples gives it a unique position that is unmatched in the current scanty information available on dietary diversity and pregnancy in Ethiopia and beyond.

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6

Chapter 6 :

Conclusions & Recommendations

Chapter Six : Conclusions and Recommendations

6.1. Conclusions

In the present thesis, the prevailing dietary pattern during pregnancy was characterized, the most frequently consumed foods were identified, sampled, and analyzed for their biochemical composition. The nutrient intakes and densities were also estimated. The association of dietary diversity, assessed using the WDDS, with maternal anemia and adverse pregnancy outcomes were investigated. The association between maternal anemia, adverse pregnancy outcomes and the consumption of specific food groups were also investigated. Using a qualitative study design, the potential barriers to adequate nutrition during pregnancy like food taboos and misconceptions were investigated.

The diets in the study area were predominantly plant-based, with very little consumption of ASFs, fruits, and vegetables. The main staple was *injera* and legume-based stews. The diet was low in zinc- and calcium-density (/100 kcal), but met the desired density for iron. Nevertheless, given the low energy intake of the pregnant women, the diet was unlikely to meet the estimated average requirements (EAR) for iron.

A dietary diversity score of ≥ 4 as assessed by the FAO's women dietary diversity score (WDDS), was associated with reduced maternal anemia, low-birth weight, and preterm deliveries. A closer look at these associations at the food group level suggests that dairy, fruits and vegetables were greatly associated with a reduction of adverse pregnancy outcomes. The consumption of ASFs in general was associated with a lower risk of maternal anemia and adverse pregnancy outcomes. This all suggests the need to diversify the diet through the promotion of the consumption of ASFs, dairy in particular, fruits and vegetables.

However, several misconceptions and taboos towards these foods were identified. This along with the fact that several pregnant women restrict the amount of food they eat in fear of having complications in delivering a bigger healthier baby may partly explains the high burden of adverse pregnancy outcomes in these settings.

6.2. Recommendations

Based on the findings of this thesis several recommendations can be made to improve the quality of diets of pregnant women and thereby reduce the risk of adverse pregnancy outcomes in rural Arsi, central Ethiopia, but also in other similar settings, nationally and internationally:

- Behavioral change communications that are culturally-adapted are needed to improving the intake of nutrient-dense foods as well as the compliance to supplements like IFA during pregnancy. An overall guideline that aims to improve the diets of pregnant women is needed.
- Behavioral change communications should aim to discourage the consumption of beverages like coffee and tea during pregnancy to not further compromise the bioavailability of minerals.
- Combating harmful traditional practices, taboos, dietary restrictions and misconceptions related to the intake of adequate and quality foods during pregnancy is urgently needed. To this end, role modeling, community conversations and other related activities could be used.
- While improving pregnant women's dietary pattern, preventing the feared obstructed labor resulting from the delivery of a bigger baby through the promotion of delivery in a health facility is needed. This would however require improvements in the efficiency of rural health facilities.
- Efforts to improve dietary diversity through the promotion of both the production and consumption of ASFs, fruits and vegetables are needed. To this end, a closer planning

and implementation of programs among nutrition, health and agriculture sectors is a necessity.

- Additional observational and ethnographic studies are needed to understand what governs food choices during pregnancy in order to help design effective food-based strategies
- Population based trials are needed to confirm the association of WDDS, maternal anemia and adverse pregnancy outcomes. In the meantime, current national and international programs on maternal, neonatal, infants and young children need to incorporate strategies to improve dietary diversity and not solely rely on IFA supplements.
- An overall national dietary guideline, including pregnant women, should be developed to help the implementation of food-based strategies in the country.

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Research Article

Reproductive and Obstetric Factors Are Key Predictors of Maternal Anemia during Pregnancy in Ethiopia: Evidence from Demographic and Health Survey (2011)

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Anemia is a major public health problem worldwide. In Ethiopia, a nationally representative and consistent evidence is lacking on the prevalence and determinants during pregnancy. We conducted an in-depth analysis of demographic and health survey for the year 2011 which is a representative data collected from all regions in Ethiopia. Considering maternal anemia as an outcome variable, predicting variables from sociodemographic, household, and reproductive/obstetric characteristics were identified for analyses. Logistic regression model was applied to identify predictors at $P < 0.05$. The prevalence of anemia among pregnant women was 23%. Maternal age, region, pregnancy trimester, number of under five children, previous history of abortion (termination of pregnancy), breastfeeding practices, and number of antenatal care visits were key independent predictors of anemia during pregnancy. In conclusion, the level of anemia during pregnancy is a moderate public health problem in Ethiopia. Yet, special preventive measures should be undertaken for pregnant women who are older in age and having too many under five children and previous history of abortion. Further evidence is expected to be generated concerning why pregnant mothers from the eastern part of the country and those with better access to radio disproportionately develop anemia more than their counterparts.

1. Introduction

Maternal death continues to be a major health and development concern globally, particularly in the developing world [1, 2]. In 2013 alone, there were an estimated 289,000 maternal deaths (210 deaths per 100,000 live births) across the globe, of which the sub-Saharan Africa region accounting for 62% (179,000) of these. During the same period, the mortality rate in developing regions (230) was 14 times higher than in developed regions (16) whilst the sub-Saharan Africa recorded the highest (510) regional MMR [2]. Astonishingly, over the 99% of these annual deaths occurring in developing countries are avoidable, as the healthcare solutions to prevent or manage complications are well known.

Regarding the major causes of maternal death, about 73% of all deaths between 2003 and 2009 were due to direct obstetric causes. Haemorrhage (27.1%), hypertensive disorders (14.0%), sepsis (10.7%), abortion (7.9%), and embolism accounted for the majority of these direct causes. On the other hand, the indirect causes contributed to over 27.5% of the maternal deaths globally and 28.6% in sub-Saharan Africa [1].

Anemia is one of the leading indirect causes of maternal mortality and it is the most common and intractable nutritional problem globally [3]. Although easily preventable and treatable, it is one of the most serious threats to the health of children and a factor in maternal mortality. In pregnant women, anemia results in an increased risk of premature delivery and low birth weight. It is also known to be an important factor in maternal death, the poor cognitive development of children, and decreased work capacity of the mother. It also decreases the health and energy of approximately 500 million women and leads to approximately 50,000 deaths in childbirth each year [4].

The World Health Organization (WHO) defines anemia as hemoglobin concentrations that are below recommended thresholds [3, 5]. The main causes of anemia are dietary iron deficiency; infectious diseases such as malaria, hookworm infections, and schistosomiasis; deficiencies of other key micronutrients including folate, vitamin B12, and vitamin A; or inherited conditions that affect red blood cells (RBCs), such as thalassaemia [6].

The prevalence of anemia during pregnancy is quite high (42%) globally and above 57.1% in Africa, signifying it as a severe public health problem in the region [3]. In Ethiopia, even if the situation seems better, the latest EDHS estimated that above 22% of women during pregnancy were found anemic [7]. On the other hand, some cross-sectional localized studies conducted at various regions of the country demonstrated that the prevalence of anemia among women of the reproductive age group in general and pregnant women in particular ranged from as low as 16.6% in the north [8] to a modest (33.2%) level in the south [9] and high up to 43.9% [10] in the eastern parts of the country.

On top of variation in the prevalence rate, the very important aspect of these local studies stipulating further investigation is the fact that none of them presented with conclusive and consistent findings on the determinants of anemia during pregnancy. A number of dissimilar and mutually exclusive determinant factors were identified in the studies. For instance, the northern study heightened hookworm infestation and HIV infection [8], whereas daily chewing of khat, restrictive dietary behavior, parity levels, and pregnancy trimesters were identified by the eastern study. Another relatively representative nationwide study [11] conducted in nine regions of the country identified other factors like chronic illnesses and deficiency of iron/folic acid as key determinants of anemia during pregnancy [11].

Generally, even if these studies have given important clues to policy makers, programmers, and other stakeholders, they mainly lack consistency and representatives to be used for national level policy making and programming by concerned bodies. Therefore, these in-depth analyses of the latest (2011) EDHS provide in-depth and explicit results on the prevalence and key proximate determinants of maternal anemia during pregnancy in Ethiopia.

2. Methods

2.1. Data Source. This study uses data from the Ethiopian Demographic and Health Survey (EDHS) conducted in 2011. The survey was conducted with nationally representative samples from all of the country's regions. The details of the sample design, including the sampling framework and sample implementation, and response rates are provided in the respective EDHS reports (http://www.measuredhs.com/).

In the DHS, there are three core questionnaires (household, women's, and male questionnaires) and nine recode files. This way of recoding is done because of two outstanding reasons: to define a standardized file that would make cross-country analysis easier and to compare data with the World Fertility Surveys (WFS) to study trends [12]. The recode files have five main and two additional digits. The first two digits of the file name correspond to the country code (e.g., ET for Ethiopia). The next two digits identify the unit of analysis (IR—women, KR—children, etc.). The fourth digit identifies the DHS phase. The fifth digit identifies the data release number and the last two digits identify whether it is a rectangular (RT) or flat (FL) file; for the hierarchical file they are left blank.

In our current analyses, we used ETIR61FL.SAV recode data files for prevalence and analyses of determinant factors. This means that we used the 2011 IR (women with completed interviews) EDHS data to describe the prevalence and determinants of anemia during pregnancy in Ethiopia.

2.2. Study Variables. The dependent variable is maternal anemia during pregnancy. According to the WHO and International Nutritional Anemia Consultative Group (INACG) [5, 13] anemia during pregnancy, after adjusting for altitude, is defined as a hemoglobin concentration of 11.0 g/dL and hemoglobin levels of 10–10.9 g/dL, 7–9.9 g/dL, and less than 7.0 g/dL were considered as mild, moderate, and severe anemia, respectively. Other cutoff points of hemoglobin concentration specific to trimesters of pregnancy are suggested by the International Nutritional Anemia Consultative Group (INACG) and others.

The selection of potential predictors of maternal anemia during pregnancy in this study is based on the literature and the availability of variables from the EDHS data sets on these potential predictors. An attempt is also made to test all potentially relevant variables existing in the EDHS data sets before concluding dropping of them; therefore, all variables which showed a statistical significance or some sort of trend during bivariate analyses are included into the analyses.

These potential predicting variables are categorized into three groups: sociodemographic characteristics, household variables, and maternal reproductive characteristics.

Sociodemographic Variables. These groups of indicators consist of both maternal and paternal (husbands') characteristics. Among the maternal characteristics, maternal age, urban/rural residence, region, educational status, and literacy level are included.

Household Variables. In this group, we included presence or absence of key household variables like electricity, radio, and television. Other variables included into this category are type of toilet facility (whether the household uses improved or nonimproved type of toilet) and sources of drinking water.

Maternal Reproductive and Obstetric Variables. Attributable to the nature of the study and availability of evidence by a wide range of literatures, a number of variables are included into this category as compared to the other two. Accordingly, pregnancy trimester, number of births in the last five years and last year, whether the current pregnancy is wanted or not, history of abortion (pregnancy termination), practice of breastfeeding, timing of first ANC visit, and number of ANC visits are all included.

Table 1: Sociodemographic characteristics of pregnant mothers and their association with anemia during pregnancy in Ethiopia, EDHS 2011.

Sociodemographic characteristic	Anemic (#)/(%)	Nonanemic (#)/(%)	Crude OR (95% CI)	Adjusted OR (95% CI)
(1) Age				
15–19	5 (12.5)	35 (87.5)	1	1
20–24	45 (19.1)	190 (80.9)	1.64 (0.61, 4.38)	1.57 (0.57, 4.35)
25–29	110 (23.7)	355 (76.3)	2.13 (0.82, 5.53)	2.35 (0.87, 6.35)
30–34	67 (27.1)	180 (72.9)	2.56 (0.97, 6.75)	2.65 (0.97, 7.260)
35–39	29 (20.1)	110 (79.1)	1.79 (0.65, 4.96)	2.04 (0.71, 5.85)
40–44	12 (30)	28 (70)	3.07 (0.98, 9.64)	3.43 (1.04, 11.28)**
45–49	2 (25)	6 (75)	1.82 (0.24, 13.70)	1.36 (0.15, 11.89)
(2) Residence				
Urban	25 (26.9)	68 (73.1)	1	1
Rural	245 (22.7)	836 (77.3)	0.77 (0.48, 1.25)	0.80 (0.43, 1.48)
(3) Region				
Tigray	16 (21.6)	58 (78.4)	1	1
Afar	7 (38.9)	11 (61.1)	2.23 (0.74, 6.71)	2.09 (0.68, 4.40)
Amhara	49 (31.2)	108 (68.8)	1.64 (0.86, 3.14)	1.62 (0.84, 3.14)
Oromia	129 (23.5)	419 (76.5)	1.11 (0.62, 1.99)	1.04 (0.57, 1.89)
Somali	29 (51.8)	27 (48.2)	3.93 (1.84, 8.39)	3.82 (1.71, 8.52)**
Ben.-Gumuz	5 (27.8)	13 (72.2)	1.47 (0.47, 4.61)	1.32 (0.41, 4.25)
SNNPR	30 (10.6)	252 (89.4)	0.44 (0.22, 0.85)	0.41 (0.21, 0.82)**
Gambella	1 (33.3)	2 (66.7)	1.79 (0.13, 23.8)	1.83 (0.11, 29.43)
Hareri	1 (33.3)	2 (66.7)	2.10 (0.14, 30.85)	2.10 (0.13, 32.89)
Addis Ababa	0 (0)	10 (100)		
Dire Dawa	3 (60)	2 (40)	4.97 (0.73, 33.64)	4.51 (0.63, 32.28)
(4) Maternal educ. status				
No education	206 (25)	617 (75)	1	1
Primary educ.	60 (18.4)	266 (81.6)	0.67 (0.48, 0.92)	0.95 (0.60, 1.51)
Sec. education	0 (0)	11 (100)	0.08 (0.002, 2.97)	0.24 (0.00, 10.82)
Higher educ.	5 (33.3)	10 (67.7)	1.31 (0.43, 4.01)	3.00 (0.66, 13.68)
(5) Maternal literacy level				
Cannot read/write	226 (23.7)	727 (76.3)	1	1
Partially read and write	25 (23.4)	82 (76.6)	0.99 (0.62, 1.59)	1.12 (0.61, 2.04)
Fully read and write	13 (13)	87 (87)	0.49 (0.27, 0.90)	0.46 (0.19, 1.11)

**Significant at P value < 0.05, OR: odds ratio.

2.3. Data Analysis. This study employed a three-stage analysis. In the first stage, univariate and bivariate analyses of the level (prevalence) of maternal anemia during pregnancy by the three categories of variables mentioned were calculated using chi-square, ANOVA, and Student's t-test.

In the later stage, binary logistic regression analyses of each variable to determine the crude odds ratio of each identified variable with maternal anemia were calculated. After making an appropriate selection of variables having a strong association with the dependent variable, we moved to the third stage of analyses of multivariate logistic regression analyses of all variables found statistically significant. SPSS version 20 software was used to run all stages of the analyses.

2.4. Data Quality Assessment. The data quality assessment report highlights its findings on misreporting, omission, and digit preference, which are common data quality problems observed in surveys and censuses in developing countries. Over all, the assessment shows that the problems do not exist to the extent that might challenge the quality of the conclusions of this study.

3. Results

A total of 1,212 pregnant women were included into the initial analyses which is 10.2% of the total women of the reproductive age group from whom data was collected in the same survey. The overall prevalence of anemia in this group was found to be around 23%, varying from 12.4% milder forms to 9% and 1.6% moderate and severe forms, respectively (Figure 1 and Table 1).

In terms of maternal age, the lowest (12.5%) and the highest (30%) prevalence of anemia were seen among teenagers (15–19) and older (40–45) women, respectively. Women in the latter group had a 3.43 more added risk of developing anemia during pregnancy as compared to the reference age group

Table 2: Selected household characteristics of pregnant mothers and their association with anemia during pregnancy in Ethiopia, EDHS 2011.

Household characteristic	Anemic (#)/(%)	Nonanemic (#)/(%)	Crude OR (95% CI)	Adjusted OR (95% CI)
(1) Presence of electricity in the HH				
No	247 (23.7)	795 (76.3)	1	1
Yes	23 (19.7)	94 (80.3)	0.77 (0.47, 1.25)	0.73 (0.39, 1.38)
(2) Presence of radio in the HH				
No	154 (21)	578 (79)	1	1
Yes	116 (27.2)	311 (72.8)	1.39 (1.05, 1.84)	1.41 (1.01, 1.88)**
(3) Presence of television in the HH				
No	260 (23.4)	849 (76.6)	1	1
Yes	10 (20)	40 (80)	0.83 (0.41, 1.68)	0.96 (0.39, 2.37)
(4) Access to safe water source				
Safe water sources	97 (22.1)	342 (77.9)	1	1
Unsafe water sources	174 (23.6)	562 (76.4)	1.09 (0.89, 1.45)	1.09 (0.81, 1.48)
(5) Sanitation facility				
Improved sanitation	**19 (25.3)**	**561 (74.7)**	1	1
Nonimproved sanitation	**251 (22.8)**	**348 (77.2)**	0.85 (0.49, 1.45)	0.81 (0.45, 1.43)
Total	271 (23)	904 (77)		

**Significant at P value < 0.05, OR: odds ratio.

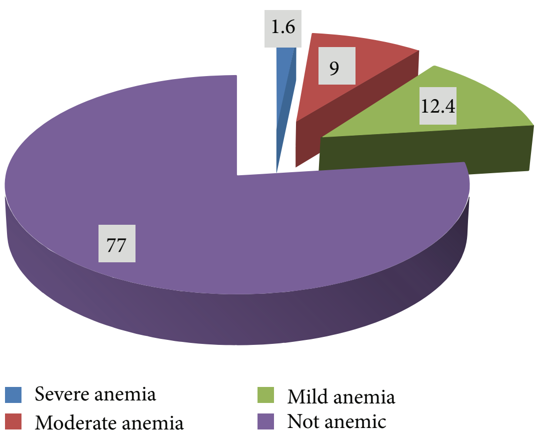

Figure 1: Prevalence and severity of anemia among pregnant mothers in Ethiopia, EDHS 2011.

(15–19) (AOR, 3.43; 95% CI, 1.04–11.28). Pregnant women aged (30–34) and (20–24) had the second highest (27.1%) and lowest (19.1%) prevalence of anemia, respectively ($P > 0.05$) (Table 1).

Pregnant women from urban setting had a nonsignificant but higher (26.9%) prevalence of anemia as compared to the rural counterpart (22.7%) (AOR, 0.80; 95% CI, 0.43–1.48). In the same way, the prevalence of anemia among pregnant women across different regions in the country ranged from as low as 0% in Addis Ababa followed by 10.6% and 21.6% in SNNPR and Tigray regions. It was also as high as 60%, 51.9%, and 38.9% in regions like Dire Dawa, Somali, and Afar, respectively (all of these regions are Muslim community dominated and are located in the eastern part of the country). The variation was significant for SNNPR (AOR, 0.41; 95% CI, 0.21–0.82) and Somali regions (AOR, 3.82; 95% CI, 1.71–8.52).

Considering the educational and literacy characteristics of mothers, even if a statistically significant difference was observed during the bivariate logistic regression analyses, none of these appeared in the final multivariate regression model. Yet, descriptive analysis shows that women with no formal education and those trained to tertiary level had a higher, 25% and 33.3%, prevalence of anemia during pregnancy, respectively. Literacy level showed a nonsignificant but a linear declining trend of anemia prevalence; that is, the higher the woman is literate, the lower is the risk of developing anemia during pregnancy ($P > 0.05$) (Table 1).

Table 2 shows that, among the household variables analyzed, all were found not to be associated with anemia of a pregnant women, but radio ownership. Pregnant women who reported lack of access to household variables like electricity, television, and safe water reported almost similar (23.4%–23.7%) prevalence of anemia which is lower than their counterparts. Contrarily to expectations and biological plausibility, however, pregnant women, who were supposed to have a lesser prevalence as they own/have access to radio and improved sanitation, reported a higher 27.2% (AOR, 1.41; 95% CI, 1.01–1.88) and 25.3% (AOR, 0.81; 95% CI, 0.45–1.43) prevalence of anemia than their counterparts, respectively (Table 2).

Regarding the obstetric factors analyzed, almost all of these showed a strong association (increased risk or protective benefit) with the outcome variable of interest, amongst which, pregnancy trimester, number of under five children, previous history of abortion (termination of pregnancy), practice of exclusive breastfeeding, and number of antenatal care visits showed a statistical significance association.

Pregnant women in their second and third trimesters of pregnancy had a 17-fold higher risk of developing anemia compared to those in the first and second trimesters (AOR, 17.05 and 17.71; 95% CI, 3.69–78.8 and 3.76–83.27),

Table 3: Selected obstetric characteristics of pregnant mothers and their association with anemia during pregnancy in Ethiopia, EDHS 2011.

Obstetric (RH) characteristics	Anemic (#)/(%)	Nonanemic (#)/(%)	Crude OR (95% CI)	Adjusted OR (95% CI)
(1) Pregnancy trimester				
First	26 (11.3)	205 (88.7)	1	1
Second	140 (27.8)	364 (72.2)	3.07 (1.95, 4.84)	17.05 (3.69, 78.8)**
Third	106 (24)	336 (76)	2.51 (1.58, 4.01)	17.71 (3.76, 83.27)**
(2) Number of under five children				
1	146 (25)	439 (75)	1	1
2	86 (18.5)	379 (81.5)	0.68 (0.50, 0.92)	0.39 (0.18, 0.84)**
3	39 (31.7)	84 (68.3)	1.40 (0.92, 2.14)	2.10 (0.52, 8.48)
(3) Ever had terminated pregnancy				
No	224 (22.6)	767 (77.4)	1	1
Yes	47 (25.5)	137 (74.5)	1.17 (0.81, 1.68)	2.63 (1.17, 5.92)**
(4) Pregnancy intention				
Wanted (then or later)	231 (22.7)	786 (77.3)	1	1
Unwanted	39 (26.5)	108 (73.5)	1.24 (0.84, 1.84)	2.39 (0.98, 5.86)
(5) Practice of breast feeding				
Ever breastfeeding	233 (23.1)	744 (76.9)	1	1
Never breastfeeding	13 (26)	37 (74)	1.15 (0.6, 2.22)	7.64 (2.28, 25.65)**
Continued breastfeeding	25 (21.9)	89 (78.1)	0.92 (0.57, 1.47)	4.05 (1.61, 10.91)**
(6) Timing of first ANC visit				
Timely (<4 months)	28 (20)	112 (80)	1	1
Delayed (4-5 months)	31 (24.8)	94 (75.2)	1.29 (0.72, 2.36)	0.85 (0.42, 1.69)
Delayed much (4–9 months)	13 (37.1)	22 (62.9)	2.26 (1.01, 5.05)	2.29 (0.82, 6.38)
(7) Number of ANC visits				
No (0) visits	130 (23.3)	429 (76.7)	1	1
Low (1-2) visits	22 (31.4)	48 (68.6)	1.53 (0.89, 2.62)	1.09 (0.50, 2.36)
Adequate (3-4) visits	34 (26.4)	95 (73.6)	1.18 (0.76, 1.83)	0.49 (0.19, 0.98)**
Frequent (4+) visits	16 (16.3)	82 (83.7)	0.64 (0.36, 1.13)	0.36 (0.08, 0.64)**
Total	271 (23)	904 (77)		

**Significant at P value < 0.05, OR: odds ratio.

respectively. In the same way, pregnant women who have two or more under five children (AOR, 0.39; 95% CI, 0.18–0.84) have previous history of pregnancy termination (AOR, 2.63; 95% CI, 1.17–5.92), who have never breastfed or continued breastfeeding to present pregnancy (AOR, 7.64 and 4.05; 95% CI, 2.28–25.65 and 1.61–10.91), and those who had adequate (3-4) and/or frequent antenatal care visits (AOR, 0.49 and 0.36; 95% CI, 0.19–0.98 and 0.08–0.64), respectively, had a statistically significant higher (increased) or lower risk of developing anemia during pregnancy (Table 3).

4. Discussion

This in-depth analysis of the latest (2011) Ethiopian demographic and health survey shows that the prevalence of anemia among pregnant women (23%) in the country is comparable to the nonpregnant women of reproductive age group (22%) and to the level of moderate public health problem. The prevalence is also lower than several other local cross-sectional studies [9, 14, 15] and previous EDHS reports [16, 17]. This shows a declining trend of anemia in the country. On the other hand, the value is far below regional and international estimates [18–20]. This shows the results of the current study should be interpreted cautiously as the data was collected for comprehensive survey of health and demographic events and the samples included in this study might not be good representative of pregnant mothers.

On the other hand, previous similar surveys [16, 17, 21], cross-sectional studies, and other reports [9, 14, 15] in the country have revealed almost similar trend. Studies of food analysis in the country have shown that, if not for bioavailability, most staple diets are rich in iron [22, 23] which might has contributed to lesser prevalence of anemia compared to the predictions and other countries of the sub-Saharan African region where the prevalence of anemia is the highest in the world.

In this study, older women were at a higher risk of anemia compared to teenagers (AOR, 3.43; 95% CI, 1.04–11.28) and the prevalence also increased with age ($P < 0.05$). This is consistent with previous local studies [8, 14]. On the other hand, pregnant mothers from some regions in the country had relatively higher prevalence of anemia during pregnancy and others quite very low. In this regard, pregnant mothers from Somali, Dire Dawa, and Afar regions have high level and those from Addis Ababa and SNNPR as well as Tigray region had the lowest level of prevalence. Those regions with high prevalence of anemia have something in common; they are located in the eastern part of the country and are dominated by Muslim community. Another study in the area [10] has estimated a similar prevalence and identified that khat chewing and restrictive dietary behaviors are the key factors associated with the unacceptably high level of anemia among pregnant mothers [10]. On the other hand, the very low prevalence level of anemia in Addis Ababa should be interpreted very cautiously as only ten cases of pregnant women were included into the analysis. This is of course contrary to previous studies in the area [11, 24].

Contrarily to scientific plausibility [20] and other positive effects of electronic communications, pregnant mothers from households owning radio have shown a statistically inverse association with the occurrence of anemia during pregnancy. This might be attributable to the high level of adult illiteracy at rural communities whereby little comprehension of radio messages or lack of adequate and tailored information about pregnancy and pregnancy related issues in the national broadcasting services exists. Generally, as this is a new and unprecedented condition, further studies on the association between the two are demanded.

Unlike sociodemographic and household variables that exhibited little or no significant association with maternal anemia during pregnancy, most of the obstetric variables studied have either increased or reduced the risk of developing anemia during pregnancy. In this regard, pregnancy trimester, number of under five children in the household, previous history of abortion, breast feeding practices, and frequency of antenatal care visits showed a significant effect on anemia during pregnancy.

Pregnant mothers in the second or third trimester had a sevenfold raised risk of anemia during pregnancy compared to during the first trimester. This is consistent with several other local [8, 10] and international [15] similar studies. It is important, however, to interpret the results as every pregnancy has three trimesters but could only be classified once with the other variables.

Having too many under five children or too frequent birth is among the key predictors of anemia in Ethiopia identified by the current analyses. This is also consistent with the findings of other studies [14, 25–27] that limiting birth or using family planning to control and space births is a key contributing factor to the prevention of anemia during pregnancy. At the same time, women who experienced abortion or terminated pregnancy before the index pregnancy were also found to have a 2.63 times higher risk of developing anemia than those who did not (AOR, 2.63; 95% CI, 1.17–5.92). This is consistent with several other studies [28, 29].

The other important aspect of the findings is the fact that breast feeding patters of mothers contributed much to the occurrence of anemia during subsequent pregnancies. Women who have never breastfed and continued breastfeeding to the index pregnancy were almost eight and four times more likely to experience anemia during recent pregnancies compared to those who have ever breastfed (AOR, 7.64 and 4.05; 95% CI, 2.28–25.65 and 1.61, 10.91). This is not explained by any study so far which prompts further study on these associations and requires careful interpretation, as the same holds true for the selection of samples is not directly related to the current analysis.

The other obstetric characteristic with protective finding to the occurrence of anemia during pregnancy is the frequency of antenatal care visits. Pregnant mothers who had at least 3-4 and above four visits had a 51% and 64% less chance of developing anemia during pregnancy than those who had less or none at all (AOR, 0.49; 95% CI, 0.19–0.98, and AOR, 0.36; 95% CI, 0.08–0.64), respectively. On the other hand, despite lesser (20% compared to 24.8% and 37.1%) proportion, pregnant mothers who timely (<4 months) started antenatal care visits were found to be nonanemic compared to those who started late (4-5 months) and too late (6–9 months); the occurrence was not statistically significant in the final logistic regression model (AOR, 0.85 and 2.29; 95% CI, 0.42–1.69 and 0.82–6.38). This is also consistent with several other studies conducted locally [11, 14, 30] or globally [31–33].

Limitations. The study has a limitation of selection of study participants and some potential variables. This is to say that even if the data is collected from representative population across the country, it did not take anemia and its determinants into account to determine sample sizes and the data collection tool lacked considering variables affecting anemia. Therefore, caution should be taken while interpreting some results.

5. Conclusion and Recommendation

Generally, even if the prevalence of maternal anemia during pregnancy declined over time, still it remains a moderate public health problem in Ethiopia. The occurrence of anemia is mainly guided by several sociodemographic (maternal age and region), household (access to radio), and many of the obstetric characteristics including pregnancy trimester, number of under five children in the household, previous history of abortion, breastfeeding patterns (never or continued breastfeeding), and frequency of antenatal care visits.

Therefore, policy makers and other concerned program managers should focus on the key variables in their future planning to deal with the high level of anemia in the country. They should pay attention to older age, those in the eastern region of the country, second and third pregnancy trimesters, having too many under five children, women with previous history of abortion, those with infrequent (≤2) antenatal care visiting pregnant mothers in the programming, and implementation priorities. Further studies are also recommended to be conducted on why some special group of mothers (those who have access to radio and those from the eastern parts of the country) are at special risk of developing the problem without a clear implication to the risk.

Conflict of Interests

The authors declare that they do not have competing interests.

Authors' Contribution

Taddese Alemu developed the analyses parameters, developed objectives, and secured support. He also undertook analysis of the data. Melaku Umeta made detailed analyses and was involved in the write-up and synthesis of the findings. He also reviewed and standardized the study. Both authors read and approved the final paper.

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Annex - B : English Version Questionnaire

QUESTIONNAIRE FOR PREGNANT MOTHERS (Baseline and End line assessment)

SECTION 0: QUESTIONNAIRE IDENTIFICATION DATA

- IR CODE _______ Keble ____________
- Woreda (District) ☐ Tiyo Village (Got) ___________
  - ☐ Dodota
  - ☐ Digelu & Tijo
  - ☐ Bekoji
- Name of the Health Center ____________

INTRODUCTION:

My name is ------------------. I am working as data collector in a survey conducted by the Food Science and Nutrition Department, Natural Science faculty, of Addis Ababa University. We are interviewing women here about food and nutrition related knowledge, attitude, and practice in order to generate information necessary for the planning of appropriate strategies (interventions) in the area and the nation at large. To attain this purpose, your honest and genuine participation by responding to the question prepared is very important & highly appreciated.

CONFIDENTIALITY AND CONSENT

We would like you to answer some personal questions that some people may find it difficult to answer. Your answers are completely confidential. Your name will not be written on this form. The nurses, doctors, and other people will not be told what you said in connection to your name. You do not have to answer any question if you don't want to and you can stop the interview at any time. However your honest answer to these questions will help us to better understand the experience of mothers related to VCT and infant feeding practices. We would greatly appreciate your help in responding to this study. The interview will take about 20 - 30 minutes. Would you be willing to participate?

If yes, proceed

If no, thank and stop here.

____

(Signature of interviewer certifying that respondent has given informed consent verbally)

Visit frequency	1st visit	2nd visit	3rd visit	4th visit	5th visit
Hg level					
GA (in wks)					
Marking					

Anthropometric data : take the following anthropometric data for each women

- Maternal Height (in cm) _________ Maternal weight (Kg) _________
- S/E Result ______
- MUAC of the woman (in cm) _________ Nutritional Category __________

SECTION I: SOCIO-DEMOGRAPHIC AND ECONOMIC INFORMATION

No	Questions	Coding categories	Skip to
101	How old are you? Maternal age in years **(Probe for best estimate)**	___________ Years	
102	What is the highest education level you completed?	Unable to read & write 1 Able to read & write 2 Grade completed ___________	
103	What is your current marital status?	Married 2 Divorced 3 Widowed 4 Separated 5	
104	What is your religion?	Orthodox 1 Protestant 2 Catholic 3 Muslim 4 Other (describe)__________ 5	
105	What ethnic or linguistic group do you belong to?	Oromo 1 Amhara 2 Gurrage 3 Others (describe) ____________ 4	
106	What is your current occupation?	Unemployed 1 Student 2 Housewife 3 Daily laborer 4 Merchant 5 Government Employee 6 Private employee 7 Other (specify) ____________ 9	

107	What is your total monthly family income (approximately)?	_________Eth. Birr No income 1 Don't know 88 No response 99	
108	How much land Does the family owns? (Land size)		
109	Family size (Total number of family members)		
110	House hold head		
111	Access to safe water (Public tap/protected spring)		
112	Which of the following electronics do you have	Radio 1 Television Telephone (Home) Telephone (Mobile) DVD Satellite Dish	

SECTION II: MATERNAL AND FAMILY CHARACTERISTICS

No	Questions	Coding categories	Skip to
201	Number of pregnancies so far (gravidity)?		
202	Number of live births so far (parity)		
203	Number of Abortions so far (Abortus)		
204	Did you have Antenatal care visit during your last pregnancy (s)	1. Yes 2. No	
205	Number of ANC visit?		
206	Practiced Exclusive Breast Feeding	3. Yes 4. No	
207	Last child's sex	1. Male 2. Female	
208	Do you have access to safe water	1. Yes 2. No	
209	If "Yes" to Q208, What is the principal source?	1. Public tap 2. Protected spring 3. Pipe water at home 4. River **5.** other source	
210	Do you have any history of caesarian delivery?	1. Yes 2. No	
211	Do you have any Nausea and/or vomiting during pregnancy?	1. Yes 2. No	
212	Are you Smoking during pregnancy?	1. Yes 2. No	
213	Do you use any illicit drugs during pregnancy		
214	History of alcohol consumption during pregnancy?		

215	How Often do you consume Coffee	1. None (Don't take coffee at all) 2. Rarely (once a week or less) 3. Seldom (once in a day) 4. Often (>= two cups per day)	

SECTION III: FEEDING PRACTICES

No	Questions	Coding categories	Skip to
301	Did you Changed (are you changing) your food intake during your last Pregnancy?	1. Yes 2. No	**2 => 303**
302	If "Yes" to Q301, How? You Changed the :- 1. Frequency of meal 2. Amount of meal per day 3. Both frequency and amount		
303	Did you avoided (are you avoiding) eating any food during your last Pregnancy?	1. Yes 2. No	**2 => 305**
304	If "Yes" to Q303, What food groups/types?		
305	Did you started (are you starting) eating additional food during your last/current Pregnancy?	1. Yes 2. No	**2 => 307**
306	If "Yes" to Q303, What food groups/types?		
307	How many meals are you consuming per day? ○ < 3 meals ○ ≥ 3 meals		
308	Health Seeking behavior (treatment) • Did you Taken drugs to prevent malaria and intestinal worms during pregnancy	1. Yes 2. No	
309	DID YOU **TAKE IRON/FOLATE** DURING YOUR LAST PREGNANCY? *(SHOW IRON/FOLATE TABLETS)*	Yes……………………………………1 No……………………………………..2 DK……………………………………3	

310	IF YES, HOW FREQUENTLY WERE YOU TAKING IT?	Daily……………………………………..1 Weekly…………………………………..2 Occasionally……………………………3 Only once……………………………....4 Other, specify…………………………...5	
311	Did You forget taking the **IRON/FOLATE?**	1. Yes 2) No	**2 => 314**
312	How many **IRON/FOLATE** Pills did you forget per month on average?		
313	What is/are the main reason/s to forget? Please, specify		
314	Did you stopped taking **IRON/FOLATE** tablets while it is not the right time to do so?	1. Yes 2) No	**2 =>401**
315	After how long taking the pills you stopped taking?		
316	What was/were the major reason for stopping?		

SECTION IV : Food Security Questions

No	Questions	Coding categories	Skip to
401	In the past 4 weeks, did you or any household member have to eat some foods that you really did not want to eat?	No…………………………………………………....1 Yes, sometimes (1-10 times)..……….……....2 Yes, Often (more than 10 times)……………..3	
402	In the past 4 weeks, was there ever no food to eat of any kind in your house?	No………………………………………………….1 Yes, sometimes (1-10 times)…..……….……...2 Yes, often (more than 10 times)……..……….3	
403	In the past 4 weeks, did you or any household member go to sleep at night hungry because there was not enough food?	No………………………………………………….1 Yes, sometimes (1-10 times)…..……….……...2 Yes, often (more than 10 times)……..……….3	
404	In the past 4 weeks, did you or any household member go a whole day and night without eating anything at all because there was not enough food?	No………………………………………………….1 Yes, sometimes (1-10 times)…..……….……...2 Yes, often (more than 10 times)……..……….3	
405	How did you overcome the food shortage? (more than one answer is possible.)	Eating less/skipping meals……………………1 Food or cash aid……………………………….2 Migrating household head…………………….3 Selling assets………………………………......4 Eating wild food…………………………..…...5 Other/specify……………………………….......6	
406	Do you own a vegetable garden?	Yes……………………………………..……..1 No……………………………………….........2	
407	If yes, how do you use the produce?	Sell all of it……………………………………...1 Sell part of it…………………………………….2 Use all for HH consumption……………………..3 Other, specify…………………………………..4	

408	Did anyone in the family benefit from food aid rations in the last one year? ***(more than one answer is possible.)***	Yes, Safety Net food rations…………..……..2 Yes, TSF food rations……………………....3 No……………….…………..…………….....4 Refused………………………….….……..…...5 Don"t know..… …………………………….…6 Other (specify program/NGO) … ………..…..7	
409	Did anyone in the household participate in public works in the last one year?	Yes…………………………………..………1 No……………………………………..…....2 DK…………………………….……………...3	
410	If yes, how many days in the last one year?	No. of days____________________	
411	Did they receive food or cash in the last one year?	No……………………………………….......1 Food………………………………….…...2 Cash……………………………………………3 Food and cash alternatively………………........4	
412	Does anyone in the household get direct support (gratuitous beneficiary)?	Yes…………………….……………...……..1 No…………………………………….…..2 DK…………………………………….…......3	
413	If anyone is a gratuitous beneficiary, do they receive food or cash?	Food…………………………………….…...…1 Cash…………………………………..…..2 Food and cash alternatively…………………....3	

SECTION V : KNOWLEDGE SCORES

No	**Questions**	**Coding categories**	**Skip to**
501	DID YOU HAVE DIFFICULTY SEEING IN DIM LIGHT DURING PREGNANCY	Yes……………………………………1 No……………………………………2 I don‟t know…………………………3	
502	CAN YOU LIST FOODS THAT ARE RICH IN VITAMIN A? (MORE THAN ONE ANSWER IS POSSIBLE)	Teff……………………………………1 Wheat ...……………………………..2 Barley....……………………………..3 Green Vegetables....………..………...4 Vegetables.....…………………………5 Fish....…………………………………..6 Meat.....…………………………………7 Egg.....…………………………………8 Milk....………………………………...9 Fruits.…………………………………..10 Butter/oil………..……………………...11 Salt………………………………......….12 Others Specify ___________............13 DK.…………………………….………14	
503	DO YOU KNOW WHAT IS MEANT BY BALANCED DIET / DO YOU KNOW WHAT IS MEANT BY VARIED DIET?	Yes……………………………………...1 No……………………………………....2	
504	MN23. WHAT ARE FOODS RICH IN IRON? **(MORE THAN ONE ANSWER IS POSSIBLE.)**	DN……………………………………....1 Teff……………………………...….2 Wheat ...…………………………..……3 Barley....…………………………..……4 Green Vegetables.........………………5	

		Vegetables.....……………..……….6 Fish....…………………………...……7 Meat....…………………………….….….8 Egg.....…………………………..…………9 Milk....…………………………………10 Fruits …………………………………11 Butter/oil……….……………………..12 Salt……………………………………..13 Others Specify ___________...........14	
505	HAVE YOU HEARD OF GOITER?	Heard ....1 Not heard 2	
506	WHAT ARE THE MAJOR CAUSES OF GOITER? **(MORE THAN ONE ANSWER IS POSSIBLE.)**	Evil eye/evil spirit…………………........1 Not eating enough food……………......2 Drinking dirty water……………….........3 Curse that come through family.........4 Not eating iodized salt…………….........5 Other, specify……………………...........6	
507	HOW CAN GOITER BE PREVENTED?	Eating sea foods………………............1 Eating iodized salt………………..........2 Eating fish…………………….............3 Eating eggs………………….............4 Drinking holy water/tsebel…..............5 Tattooing (Nikisat)………….................6 Other, specify………………………....7	

1) Nutrition Knowledge Score

1. How many foods groups do you know? What are they? can you please tell us..?
2. Can you list some foods that are the main sources of energy for the body?
3. Can you list some foods that are the main sources of body-building substances?
4. Can you list some foods which are protective from diseases?

2) Health and healthcare Knowledge Score

What are the health and healthcare knowledge that maximizes healthy pregnancy and child bearing?

A. Check if the mother knows each (Don't probe)

Eating well (good quality foods).		Do not Smoke		Take iron tables or iron syrup	
Avoid drinking alcohol		Attend ANC-care clinic		Check HIV Status	
Protect from malaria with nets/drugs		Other (Avoid stress/exercise) (equally-weighted sub-scale)			

B. When should a pregnant woman start going to ANC clinic?

C. How many times to attend ANC

D. List 3 activities that occur at ANC clinic (equally-weighted sub-scale)

SECTION V : ATTITUDE SCORE

For the following Questions, just Read the case and ask the mother if she agrees or disagrees:

1. **Theme: Importance of dietary diversity**

   "Tolosa is upset with his wife W/ro Werknesh, because instead of giving him Porridge every day, some days she makes 'Enjera with Shiro', other days sweet potato, and once a week meet. These foods do not provide the same energy as Porridge. Tolosa feels it is best to follow tradition and eat Porridge every day".

   Do you agree with Tolosa?")

2. **Theme: Understands nutritive value of foods**

   "*Fatuma feeds everyone in her household sweet potato for breakfast because it is more nutritious than bread."*

   Is Emiyu's thought correct?

3. **Theme:** Proper nutrition during pregnancy

   "Derartu has just found out that she is pregnant. She is going to try and avoid gaining too much weight during her pregnancy because she knows that if the baby is large she will have a difficult delivery."

   Do you also agree with Derartu?

4. **Theme: Decrease excessive workload during last month of pregnancy**

   "W/ro Danse is in her 9th month of pregnancy. She has had several children before. Every day she goes to the family farm to labor and stay until evening. However, this time, her husband says she should stop cultivating the last month of her pregnancy and just do housework. He will hire some extra labor to help in the field. W/ro Dansie says the money could be spent on other things.

   Do you agree with the husband that it is important to cut down on the amount of hard work you do during the last month of pregnancy?"

5. **Theme:** Attending ANC clinic at 6 months is fine

   "Ayantu is 4 months pregnant. Her friend Abebech is encouraging her to go to the ante-natal clinic. But Ayantu says she is busy and usually goes in her 6th month which is soon enough to get all the important treatments needed before the baby comes.

   Do you agree with Ayantu?"

SECTION VI: PRACTICES, DIETARY DIVERSITY

Household food consumption for 12 groups of foods (1 item per food group type)

Dietary Diversity Questionnaire

Please describe the foods (meals and snacks) that you ate or drank yesterday during the day and night, whether at home or outside the home. Start with the first food or drink eaten in the morning.

Write down all food and drinks mentioned. When composite dishes are mentioned, ask for the list of ingredients. When the respondent has finished, probe for meals and snacks not mentioned.

Breakfast	*Snack*	*Lunch*	*Snack*	*Dinner*	*Snack*

[Household level: consider foods eaten by *any member of the household*, and *exclude* foods purchased *and* eaten outside the home]

When the respondent recall is complete, fill in the food groups based on the information recorded above. For any food groups not mentioned, ask the respondent if a food item from this group was consumed.

Question number	Food group	Examples	YES=1 NO=0
1	CEREALS	corn/maize, rice, wheat, sorghum, millet or any other grains or foods made from these (e.g. bread, noodles, porridge or other grain products) + *insert local foods e.g. ugali, nshima, porridge or pastes or other locally available grains*	1 (millet porridge and rice)
2	VITAMIN A RICH VEGETABLES AND TUBERS	pumpkin, carrots, squash, or sweet potatoes that are orange inside + *other locally available vitamin A rich vegetables (e.g. red sweet pepper)*	1 (pumpkin)
3	WHITE ROOTS AND TUBERS	white potatoes, white yams, white cassava, or other foods made from roots	0
4	DARK GREEN LEAFY VEGETABLES	dark green/leafy vegetables, including wild ones + *locally available vitamin A rich leaves such as amaranth, cassava leaves, kale, spinach etc.*	1 (cassava leaves)
5	OTHER VEGETABLES	other vegetables (e.g. tomato, onion, eggplant), including wild vegetables	1 (onion and tomato)
6	VITAMIN A RICH FRUITS	ripe mangoes, cantaloupe, apricots (fresh or dried), ripe papaya, dried peaches + *other locally available vitamin A rich fruits*	1 (mango)
7	OTHER FRUITS	other fruits, including wild fruits	0
8	ORGAN MEAT	liver, kidney, heart or other organ meats or blood-based foods	0
9	FLESH MEATS	beef, pork, lamb, goat, rabbit, wild game, chicken, duck, or other birds	0
10	EGGS	chicken, duck, guinea fowl or any other egg	0
11	FISH	fresh or dried fish or shellfish	0
12	LEGUMES, NUTS AND SEEDS	beans, peas, lentils, nuts, seeds or foods made from these	1 (groundnuts)
13	MILK AND MILK PRODUCTS	milk, cheese, yogurt or other milk products	0
14	OILS AND FATS	oil, fats or butter added to food or used for cooking	1 (oil in sauce from lunch and dinner)
15	SWEETS	sugar, honey, sweetened soda, sweetened juice or sugary foods such as chocolates, candies, cookies and cakes	1 (sugar in tea)
16	SPICES, CONDIMENTS, BEVERAGES	spices (black pepper, salt), condiments (soy sauce, hot sauce), coffee, tea, alcoholic beverages or *local examples*	1 (fish powder and tea)
Household level only	Did you or anyone in your household eat anything (meal or snack) OUTSIDE the home yesterday?		
Individual level only	Did you eat anything (meal or snack) OUTSIDE the home yesterday?		1

Annex D : Amharic version Dietary diversity questionnaire for grouping food items

የጥያቄ ተ.ቁ	የምግብ ቡድን	ምሳሌዎች	አዎ= 1 & የለም= 2
1	**እህልና የእህል ዘሮች**	በቆሎ ፣ ሩዝ ፣ ስንዴ ፣ ማሽላ፣ ዳጉሳ እና ማንናውም ከእነዚህ የሚዘጋጅ የምግብ ዓይነት (እነጀራ ፣ ገንፎ ፣ ቂጣ ...ወዘተ)	
2	**በቫይታሚን «ኤ» የበለጸጉ አትክሎችና ስራስሮች**	ካሮት ፣ ቀይሰር ፣ ስኳር ድንች ፣ ዱባ ፣ ሚጥሚጣ	
3	**ነጭ ሥራ-ሥሮችና ግንዶች**	ድንች ፣ ቦዬ ፣ ካሳቫ ፣ እና ሌሎች ከስራስሮች የሚሰሩ ምግቦች	
4	**ቅጠላቸው የሚበላ ደማቅ አረንጓዴ ቅጠላቅጠል ያላቸው ተክሎች**	ጎመን ፣ ሳማ ፣ ጥቅል ጎመን	
5	**ሌሎች አትክልቶች**	ቲማቲም ፣ ስንኩርት ፣ እና የዱር አትክቶችም ጭምር	
6	**በቫይታሚን «ኤ» የበለጸጉ ፍራፍሮዎች**	ማንጎ (የበሰለ) ፣ ፓፓያ ፣ ግሸጣ ፣ ኮክ ፣ እና ሎሎችም	
7	**ሌሎች የዱር ፍራፍሬዎች**	ሌሎች ፍራፍሬዎች በተለይ የዱር ሆኑ (እንጆሪ ፣ ቀጋ ፣ ኮሽም)	
8	**ስጋ (የሰውነት ክፍል) (Organ meat)**	ጉበት ፣ ኩላሊት ፣ ልብ ፣ ደም ወይም ሌላ አካል	
9	**ስጋና የስጋ ውጤቶች**	የበሬ ስጋ ፣ የአሳማ ስጋ ፣ የበግ ስጋ ፣ የፍየል ስጋ ፣ የዶሮ ስጋ እና ከእነዚህ የተሰሩ ማናቸውም ምግቦች	
10	**እንቁላል**	የዶሮ እንቁላል ፣ የዳክዬ ፣ የቆቅ ፣ የጅግራ ...ወዘተ እንቁላል	
11	**አሳ**	ትኩስ (ፍረሽ) አሳ ወይም ደረቀ ወይም የባህር አሳ	
12	**ጥራጥሬ**	ባቄላ ፣ ሽምብራ ፣ አተር ፣ ኦቾሎኒ እና ከእነዘህ የሚሰሩ ምግቦች በሙሉ	
13	**ወተትና የወተት ተዋጽዖዎች**	ወተት፣ አይብ፣ ዕርጎ ፣ አሬራ ፣ ወይም ሌላ የወተት ተዋጽዖ	
14	**ዘይትና ቅባቶች**	ዘይት ፣ ቅባት ወይም ቅቤ ተጨምሮባቸው ተሰሩ ምግቦች	
15	**ጣፋጮች**	ስኳር ፣ ማር ፣ ጣፋጭ መጠጦች ፣ ጁስ ፣ ዌም ጣፋጭ ምግቦች እንደ ብስኩት ፣ ቸኮሌት ፣ ኬክ .... የመሳሰሉት	
16	**ቅመሞች ፣ ማጣፈጫዎችና መጠጦች**	ቅመሞች (ጨው ፣ በርበሬ) ፣ ማጣፈጫዎች (ሱጎ) ፣ መጠጦች (ቡና ፣ ሻይ ፣ አልኮል ...ወዘተ)	
	ከቤት ውጪ (ምግብ ቤት ፣ ጎረቤት ፣ ድግስ ቤት....ወዘተ) የተበገቡት ማንናውም ምግብም ሆነ መጠጥ አለ?		
Total food variety score (FVS)			
Total Dietary diversity score (DDS)			

Annex E: Monthly Follow-Up Questionnaire For Pregnant Mothers

- QUESTIONNAIRE ID _______ Keble ____________
- Adress (Phone) ______________
- Woreda (District) --------------
- Village (Got) ___________ Mobile __________________
- Name of the Health Center/Hospital ____________

Summary parameters	1st visit	2nd visit	3rd visit	4th visit	5th visit
Hg level					
Date					
IFA given					
IFA taken					
IFA left					
Reason for not completing IFA					
MUAC of the woman (in cm)					
Maternal weight (Kg)					
Baby					
• Birth weight					
• Birth length					
• Head circumference at birth					
• Chest circumference					

SECTION VI: PRACTICES, DIETARY DIVERSITY

Write down all food and drinks mentioned. When composite dishes are mentioned, ask for the list of ingredients. When the respondent has finished, probe for meals and snacks not mentioned.

Breakfast	*Snack*	*Lunch*	*Snack*	*Dinner*	*Snack*

Annex F : Qualitative Data Collection Questionnaire

Introduction and Consent

Thank you very much for setting aside time to talk with us today. I am. ___________ currently studying nutritional practices of pregnant women in rural communities of Ethiopia, focusing on Arsi zone. The purpose of this Study is to build a comprehensive story of pregnant mothers perceptions, knowledge's and attitudes towards their feeding during pregnancy by employing different research and analysis methods and tapping into the collective wisdom of all the partners, pregnant women, elderly, health professionals and other deemed relevant. donors, implementers and government agencies. In addition to talking about specific programs, your experience and perceptions about the importance of changing social and dietary conditions; the influx and distribution of traditional practices and drivers of key decision that were taken can provide valuable lessons for Ethiopia and other countries.

By meeting with you as a researcher in maternal nutrition policy and programs, we hope to gain a better understanding of:

WHAT are the perceptions of the community about maternal nutrition during pregnancy?

WHAT are the taboos and norms in the community?

HOW they behave and practice feeding and caring for the mother

WHERE and WHEN they these are practiced?

We hope that you had an opportunity to fill in the detailed chart that we sent out before this interview and we can use that information to guide our further discussion.

Before we begin, we want to let you know that any information or examples we discuss during this interview will not be attributed to any specific person or institution to maintain your privacy. All quotes used in the evaluation report will be attributed to a general stakeholder group not by individual, and all identifying information will be removed. You are free to choose not to respond to any of our questions or stop the interview at any time. This interview will take about one hour. If you don't mind, we would like to record this conversation, solely for the purposes of listening attentively now and taking notes alright?

Before we begin, do you have any questions?

PART I: KEY INFORMANTS BACKGROUND INFORMATION

1. Name of informant ________________________

2. Sex a) Male b) Female

3. Age_________ Years

4. Profession / Occupation ____

5. Educational status _____________________________________

5. Name of the Organization in which You are working __________________

- Department/unit in the organization _______________________________
- Physical address of where you work _______________________________
- Email _________ Telephone ____________________________________

6. Work Experience (For health professionals)

- Total Working Experience (in any institution)________________________
- Years worked in this organization _________________________________
- Years of Experience working on nutrition and related ________
- Work role and responsibilities in current organization

PART II: PERCEPTION AND VIEWS OF MOTHERS, HUSBANDS, HEALTH WORKERS, ELDRLY ABOUT MATERNAL NUTRION DURING PREGANCY

Theme	Questions to discuss with probes	
Nutrition Knowledge Score	• Knowledge of food groups o What are the main sources of energy? o Main source of body-building foods o Main sources of protective foods	
Dietary Diversity	• Household food consumption of groups of foods o How many foods groups do you know? o What are they? o What to eat during pregnancy? o What to avoid? why?	
Food intake	• Eating less, more or the same amount of food during pregnancy o How much to eat? Why? o When to eat more? less?	
Weight gain during pregnancy	• How much weight to gain? o estimated size in? o Potential benefits and risks? o who teaches this?	
Seeking treatment	• Taken drugs to prevent malaria and intestinal worms during pregnancy	
Health and healthcare Knowledge	• Do you know about the following health care practices? tell me a little more about each? o Eating well (good quality foods). o Avoid drinking alcohol o Do not Smoke o Attend ANC-care clinic o Take iron tables or iron syrup o Protect from malaria with nets/drugs	

	○ When should a pregnant woman start going to ANC clinic? ○ How many times to attend ANC ○ Activities that occur at ANC clinic	
Attitude	• What is your feeling about the following? ○ Importance of dietary diversity ○ Understands nutritive value of foods ○ Proper nutrition during pregnancy ○ Decrease excessive workload during last month of pregnancy ○ Attending ANC clinic at 6 months is fine	
Taboos	• What foods to avoid during pregnancy? why? • What are the cultural believes and practices in the community? • Underling reasons?	

THANK YOU VERY MUCH FOR YOUR TIME AND INSIGHTS.

Annex G : Afan Oromo version of the questioner

Mata duree : Waraqaa gaaffii dubartoota ulfaaf

Kutaa 0: Odeeffannoo eenyummaa waraqaa gaaffii kana

001 Lakk.eenyummaa waraqaa gaaffiii ___________

002 Naanno: **Oromiya**

003 Aanaa_______________________________________

004 Ganda ______________

005 Geree fi Goxii ________

006 lakkoofsa manaa_____

Seensa :

Maqaan koo ------------------. Dha.Ani hojiin koo odeeffannoo nama gaafachuudhaan sassaabuudha innis dipartimentti saayinsii nyaataa fi sirna sooratаа Fakaltii saayinsii uumamaa yuniversitii Finfinnee dhaan deeggarama.Nuti kan dubartoota gaafachaa jirrn waa'ee nyaataa fi sirna soorataa irratti beekumsa ilaalcha fi shaakala isaa barbaachisan uumuu fi karooraa fi xiyyeeffannoo akka naannoo kanaatti fi akka biyyaatti guddisuuf .Dhimma kan fiixaan baasuuf ,gargaarsii fi hirmaannaan kee gaaffii kana deebisuun ati nuuf gootuu baayee barbaachisaa fi galatoonifamuu kan qabudha.

Ichiiti eeguu Fi Hayyamamummaa

Gaaffii dhuunfaa yookan waa'ee mataa keetii sii gaafannuuf deebii akka ati nuuf kennituuf si affeerra. Deebii kee namni biraa hindhagahu yookan ichiiti kee guutummaatti ni eegna . Maqaan kee waraqaa kana irratti hin barreeffamu.Narsooni ,Doktorootatti ykn nama biraatti gaaffiiishee gaafanneera jenne hin himni.Gaaffii kana deebisuuf fedhii hin qabdu taanaan dhiisuu dandeessa ykn sa'atii barbaadetti dhaabuu dandeessa. Garuu gaaffii kana deebisuuf gargaarsaati nuuf gootuu muuxannoo haadholiin VCT fi shaakala isoollee isaanii nyaachisuuirratti qaban beekuuf nufayyada.Gargaarsa ati deebii kana deebisuuf nuuf gootuuf guddaa si galateeffanna.Gaaffii fi deebiin kun daqiiqaa 20-30 fudhataa Irratti hirmaachuu ni barbaadda mitii?

Yoo isheen ,eyyeen jette itti fufi.

Yoo isheen hin barbaadu jette moo asirratti dhaabi.

(Mallattoon nama gaaffii gaafatee kanaa isaa deebii deebisan sanaaf ichiti isaa akka eegeetti ilaalama)

Kutaa I: Seenaa Jireenyaa fi odeeffannoo haala dinagdee

Lakk.	Gaaffii	Codii Gostootaa	Irra darbi
101	Umuriin kee hagami ? **(Tilmaamaa)**	____________ waggaa	
102	Sadarkaan barumsaa ati xumurte hoo?	Barreessuu fi dubbisuu hin dandasuu 1 Barreessuu fi dubbisuu ni dandeesi 2 Kutaa xumurte ____________	
103	Haala fuudha fi heeruma keetii kan yerooamma?	Hin heerumnne 1 Heerumeera 2 Walhiikneera 3 Abba manaati na jalaa duhe 4 Gargar baaneerra 5	
104	Amantaan kee hoo?	Orttodosii 1 Proteestaantii 2 kaatolikii 3 Muslimaa 4 Kaa biro (Ibsi)___________ 5	
105	Sabummaan kee hoo?	Oromo 1 Amaaraa 2 Guraage 3 Kan biroo (ibsi) _____________ 4	
106	Yeroo ammaan tana hojiin kee maali?	Hojii dhabeesse 1 Barataa 2 Haadha manaa 3 Hojjettuu manaa 4 Dafqaan bulaa 5 Daldalaa 6 Hojjeta mootumma 7 Hojjeta dhuunfaa 8 Kan biroo (ibsi) _____________ 9	
107	Guutummaan galii keetii ji'atti meeqa	__________Eth. Birr	

	(Tilmaamaan)?	Galii hin qabu 1 Hin beeku 88 Deebii laachu dhisu 99	
108	Warri kee lafa hammami qabu? (Lafa safaru)		
109	Baayinni warra keessani meeqa (walii gala nama wajji galan)		
110	Hoogganaan mana keessanii eenyu		
111	Bishaan qulqulluu ni argattuu (Bonbbaa Hawaasaa /Burqaa sirritti eeggamee)		

Kutaa II: Haala maataa fi Haadhaa

Lakk.	**Gaaffii**	**Codii Gostootaa**	**Irra darbi**
201	Si'a meeqa Ulfoofte?		
202	Baayinni ijoollee keetii kan lubbuun jiranii meeqa?		
203	Ulfaa ofirraa baastee haammami		
204	Hordoffii da'umsa duraa gooteetaa hamma ulfaa isa dhumaatti ?	5. Eyyen 6. Miti	
205	Baayinni Hordoffii da'umsa duraa hagami?		
206	Hamma ji'a jahatti mucaa kee harma haadhaa qofa hoosifte	7. Eyyen 8. Miti	
207	Saalli mucaa keetii inni/ isheen dhumaa maalii	3. Dhiiraa 4. Dhalaa	
208	Bishaan qulqulluu ni argattu?	3. Eyyen 4. Miti	
209	Yoo Eyyen jette Maddi isaa Maalii Maal irratti?	6. Boonbaa 7. Bishaan lafa keessa 3. Bishan boolla iddo manaa 4 .Laga **9.** Kan biroo	

210	Seenaa da'umsa kee ilaalchiise kanan duraa yamuu deesitii gargaarsa akka siibilaa dhaan siif harkkifameeraa?	3. Eyyen 4. Miti	
211	Wayitii ulfaatti singiggoo fi oldeebisan ykn balaqqama qabda turte?	3. Eyyen 4. Miti	
212	Wayitii ulfaatt isa dumaa sigaaraa ykn tinboo xuuxxee beekta?	3. Eyyen 4. miti	
213	Wayitii ulfaatti akka qorica wayi nama dammaqsan fudhattee beektaa?		
214	Isa dugaamee nama macheeyisuu dhugdee beektaa?		

Kutaa III: Shaakala haala nyaata

Lakk.	Gaaffii	Codii Gostootaa	Irra darbi
301	Haala nyaata keetii wayitii ulfaa gara dumaatti jijjirteettaa ?	3. Eyyen 4. Miti	
302	Yoo eyyen jette , gaaffii 301,akkamii • Guuyyaatti yeroo meeqaa deddeebitee nyaattaa • Yammu nyaattu moo haammamii • Irra deddeebii fi haammamii nyaattuu		
303	Wayitii ulfaa keessatti nyaata kamillee irraa of qusatte beekta?	3. Eyyen 4. Miti	
304	Yoo eyyen jette gaaffii 303, gosa nyaata isa kami?		
305	Wayitii ulfaa keetii duuraa ykn kan itti jirtuu gosa nyaata garaa garaa nyaatteetta ?	1. Eyyen 2. Miti	
306	Yoo eyyen jette gaafffii 305 irratti ,nyaata gosa Kami ?		
307	Guyyaatti almeeqa nyaatta nyaata ? ○ < 3 nyaata ○ ≥ 3 nyaata		
308	Amala mana yaalaa deemtee qoricha fudhachuuqabda ? • Wayiti ulfaa busaa fi raammoo garaa ofirraa ittisuuf qoricha fudhatteettaa ?	1. Eyyen 2. Miti	

SECTION IV : Galmee Beekumsa (Qabxii Beekumsa)

1) Galmee beekumsa waa'ee sirna soorataaNutrition.

5. Gosa nyaataa meeqa beekta ? Isaan eenyu faa dhaa? Natty himuu dandeessaa?
6. Gosoota nyaataa qaama keenyaaf aannisaa kennan natty himuu dandeessaa?
7. Gosoota nyaataa qaama keenyaa ijaaran tarreessuu nidandeessa?
8. Gosoota nyaataa qaama keenyaa dhukkuba adda adda irraa nu eegan natti himuu dandeessaa?

2) Gallmee beekumsaa haala fayyaa irratti

Beekumsa fayyaa kan fayyummaan ulfaa fi mucaan akka fayyaan dhalatuuf gargaara maali ?

E. Haati akka kana beektu mirkaneessi ?
    - Sirriitti nyaachuu (qulqullummaa isaa kan eeggate)
    - Dhugaati dhuguu dhiisuu
    - Sigaaraa xuuxuu dhiisuu
    - Hordoffii da'umsa dura buufata fayyaatti fudhaachuu
    - Iran fudhaachuu (kinini dhaa ykn shiropii)
    - Busaa irra of eeguu (agoobaratti gargaaramuun ykn qorichaan)
    - HIV qoratamuu
    - Kan biro(dhphachuu dhiisuu ykn cannaqamu dhisun/ isportii hirisuu) (Kallattii hundaan of madaaluu)
F. Dubartiin ulfaan yoom mana yaalaa deemtee hordoffii da'umsa duraa eegaluu qabdi ?
G. Al meeqa hordoffiif deemu gabdi?
H. hordoffii da'umsa duraa keesstti waanneen sadii mulataan tarreessi

Kutaa V : Galmee / Qabxii ilaalcha

Gaaffiilee armaan gadiif ,dubbisiitii haadha warra gaafadhu yoo isheen walii galla ykn walii hin gallu jette.

1.Faayidaa nyaata gosa garaa garaa

"Tolasaan haadha warra isaa Adde Workinashitti dallaneera sababiin isaa immoo utuu inni yeroo hundaa marqaa nyaachuu barbaaduu , isheen immoo gaafa tokko tokko biddeena shiroo dhaan ,dinnicha fi foon torbanitti al tokko kennitiif .Nyaani kun aannisaa walqixa marqaa waliin hin qabu.Tolasaan haala yeroo duriitiin callisee yeroo hundaa marqaa nyaachuu barbaada.

Yaada Tolasaa ni deggartaa?

2 Maalummaa nyaata sirriitti hubachuu

"*Faatummaan yeroo mara ciree irratti nama mana isaanii jiran hunda isaanii dinnicha hojjettee nyaachifti"*

Yaadni Faatummaan sirriidhaa?

3. Wayitii ulfaatti nyaata barbaachisa

"Deraartuun ulfaa tahuu isee beekteettii ulfaatina ishee wayitii ulfaatti gara malee akka hin daballeef ni yaalti ykn ni barbaaddi , sababiin isaa immoo mucaan ishee guddaa yoo tahe ,rakkooti yeroo da'umsaatti namudata jetteetti.

Yaada ishee ni deggertaa?

4. Wayiti ulfaa gara dhumaatti hojii baayisuu dhiisuu

"Adde Danseen ulfaa ji'a sagalidha kana dura ijoollee baayee qabdi yeroo hundaa gara dirree qonnaa hojjechuu dhaan deemtee hamma galgalaatti turti garuu ,yeroo kanatti abbaan manaa ishee akka isheen hojii qonnaa dhiistee hojii mana keessaa hojjettuuf itti hime .humna biraitti dabaluun hojjechuu jalqabe birriin qaxare .Adde Danseen birriin waan biraa irra oola jette

Yaada abbaa manaa ishee ni deggartaa baayina hojii hirisuuf kaninni wayiti ulfaa gara dhuummaatti hojjete?"

9. **Hordoffii da'umsa duraa ji'a jahaaffaatti buufata deemanii tajaajila fudhachuun gaarii dha.** Attending ANC clinic at 6 months is fine

"Ayaantuun ulfaa ji'a 4 dhaa .Hiriyyaan ishee Ababach gara mana yaalaa hordofii da'umsa duraa akka isheen fudhattuuf jajjabeessiti garuu Ayyaantuutti hojiiti itti baayata gara ji'a 6 ffaatti deemen qoricha naaf barbaachisa utuu mucaan hin dhufiin fudhadhe jette.

Yaada Ayyaantuu ni deggartaa?"

Kutaa VI: Shaakalaa Nyaatamman Gosa garaa garaa

Nyaata mana Keessatti nyaatamu kan gosa 12 qabatu

Waraqaa Gaafii Nyaatamman Gosa garaa garaa

Nyaata kaleessa galgala ,guyyaa manatti ykn alatti nyaattee tarreessi .Nyaata jalqaba ganama nyaatte ykn dhugde waliin eegali.

Gosa nyaataa fi dhugaatii armaan gaditti ibsaman hunda isaanii barreessi.qabiyyeen isaanii yeroo ibsaman qabiyyee isaanii tokko tokkon ibsi jedhii gaafadhu.

Ciree	*Turtii (Makisesii)*	*Laaqana Lunch*	*Turtii (Makisesii)*	*irbaata*	*Turtii (Makisesii)*

{Sadarkkaa manatti Hubatamuu : Namoonni mana isaan keessaa galan hunddu nyaataa mana alaa kan nyaatamuu hingashiin kan mana keessaa nyatamu ilaalchisuu.}

Namoonni gaafataman gaaffii kana gubbaa jiiruu guutan boodaa nyaata caqasaman gosa gosa dhaan gutuu .nyaata gosa kamiiyyuu kan hin caqasamiin jiruu taanaan ,nyaataa gosa kana fayyadamuu isaanii gaafachu .

Lakk.gaaffii	Gosa nNyaataa	Fakkeenya	Eyyeen=I Miti = 0
1.	Kanneen firiin isaanii nyaatamu	Boqolloo ,Ruuzii , Qamadii , Boobee , nyaata kanneen warra kana irraa hojjataman ,marqaa ,pasta fi kan biroo	
2.	Nyaata Viitammin badhaadhaanii fi kanneen jirhni isaanii nyaatamu	Kaarotii , cemcemee , mosee fi kanneen biroo	
3.	Hundee adii fi isaanii warra nyaataman	Dinnicha adii kaasaavaa adii fi kanneen biroo	
4.	Kuduraalee baaka	Baala kaasaavaa ,ispinachi	

	magarisa qaban		
5.	Kuduraalee kan biro	Timatimi , qullubbii	
6.	Firiiwwaan viitaminiin badhaadhaan	Maangoo , paapayaa	
7.	Firiilee biroo	Firiilee lagatti biqilan	
8.	Qaama foonii ykn foon diiminaaf kan erga qalamanii nyaataman	Tiruu ,Onnee ,Kalee fi kan biroo	
9.	Foon dheedhiif kan tahan	Sangaa, Rehee ,hoolaa	
10.	Hanqaaquu	Indaaqqoo ykn lukkuu ,daakkiyyee, sololiyaa fi kan biroo	
11.	Qurxummii	Qurxummii haaraa ykn isa gogaa	
12.	Akuletarii Nuugii fisanyiin isaanii kan nyaatamu	Baaqelaa atarii saalixii , nuugii fi kan biroo	
13.	Aannanii fi bu'aa aannanii	Aannaan , baaduu ,itittuu ykn kan biroo	
14	Coomaa fi zayitii	Zayitii , dhdhaa fi kanneen biroo	
15.	Kan miyaawoo tahan	Sukkaara , damma ,chokolatii ,karameella , cuunfaawwan miyaawoo fi kaneen biroo	
16.	Dhugaatii dhandhama ggaarii kanneen qeban	Shayii , buna , dhugootii kan biroo	
	Knneen sadarkaa manaatti qophaahan	Kaleessaati ykn mana keessan keessa nyaata alaa kan nyaate jira ?	
	Sadarkaa nama dhuunqactti	Kaleessaati ate nyaatanyaatteetta?	